Spring Garden Checklist

LESLIE YOST, HORTICULTURIST

'Sensation Lilac' provides vibrant flowers in the spring.

'Grefsheim Spirea' is an excellent spring flowering shrub that does well with renewal pruning every few years.

'Beauty Bush' is an attractive, large spring flowering shrub that benefits from frequent renewal pruning following flowering.

Spring has sprung! Use this checklist to create the most ideal conditions for your garden and landscape.

- ☐ Pull mulch away from planting beds or work it into soil if it's well composted.
- ☐ If you didn't divide perennials in the fall, it can easily be done before new growth appears. Once the new growth appears, you can still divide, but it just won't be as tidy.
- ☐ Clean up any debris from plant dieback.
- ☐ Review garden plans and double check catalog order ship dates.
- ☐ Walk through the landscape to see if plants or hardscape materials have heaved. Replant or replace anything that is out of place.
- ☐ Prune spring flowering trees and shrubs after they complete their bloom cycle. Pruning sooner will remove this spring's bloom buds.
- ☐ Vegetable gardeners will want to put out onions, lettuce, radishes, and other cool season crops early in the spring.
- ☐ Start annual seeds inside for planting in the garden once the threat of frost passes.
- ☐ Bring out any tender rhizomes and bulbs, such as canna lilies or gladiolas, that were stored for the winter.
- ☐ If necessary, fertilize and put down a pre-emergent herbicide on your lawn.
- ☐ As the season warms and the threat of frost passes, enjoy planting all the wonderful selections you've made from catalogs and your spring shopping.
- ☐ Don't forget to mulch! Mulching helps reduce weeds and maintains soil moisture.
- ☐ Begin a gardening journal. Marking the date and weather conditions of gardening tasks in a notebook this year will help you decide when to tackle some of your gardening chores next year.

Enjoy this wonderful time of year!

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Dwarfism Genes for Modifying the Stature of Woody Plants: A Case Study in Poplar

BY ELIZABETH ETHERINGTON, HARISH GANDHI, VICTOR BUSOV, RICHARD MEILAN, CATHLEEN MA, KEVIN KOSOLA, AND STEVEN H. STRAUSS

Farmers and arborists have been selecting trees with a diversity of shapes and sizes for millennia. Generally, the tendency has been to choose smaller, narrower trees that can most easily fit in fencerows, around crops, and in urban yards where space is at a premium. Smaller trees often have other advantages as well, such as early and heavy flower and fruit production, increased wind-firmness, and higher general stress tolerance.

A similar trend has occurred in agricultural crops over the last 50 years, where cereals have been bred with semi-dwarfism genes to keep them short and stout to avoid lodging and to maximize allocation of energy to seeds rather than stems. These "green revolution" varieties of rice and wheat have had dramatic benefits for crop yields and have been credited with preventing the starvation of many millions, and improving national economies (Silverstone and Sun 2000).

The interest in semi-dwarf varieties has led to considerable activity in basic science toward identifying the genes that are responsible for control of plant height. This creates the potential for gene-directed "trait engineering,"[1] a complement to the purely form- and selection-based approaches common to traditional breeding. The extraordinary power of "genomics science" has allowed a number of the genes, and the associated physiological mechanisms for genetic control, to be described in detail. The genomic paradigm relies on model organisms to speed analysis of basic mechanisms and discovery of genes, uses sequencing and analysis of genes by the thousands via "gene chips" and other high-tech tools, and references genetic information across all plant species by a computer in the blink of an eye. The genes for height control that have received the most attention are those from the class of hormones known as gibberellic acids (GAs). The active forms of GA promote the elongation of plant cells. When active GA synthesis, or signaling caused by GA, is disrupted, cells do not fully elongate and plants remain short and stocky. When the activity of genes that degrade active GA are elevated, plants similarly remain short (Salamini 2003).

Figure 1. Field view of pairs of trees of the same gene insertion type during the second year after planting showing strong dwarfism (foreground) or changes in crown shape (to left of the 1.5-m woman).

