CHAPTER 14
Breeding strategies for the 21st Century: domestication of poplar

H.D. Bradshaw. Jr.. and Steven H. Strauss

Introduction

The goal of plantation culture is the economically and ecologically sustainable production of wood to meet a growing worldwide demand. At the end of the 20th Century, poplar culture has reached a fork in the road to the future, and both paths must be followed in order to achieve the goal of sustainability. One branch of the road leads to poplar forestry, where natural or extensively managed stands have multiple functions in addition to wood and fiber production, such as watershed protection, maintenance of biodiversity, and recreation. Aspen in the boreal ecosystem of North America is an example of a species for which a poplar forestry management strategy seems appropriate. This form of forestry strongly supplements the ecological preservation functions of biological and riparian reservations that include poplars as key species.

The other branch leads to poplar agriculture, where "tree farms" are intensively managed with the dominant goal of producing the maximum volume and quality of wood, fiber, and biomass for energy on the smallest possible land base. The best agricultural practices will be used to increase economic yields, including planting on the best available sites; fertilization and irrigation; control of weeds, pests, and diseases; and genetic improvement through conventional breeding, interspecific hybridization, vegetative propagation (cloning), and biotechnology. Examples of poplar agriculture in North America are the large-scale hybrid poplar fiber and energy farms established in the past 20 years, primarily by the forest products industry and government agencies. Increasing the productivity of plantation forests could have many benefits, including providing an alternative crop for farmers and sparing native forests from overharvesting. In this chapter, we

H.D. Bradshaw, Jr. College of Forest Resources, University of Washington, Seattle, WA 98195, U.S.A.

Steven H. Strauss. Department of Forest Science, Oregon State University, Corvallis, OR 97331, U.S.A.

will focus upon breeding strategies designed for poplar agriculture, where the greater potential for yield increases justifies the expense and effort required to implement state-of-the-art breeding technology.

Predicting the future course of technology has led to more embarrassments than successes. Nevertheless, we venture the forecast that the 21st Century will be remembered by the forest products industry as the Era of Tree Domestication, and that poplar will lead the way. Just as poplar farmers have adopted an agricultural paradigm for cultural practices such as fertilization, irrigation, and weed control, poplar breeders will follow the agricultural example in genetics, beginning with the most fundamental process in crop development: *domestication.*

Plant domestication

Crop domestication is among the greatest technological advances in human history (Diamond 1997), enabling the efficient, reliable production of food on a small land area. The domestication of agricultural crops from their wild ancestors was begun by prehistoric farmers 5000-10 000 years ago, and continues today as a result of ongoing breeding programs. Few people realize how different domesticated crop plants are from their wild relatives. Whereas a wild plant struggles for existence in a complex ecosystem, expending much of its energy sensing its environment and competing with neighboring plants for access to light, water, and nutrients, the environment of a cultivated crop is simplified and optimized by the farmer to allow the domesticated plant to devote its energy almost exclusively to the production of useful structures such as seeds, tubers, or fruits. The process of domestication greatly improves the yield and quality of the crop for human use, and simultaneously reduces or eliminates the ability of the plant to survive in its original wild state. The crop depends upon the farmer for survival as much as the farmer depends upon the crop for food and income.

The conversion of wild plants into domesticated crops involved radical changes (mutations) in the genes of crop ancestors. It is only in the past 15 years, with the development of modern molecular genetic methods to examine the detailed structure of plant DNA, that we have begun to discover the number and identity of genes that were mutated during the course of plant domestication.

For example, the conversion of wild mustard flowering stalks into the striking "flower-heads" of cauliflower appear to have involved mutations in just two master genes that control flower and fruit development (Smyth 1995). Dwarf and semi-dwarf rice and wheat strains, the foundation of the Green Revolution which doubled grain yield between 1960 and 2000, are the result of a single mutation (Peng et al. 1999). Based upon the work of George Beadle (Beadle 1972) and John Doebley (Doebley and Stec 1991) we know that mutations in just five major genes transformed the inedible seed-head of teosinte (the wild ancestor of corn) into an "ear" of corn (Fig. 1). Maize (corn) has at least 50 000 different genes, but only five of them had to be altered by mutation to transform teosinte from a