To study how these kinds of genes might be used to control the stature and form of a tree, we inserted a variety of GA-related genes into a test variety of a hybrid poplar (*Populus tremula* x *P. alba*). This poplar variety was selected because healthy, uniform plants can be readily obtained from cells that have been genetically modified in tissue culture using the gene-transfer agent *Agrobacterium*. *Agrobacterium* is widely used because it is a natural plant genetic engineer that is effective on a wide range of plant species. Most of the genes were obtained from the model plant *Arabidopsis*, but one also originated from pea and from poplar itself. Most of the genes were strongly active throughout the plant because a generic kind of a gene expression controlling element was used (the *35S* promoter). The products of these genes metabolized active GA faster than usual (*2-oxidase*), or inhibited the cellular signal needed for GA to activate the metabolic pathways required for cell growth (*gai*, *rgl2*).

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We also used two "milder" gene forms, one with the native, wild-type *GAI* gene promoter and a mutant version of the strong GA inhibitor gene gai and another with the native *GAI* gene promoter and the native *GAI* gene. In total we used seven distinct kinds of GA-inhibiting genes and generated more than 160 different types of insertions

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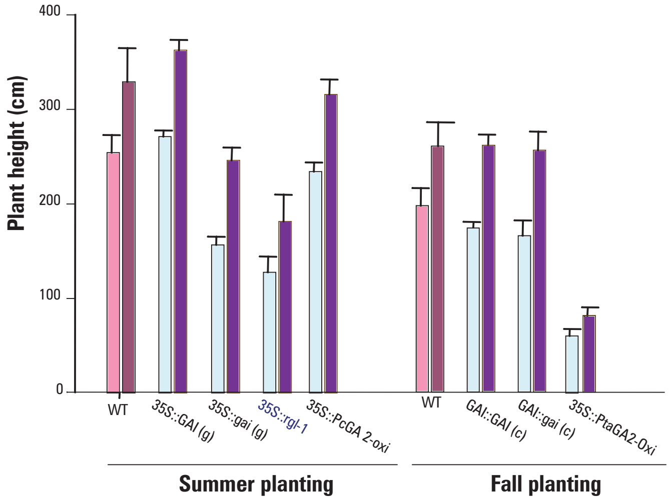

Figure 2. Genes differ greatly in the strength of dwarfism they impart. Pairs of bars are based on measurements taken in 2004 and 2005; trees were planted in summer or fall of 2003. Brackets are standard errors of the mean, g=genomic DNA, c=cDNA source.

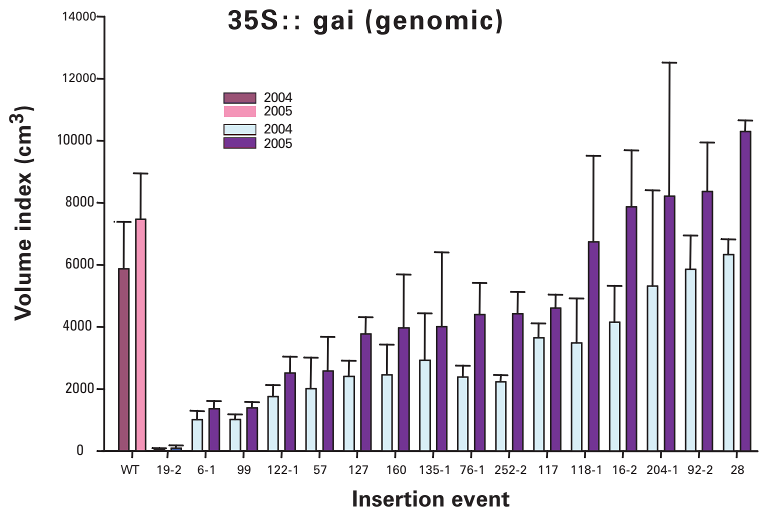

Figure 3. The gai gene gave a wide range of dwarfism among insertions, allowing the extent of semi-dwarfism to be "customized" by breeders. WT = wild-type.

of these genes into poplar DNA (each insertion, even with the same gene, can produce somewhat different effects). In 2003, we planted more than 600 genetically modified trees in the field after authorization from USDA and assessed them several times for variation in size and appearance. Identical ramets (i.e., copies of the same variety) of each gene insertion type were planted in pairs in the field to assist in visualizing their genetic differences.