Fig. 1. The transition from inedible wild teosinte (A) to primitive maize (B-D) required mutations in just five major domestication genes. Modern maize is shown on the right (E). Photo courtesy of John Doebley.
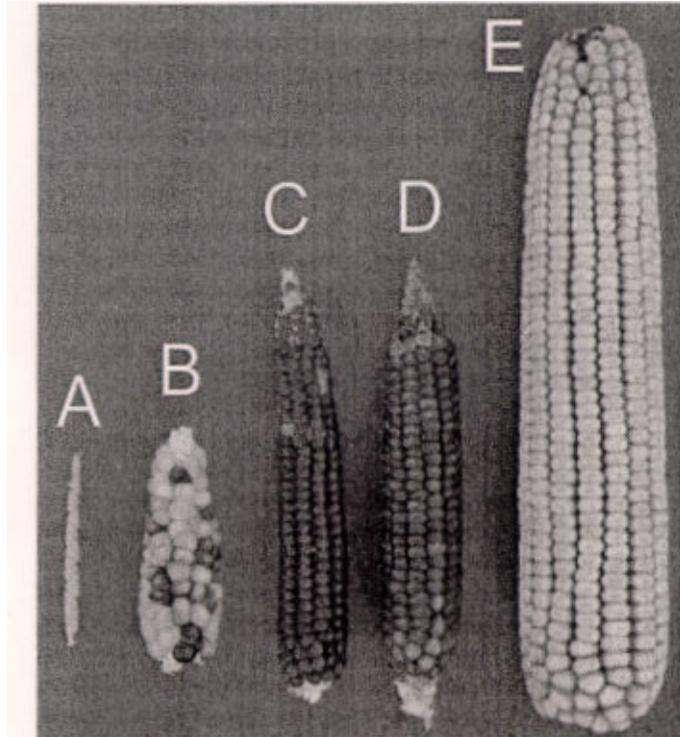
weedy grass growing exclusively in the central highlands of Mexico into the most important agricultural crop across North America.

A genetically similar, though less visibly dramatic, domestication history has occurred in many other crops. Interestingly, although a few trees used in horticulture (e.g., apples, olives, cherries) have been domesticated to a significant degree, none have included forest trees grown for wood. There are several reasons for the lack of forest tree domestication. First, until the 20th Century forest trees were grown mostly under natural or semi-natural conditions, so that "domestication genes" would be a disadvantage to them. It is only under the benign conditions of a farm that domesticating mutations have value. In contrast, crops have been grown in an agricultural setting for the past 100 centuries, allowing a long time period for domestication genes to be recognized and perpetuated through selective breeding. Second, the generation time of forest trees grown under near-wild conditions is very long, commonly 10-30 years from planting a seed until the tree produces its own seeds, so that progress by conventional breeding is slow when compared with annual crops. Wheat, rice, corn, soybeans, and other crop plants have been bred by humans for many thousands of generations. A major facet of

domestication of fruit trees was selection for earlier and more intense reproduction, which then would feed back to speed further domestication. In contrast, the most advanced forest tree breeding programs are only in their third or fourth breeding cycle. Third, many "domestication" mutations are genetically recessive, and so can only be observed by careful inbreeding. Most tree species are strictly outbreeding (cross-pollinating), and so recessive domestication genes remain hidden. Many crop plants are naturally self-pollinated, or can be easily inbred, making it simple to discover beneficial recessive mutations.

If we accept the idea that trees can be domesticated to dramatically improve their yield and quality under the intensive agricultural systems typical of modern farming, and that domestication may require mutations in just a handful of critical genes, we are left with four important questions:

1. What will a domesticated tree look like?

2. How will we identify domestication genes?

3. How will domestication genes be deployed in breeding programs and production plantations?

4. What consequences will domestication genes have for poplar agriculture?

Characteristics of a domesticated tree

Before attempting to describe the (future) appearance of a domesticated poplar, it is advisable first to ask why wild forest trees have the appearance that they do. Wild trees have been shaped by the force of Darwinian natural selection, not by the hand of humans as crops have. The primary commercial interest in forest trees is for the wood they produce. Why do wild trees produce wood? The answer is: "So that they can grow tall." Without the strength and stiffness that wood provides to the stem, herbaceous plants can never grow as tall as a tree because they would collapse under their own weight. Why do wild trees grow tall? They do so because there is competition among plants for sunlight to power photosynthesis and thus provide resources for sexual reproduction. Trees are Nature's ultimate solution to the problem of competition for light. Only the dominant trees in the forest canopy are able to produce the maximum number of seeds and (or) pollen, and thus pass on their genes to future forests. Reproductive output, and not wood production, is the currency of natural selection in wild trees. Wood is just a tree's way of making seeds, and a wild tree makes as little wood as is necessary to achieve its reproductive goals.

Domesticated trees, on the other hand, will be expected to channel their photosynthetic effort into the production of wood, and not into reproduction. Domesticated trees growing in a plantation do not need to compete with each other or with weeds for access to light, since the farmer will plant the trees with enough space

between them, and will control weeds. In a clonal plantation, each neighboring tree is genetically identical, so it makes no sense for the trees to compete with copies of themselves.

Domesticated trees will therefore have a greater stem diameter in proportion to their height than wild trees do. In physiological terms, the sink strength of the vascular cambium will become greater relative to the sink strength of the apical meristem. Thicker, stiffer stems will result in increased wood quality (less reaction wood), larger piece sizes, less waste during sawing, and a greatly increased harvest index (ratio of harvestable product to total biomass) because of the reduced need for photosynthate allocation to structural roots. Because of their inherently stiffer stems and reduced need for longevity compared to wild trees, it will be possible to reduce significantly the proportion of lignin without causing stem form or bending strength to be compromised. The reduction in lignin amount, which is energetically very costly to synthesize, is likely to increase cellulose yield and tree growth rate (Hu et al. 1999). Changes in lignin structure can also reduce the intensity, and thus the cost, of chemical and mechanical treatment during pulping (Baucher et al. 1996).

In a domesticated tree, the number, length, and diameter of branches will be reduced, while maintaining useful dimensions (4-8 feet) of internodes (clear wood) between whorls of branches. This will improve wood quality by producing fewer knots, and may improve productivity by increasing water flow to the leaves (Tyree et al. 1983; Tyree 1988). The domesticated tree will carry the minimum number of leaves necessary to support rapid growth. Crown geometry will become optimized for efficient use of space. Changes in branching and canopy geometry are a common feature of domesticated plants, including maize and the other cereal crops where increased leaf angles have accompanied the shift to very high planting densities (Duvick 1992).

Domesticated forest trees would flower late or not at all, making available additional resources for vegetative growth. A majority of the increased productivity during domestication of crop plants has come from increased allocation of photosynthate to reproductive tissues (Evans 1980). In forest trees, we desire the opposite result: increased vegetative stem mass at the expense of reproductive organs and other vegetative parts. Many of the tree species grown under intensive culture, including poplars, are early successional species that are adapted to grow rapidly in full sunlight while colonizing a new site. After a short time, these pioneer species begin to reproduce vigorously, and continue dispersing copious quantities of seeds and pollen for the remainder of their lives. The combination of rapid growth potential and heavy flowering should enable a significant increase in tree growth rate if flowering could be postponed until after harvest, or prevented entirely (Strauss et al. 1995). Because poplars grown in tree farms are vegetatively propagated, and often composed of hybrids that are not used for further breeding, elimination of flowering in plantations would not pose a difficulty for poplar agriculture. However, preventing flowering in a controlled, specific

manner will be very difficult to achieve without genetic engineering (discussed below).

Many species of poplars, particularly the aspens and related taxa, are difficult to propagate vegetatively via rooting or other means. The loss of competence for regeneration with age and tissue differentiation is almost universally observed in forest trees, greatly limiting the economic use of cloning for tree improvement. However, the amenability of trees to vegetative regeneration usually shows great genetic diversity within and among species, suggesting that genes can be isolated that strongly modify competence for regeneration. The addition of such genes via transgenesis, preferably subject to regulation by an inducible promoter (one that causes expression in response to an exogenously applied substance), could greatly increase the rate of genetic gain via clonal propagation.