Large modifications to size and form

Though most were not from poplar, the majority of genes we inserted had a dramatic dwarfing effect on tree growth. This shows that the genetic mechanisms we targeted—fundamental GA metabolism and signaling—are highly conserved among plants species that differ greatly in form and evolutionary history. This was obvious in the field, where there were many pairs of trees with the same DNA insertion that had similar and dramatic reductions in size or changes in crown form (Figure 1). The intensity of dwarfing varied greatly, from genes that gave rise to trees that appeared essentially the same as wild type, to those for which nearly all regenerated trees were extremely small, making it difficult to find them if weed control was not very intensive (Figure 2). Trees ranged in height from 3 m to shorter then 20 cm. The poplar *GA2-oxidase* gene had the most dramatic dwarfing effect, followed by the *rgl1* gene from *Arabidopsis*. The highly dwarfed trees also regenerated in tissue culture very slowly, making the process of tissue culture regeneration difficult and slow. Due to the strong negative effect of high *gai* overexpression, only 11 gene insertions with the *35S::gai* transgene were recovered.

Some forms of the *gai* gene gave a wide and near continuous range of semi-dwarfism, enabling intermediate dwarf trees to be identified to reduce tree size, but not produce highly reduced "bushes" that are slow growing and difficult to manage in a landscape (Figure 3). The wild-type *GAI* genes had no substantial effects on height growth, regardless of the promoter employed; however, these trees did flower precociously in the largest numbers, as discussed below.

Trees planted in the field during the summer of 2003 were analyzed for mean internode distance by measuring the number of nodes in a section of the stem. The association of internode distance with height was strong (Figure 4). This shows that, as expected, the main effect of GA inhibition was to reduce cell elongation, rather than cell division. The higher cellular and leaf-node density in GA-modified trees may affect other physiological traits, such as leaf area index, water flow through the stem, and crown photosynthesis. The dense crowns may also be of increased ornamental value.

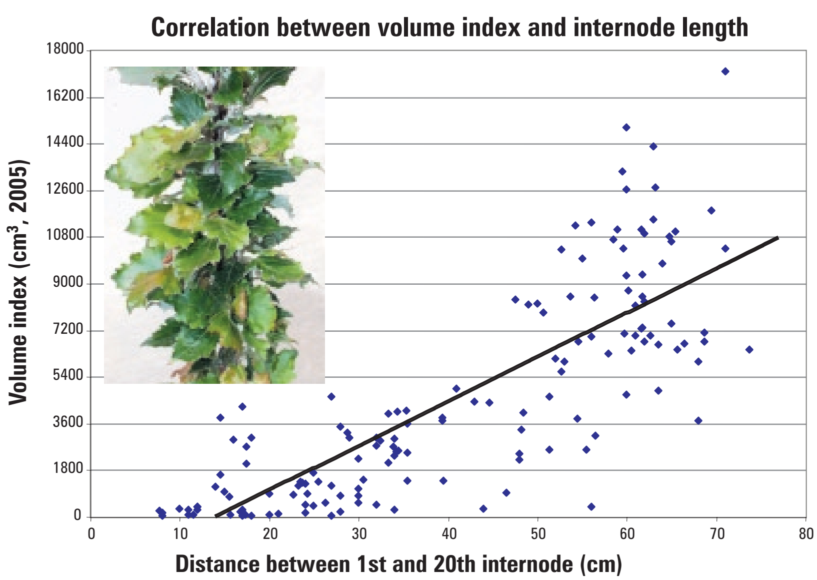

Figure 4. Correlation between plant size and internode length was strong, giving dense and bushy trees (inset).

Figure 5. Examples of variation in leaf morphology observed, including during leaf-out (left); dark green, pubescent foliage (upper right); and small-leafed, sage color foliage (lower right).

Figure 6. Precocious and unusual upright catkins formed during late summer on semi-dwarf poplars.