Discovery of domestication genes for poplar

Three experimental elements may be integrated to discover domestication genes. First, thousands or even millions of poplar seedlings could be tested for rare domestication traits arising as the result of a mutation in a major domestication gene. This random, hit-or-miss approach was used successfully to domesticate every modern crop plant, and so might be considered the "traditional" method of domestication. The principal disadvantage of this method is that progress is very slow and sporadic. Because of the long generation interval of trees, and their cross-pollinating mating systems, rare recessive mutations are extremely difficult to find. A directed program of inbreeding is likely to be required to expose recessive mutations; however, inbreeding will result in plants of poor form and low vigor due to the high genetic load of trees, making domestication alleles difficult to discern. Domestication by this traditional route could take many decades or centuries, even with the rapidly growing knowledge of plant genetics and the amenability of poplar to biotechnology.

The other two approaches to poplar domestication depend entirely upon the use of genetic transformation (i.e., asexual gene transfer), also known as transgenic technology, to overcome the severe limitations of more traditional domestication methods. The second technique for identifying domestication genes in poplars is to use the rapidly growing genetic information database surrounding model herbaceous plants such as the tiny mustard weed *Arabidopsis. Arabidopsis is* the first plant to have all of the DNA in its chromosomes completely sequenced, giving us an unabridged list of all the genes in a higher plant. Its small genome, small size, rapid life cycle, and amenability to transformation have also made *Arabidopsis* the plant species of choice for cloning genes that regulate plant development. There is a large and growing collection of genes known to affect such traits as the response to competition, flowering, stem growth, disease resistance, and tolerance to abiotic stresses (e.g., cold and drought).

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This wealth of "candidate genes" for domestication from *Arabidopsis* and crops can be used directly to inform efforts to isolate similar kinds of genes (homologs) from poplar and other forest trees (Table 1). This will be most efficient once a large set of expressed sequence tag (EST) sequences are available (Sterky et al. 1998), enabling direct identification of the desired genes via computer searches. However, it is also feasible to isolate new genes directly from poplar DNA based upon sections of the genes that are highly conserved among species. After isolation of each of the candidate domestication genes, their function must be tested in poplars. In trees this can only be done by testing for production of a "mutant" phenotype via transformation. Typically, the effects of hyper-expression and suppression of the gene are studied to understand its function. The gene can also be transformed under the control of its own, natural promoter to assess its subtler effects. Because transgenes are inserted randomly into the genome, study of a population of transgenics is akin to studying a range of alleles (gene variations). A gene that is deleterious when completely knocked-out or highly expressed could still be useful when a less dramatic change in its expression is engineered.

A third strategy for finding domestication genes in trees requires direct, random mutagenesis. Because of the difficulty of inbreeding and the delayed flowering in trees, methods that cause dominant mutations are desirable, as they can be discerned directly after transformation. Activation tagging (reviewed by Weigel et al. 2000) is a method whereby a strong promoter or enhancer of gene expression is randomly inserted into a genome to hyper-activate nearby genes, causing dominant mutations (Fig. 2). Because the frequency of gene activation is low, it requires that a very large population of transgenic trees be produced and screened for domestication traits in a field trial (e.g., tens of thousands to hundreds of thousands of unique transgene insertions). Once traits of interest are observed in the mutated trees, the responsible gene can be readily isolated because the promoter and associated DNA act as a tag to facilitate recovery from the genome. The recovered DNA is then sequenced and re-transformed to verify that it is the cause of the domestication trait. In *Arabidopsis,* activation tagging resulted in the isolation of a number of regulatory genes that were missed with other mutagenesis methods. Although most domestication alleles identified in crops have been

Table 1. Genes with potential for forest tree domestication.

Trait	Gene function	Gene identified in:	Example
Height growth	Hormone synthesis	*Arabidopsis*	Lester et al. 1997
Height growth	Transcription factors	Rice, wheat, maize	Peng et al. 1999
Stem thickness	Unknown	*Arabidopsis*	Talbert et al. 1995
Lateral branching	Transcription factors	Tomato, maize	Doebley et al. 1997
Competition sensing	Phytochrome	*Arabidopsis*, tobacco	Smith 1995
Flowering	MADS box	*Arabidopsis*	Michaels and Amasino 1999

Fig. 2. A schematic diagram of how activation tagging works. A gene enhancer lacking a transcription start site is randomly inserted into the genome. When it inserts sufficiently near to a gene to enhance expression over wild-type levels, it can lead to a novel phenotype of biological or economic interest. The enhancer element and associated DNA then provides a molecular "tag" with which to isolate the gene.
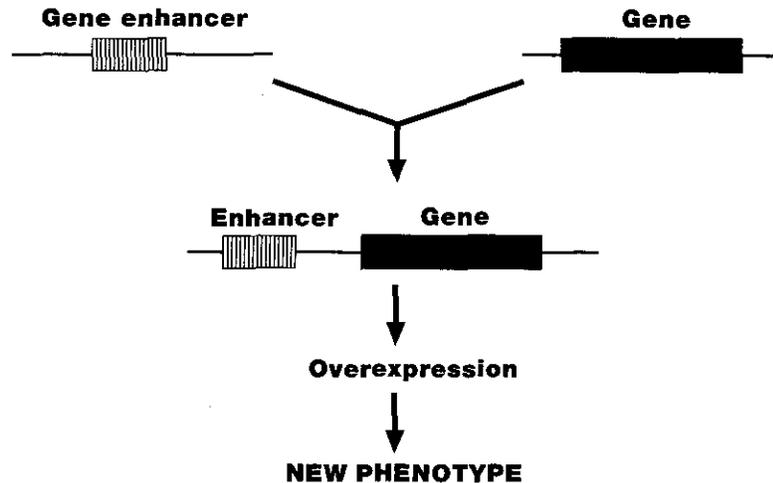
recessive in gene action, the identification of dominant alleles via activation tagging would be especially valuable for trees because of the difficulties of inbreeding-based genetic improvement systems.

Genetic engineering as a core technology for the "Gene Revolution" in poplar culture

Domestication genes are expected to be alleles of wild-type genes. Because these alleles would have deleterious effects on tree fitness in the wild, they should be very rare in germplasm collections from natural populations. However, even if they could be discovered in wild collections and indexed using genetic markers, it would take numerous generations to introgress them into the number of breeding lines required for deployment in production populations. With the delayed flowering of trees, and the extreme genetic diversity within and among regional, national, and international breeding programs, a broad domestication program using traditional breeding would require centuries to achieve, even in poplars. Unless flowering can be greatly and routinely accelerated (see below), genetic transformation appears to be the only option for the use of domestication genes in forestry.

A key advantage of transformation is that new genetic functions can be introduced into highly productive genotypes with minimal disruption of its genetic qualities. In contrast, the introduction of new genes via breeding requires a

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complete shuffling of the genotype, and is limited to those genes available in the gene pool of the species and its interfertile relatives. Because of mutations that are introduced during transformation and associated in vitro culture (somaclonal variation), it is necessary to screen a number of "progeny" (lines) from transformation experiments to identify those with normal growth and stable, desirable expression of the new trait. This can take several growing seasons. The commercial successes of genetic engineering, and the production of many normally growing transgenic poplars in field trials around the world (Meilan et al. 2000), suggest that finding normal, stably-performing transgenic trees is not a substantial obstacle.

The deployment of domestication transgenes in trees will raise very different concerns than have the first generation of transgenes. The first wave of transgenic trees largely consisted of herbicide- and insect-resistant varieties. These two traits raise concerns about the potential for increasing the "weediness" of transgenic poplars. In contrast, domestication genes should handicap trees if they spread to a natural environment, reducing concerns that transgenic poplars might disrupt ecosystems due to their increased vigor, or reduce options for weed control by farmers.