In addition to crown density, another feature of potential ornamental value is the striking variation in foliage color and leaf shape that we observed. Some of the genes employed gave rise to trees that had colorful foliage during leaf flush, a high degree of pubescence, or very diminutive leaves with a strong sage type of coloration (Figure 5). Most of the semi-dwarf plants in the study had very dark green leaves at maturity compared to wild type. This suggests that the use of GA-modifying genes directed specifically at leaf development could impart even more striking variation in morphology of ornamental value.

Early Flowering

During August 2005 we noticed that trees from 13 gene insertions were flowering at an abnormal time of the season (late summer), earlier than usual in their life span, and that the catkins had an unusual upright morphology (Figure 6). Additional flowering was observed during normal spring flowering in 2006, their third growing season. Controls had also begun to flower at this time, but the transgenics had significantly greater numbers of flowers than did the non-transgenic trees. All of the flowers appeared normal and female, as expected for this variety. Although dwarf trees are not expected to pose any significant environmental risks, due to regulatory requirements all catkins were manually removed from the field trial prior to catkin dehiscence. The wild-type *GAI* genes driven by the *35S* promoter gave rise to the highest frequency of flowers; approximately 80% of the 57 events in two *GAI*-based constructs included at least one flowering tree. In contrast, the strongly dwarfed *35S::rgl1* and *35S:poplar-GA-2 oxidase* trees flowered very little. This suggests that the early onset of flowering results from a combination of specific GA inhibition and growth to reach a minimum size.

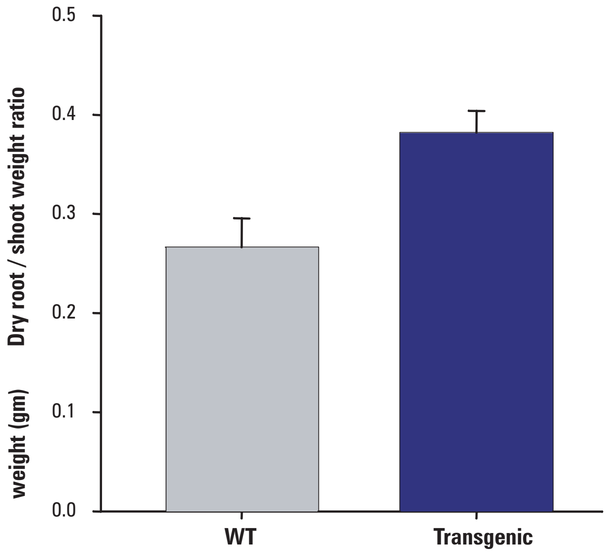

Figure 7. Increase of root:shoot biomass ratio in semi-dwarf poplars from a raised-bed study.

Strong Root Development

We noticed during initial tissue culture studies that the dwarf trees in culture boxes had very strong root development. This observation was published last year in the journal *Planta* (Busov et al. 2006). We followed up on this observation by growing plants in a raised bed. We planted 15 trees from each of the 7 different constructs and a non-transgenic control and harvested trees at monthly intervals, estimating shoot and root weights. We found that the semi-dwarf trees indeed had a statistically higher root:shoot ratio (Figure 7). The transgenics were 25% smaller than the controls in height, but had an investment in roots (vs. shoots) that was 30% greater than the controls. The preferential allocation to roots over shoots may be a large reason why an increase of stress tolerance, or recovery from poor health, has been attributed to trees treated with the GA inhibitor paclobutrazol