Domestication transgenes could still harm wild populations, however, if not deployed wisely. If domestication transgenes do not include those which prevent sexual reproduction, the use of large populations of transgenic poplars near small, fragmented populations of wild poplars could result in a loss of viability in the wild stands over time. Genetically unfit pollen and seeds from plantations might numerically overwhelm the reproduction of small wild populations, driving them toward local extinction. The combination over the landscape of healthy wild or extensively managed populations, with highly productive agriculture-style plantations that do not produce sexual progeny, is therefore highly desirable. This kind of landscape integration is also likely to be most effective at providing habitat and other ecosystem services while allowing highly efficient wood production.

Goals for poplar domestication

What are the targets for domestication in forest trees that we can foresee in the next century? The goals would be highly diverse, depending on management objectives. However, we suspect that transgenic plantations would embody several of the trait improvements described below:

> 1. *Increased yield.* The ultimate goal of domestication is to produce more wood fiber per unit area and time at less expense. This should be possible by allocation of more photosynthate to stems versus roots, branches, and reproductive tissues; less investment in costly biochemicals such as lignin above the levels needed for structural stability and herbivore protection in tree farms; and improved physiological efficiency due to changes in

height and crown architecture that maximizes photosynthesis per unit area. If fundamental aspects of development can be controlled, such as the timing of flowering and the juvenile-mature transition, it will be possible to speed genetic gain by allowing more rapid breeding and less costly clonal propagation. By combining domestication transgenes with genes for agronomic traits such as herbicide and pest resistance, the yield and environmental benefits of these genes (e.g., reduced water usage, reduced use of undesirable pesticides and herbicides) should be more readily achieved with fewer undesired side effects - and thus, hopefully, with greater public acceptance.

2. *Tailoring of wood properties to specific end uses.* Wood that is used for structural purposes, pulp, or energy has very different chemical and physical requirements. For example, by changing the amount and chemistry of lignin in wood, its energy content, digestibility, and amenability to processing into biofuels such as ethanol can be altered markedly. The rapidly expanding catalog of genes expressed in xylem and cambium as a result of genomic sequencing projects should make it possible to alter traits such as fiber length, cell wall thickness, and microfibril angle, enabling major changes in wood and pulp strength, density, and dimensional stability.

3. *New products and ecological functions.* Breeding and hybridization has on many occasions given rise to crops with novel properties that determine their use (e.g., the various forms of wheat, rice, and oil crops). Transformation can speed this process; however, it can also enable entirely new biochemical products, or metabolic processes, to be introduced. For example, production of cellulase by trees in bioenergy plantations, or xylanase for trees in pulp plantations, could be a co-product that increases the value of the trees and aids their eventual processing (Morrison et al. 1999). These products could be produced continuously, or synthesized on demand via induced gene expression systems. By engineering genes into trees that facilitate the removal of heavy metals and other pollutants from soils, their value for bioremediation of polluted soils (e.g., Rugh et al. 1998) and biofiltration of farm runoff could be greatly enhanced.

Conclusion

The combination of new knowledge from genomics and molecular biology, with the established feasibility of genetic engineering in poplar, make it an ideal genus with which to demonstrate the power of biotechnology for domesticating trees to meet human needs. Because of the recalcitrance of trees to traditional genetic manipulation, without genetic engineering domestication would proceed extremely slowly and many goals would be effectively unobtainable. Whereas the domestication of the major crops required thousands of years, we expect that genetic

engineering will allow the domestication of trees to be accomplished within a few decades. This Gene Revolution may be essential to meeting the wood, fiber, energy, and environmental needs of the rapidly growing world.

Acknowledgements

References

Beadle, G.W. 1972. The mystery of maize. Field Mus. Nail. Hist. Bull. 43: 2-11.

Baucher, M., Chabbert, B., Pilate, G., van Doorsselaere, J., Tollier, M.-T., Petit-Conil, M., Cornu, D., Monties, B., van Montagu, M., Inze, D., Jouanin, L., and Boerjan, W. 1996. Red xylem and higher lignin extractability by down-regulating a cinnamyl alcohol dehydrogenase in poplar. Plant Physiol. 112: 1479-1490.

Diamond, J. 1997. Guns, germs, and steel: the fates of human societies. W.W. Norton, New York. 480 pp.

Doebley, J., and Stec, A. 1991. Genetic analysis of the morphological differences between maize and teosinte. Genetics, 129: 285-295.

Doebley, J., Stec, A., and Hubbard, L. 1997. The evolution of apical dominance in maize. Nature (London), 386: 485-488.

Duvick, D.N. 1992. Genetic contributions to advances in yield of U.S. maize. Maydica, 37: 69-79.

Evans, L.C. 1980. The natural history of crop yield. Am. Sci. 68: 388-397.

Hu, W.-J., Harding, S.A., Lung, J., Popko, LL., Ralph, J., Stokke, D.D., Tsai, C.-J., and Chiang, V.L. 1999. Repression of lignin biosynthesis promotes cellulose accumulation and growth in transgenic trees. Nature Biotech. 178: 808-812.

Lester, DR., Ross, LL, Davies, P.J., and Reid, LB. 1997. Mendel's stem length gene *Le.* encodes a gibberellin 3-beta-hydroxylase. Plant Cell, 98: 1435-1443.

Meilan, R., Ma, C., Cheng, S., Eaton, LA., Miller, L.K., Crockett, R.P., DiFazio, S.P., and Strauss, S.H. 2000. High levels of Roundup and leaf-beetle resistance in genetically engineered hybrid cottonwoods. In Hybrid poplars in the Pacific Northwest: Culture, Commerce and Capability. *Edited by* K.A. Blamer and LL Johnson. Washington State University Cooperative Extension, Pullman. In press.

Michaels, S.D., and Amasino, R.M. 1999. FLOWERING LOCUS C encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell, 11: 949-956.

Morrison, D.L., Coleman, G., Dale, B.E., Finizza, A.J., Hall, R., Johnson, D., Nichols, R., Sperling, D., and Strauss, S.H.. 1999. Review of the research strategy for biomass-derived transportation fuels. National Research Council (U.S.A.), Board on Energy and Environmental Systems. National Academy Press, Washington, D.C. 48 pp.

Peng, J., Richards, D.E., Hartley, N.M., Murphy, G.P., Devos, K.M., Flintham, J.E., Beales, J., Fish, L.J., Worland, A.J., Pelica, F., Sudhakar, D., Christou, P., Snape, JW., Gale, M.D., and

Harberd, N.P. 1999. "Green revolution" genes encode mutant gibberellin response modulators. Nature (London), 400: 256-261.

Rugh, C.L., Senecoff, J.F., Meagher, R.B., and Merkle, S.A. 1998. Development of transgenic yellow poplar for mercury phytoremediation. Nature Biotechnol. 16: 925-928.

Smith, H. 1995. Physiological and ecological function within the phytochrome family. Ann. Rev. Plant Physiol. Plant Mol. Biol. 46: 289-315.

Smyth, D.R. 1995. Origin of the cauliflower. Carr. Biol. 5: 361-363.

Sterky, F., Regan, S., Karlsson, J., Hertzberg, M., Rohde, A., Holmberg, A., Amini, B., Bhalerao, R., Larsson, M., Villarroel, R., Van Montagu, M., Sandberg, G., Olsson, O., Teeri, T.T., Boerjan, W., Gustafsson, P., Uhlen, M., Sandberg, B., and Lundeberg, J. 1998. Gene discovery in the wood-forming tissues of poplar: Analysis of 5,692 expressed sequence tags. Proc. Natl. Acad. Sci. U.S.A. 95: 13330-13335.

Strauss, S.H., Rottmann, W.H., Brunner, A.M., and Sheppard, L.A. 1995. Genetic engineering of reproductive sterility in forest trees. Molec. Breed. 1: 5-26.

Talbert, P.B., Adler, H.T., Parks, D. W., and Comm, L. 1995. The *REVOLUTA* gene is necessary for apical meristem development and for limiting cell divisions in the leaves and stems of *Arabidopsis thaliana.* Development, 121: 2723-2735.

Tyree, M.T. 1988. A dynamic model for water flow in a single tree: evidence that models must account for hydraulic architecture. Tree Physiol. 4: 195-217.

Tyree, M.T., Graham, M.E.-D., Cooper, K.E., and Bazos, L.J. 1983. The hydraulic architecture of *Thuja occidentalis.* Can. J. But. 61: 2105-2111.