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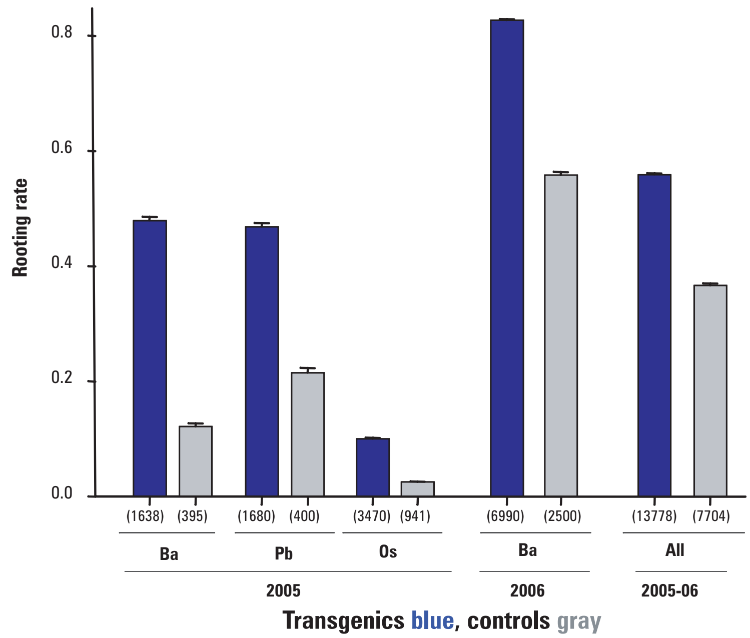

Figure 8. Improved rooting percentage of sticks from dormant trees in three nurseries (Ba, Pb, Os) over two years. Sample sizes given below bars, transgenics to left for each pair (blue), controls to right (gray).

(Watson 2000). The significant increase in root proportion suggests that these dwarfing genes may be useful when extensive root development is needed, such as for bioremediation of polluted soils, in very windy environments, or where there is limited soil moisture.

We also found that, when propagated at the University or at commercial nurseries, the semi-dwarf transgenic plants rooted at a much higher rate than wild-type plants (Figure 8). Based on our large sample size of 18,014 cuttings, we found that the transgenics rooted at a rate that was approximately 50% higher than for the non-transgenic controls. The mutant *gai* gene driven by the wild-type *GAI* promoter had the highest rooting rate. The *rgl1* and poplar *GA2-oxidase* genes, both driven by the *35S* promoter, had the poorest. Thus, it appears that one consequence of semi-dwarfism—usually desirable for the nursery industry—is plants whose root activity, both growth and redifferentiation, is improved.

Our results suggest that trees with semi-dwarfism genes directed at the GA pathway could be used to bolster the diversity of sizes, forms, and physiological properties of trees.

Prospects and Challenges

Our results suggest that trees with semi-dwarfism genes directed at the GA pathway could be used to bolster the diversity of sizes, forms, and physiological properties of trees. This may be of value to arborists, horticulturists, foresters, landscapers, and homeowners. With the rapid growth of genomics, there are many new possibilities for choosing and controlling genes in order to more precisely modify target traits such as crown width or root growth. Thus, we have just gotten a look at the "tip of the iceberg" in the studies described above.

The main scientific challenges are the need for more research into effects of different genes in the field and for improved gene-transfer methods so these kinds of genes can be readily produced in the most important landscape species and varieties, not just poplars. Current gene-transfer methods are not up to the task of efficiently transferring genes into the diversity of species and varieties used by the nursery industry. On the social side, because GMO methods were used, there are considerable and costly governmental (regulatory) hurdles, and market issues, given that some consumers are likely to be unwilling to purchase trees produced via GMO processes. Some of the genes and gene-transfer processes are also patented, requiring special licenses and fees to use them.

From an environmental viewpoint, dwarfed trees are unlikely to be a threat to spread because they will compete poorly with normal or wild trees. In fact, one advantage of the use of semi-dwarfism genes is that they should retard the spread of the exotic, and potentially invasive, trees that are commonly sold by nurseries. The genes would also provide a strong barrier to spread of more ecologically novel genes with which they may be intentionally linked by genetic engineers, such as genes for pest resistance derived from distant species. Given their potential value and safety, it seems likely that semi-dwarfism genes will find use in ornamental trees in the not-too-distant future.

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Footnote

1. We use the terms trait engineering, genetic engineering, transformation, GMO, and transgenic to refer to the use of recombinant DNA and asexual methods to modify and insert genes into plants. For the studies described here, plant genes are being used to modify native plant physiological processes (in contrast to the widely-grown GMO soy and maize crops).