

A SHORT ROUTE TO GENE DISCOVERY IN TREES: CONTROL OF STATURE THROUGH GIBBERELLIN CATABOLISM

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Manipulation of plant stature has long been a major goal in agronomy, horticulture, and silviculture. Dwarf and semi-dwarf "Green Revolution" varieties in wheat and rice contributed to dramatic increases in cereal crop yields. The new varieties were shorter, more resistant to damage by wind and rain (lodging), and responded better to nitrogen fertilizers by increasing grain yield rather than straw biomass. Dwarfing in fruit trees has allowed dense field cultivation, facilitated mechanized maintenance, increased efficiency of fruit collection, and allowed more precise pesticide application, reducing spray drift¹. Most of the dwarfing rootstocks induced precocious and profuse flowering. Dwarf ornamental cultivars have also been developed in numerous tree and shrub species, and can provide added safety and low-cost maintenance.

Control of plant stature and form has previously required the use of plant growth regulators or classical plant breeding. Plant growth regulators are exogenously applied to promote or retard elongation, often through chemical alteration of gibberelic acid (GA) biosynthesis. However, stature control through 'anti-GA' plant growth retardants requires repeated application of synthetic chemicals that are costly, variable in effectiveness, and can have un-desired environmental consequences, or at least perceptions thereof.

Dwarfing alleles that do not produce deleterious consequences for plant vigor, particularly dominant forms most useful in horticulture, are very rare in natural gene pools because they are readily eliminated by natural selection. Healthy dwarf plants, especially in the variety of backgrounds needed for large-scale commercial deployment, are therefore difficult to obtain through classical breeding. Transgenic manipulation of GA levels or signaling through the insertion of dominant dwarfing genes could therefore provide an important alternative approach to control of plant stature².

Research in the last several years has clearly demonstrated that dwarfism is commonly associated with deficiencies in GA levels or signaling. Gibberellic acids are tetracyclic diterpenoid growth regulators that play a critical role in plant development. The level of bioactive GAs is precisely controlled by several mechanisms, including transcriptional regulation of the genes encoding enzymes from both biosynthetic and catabolic pathways. By modifying transcriptional regulation of genes controlling GA flux, it is possible to modify developmental processes regulated by GA and thus plant stature and form.

The roles various enzymes play in the regulation of bioactive GA levels vary widely. A small number of enzymes near the final biosynthetic and catabolic steps appear to determine the ultimate level of

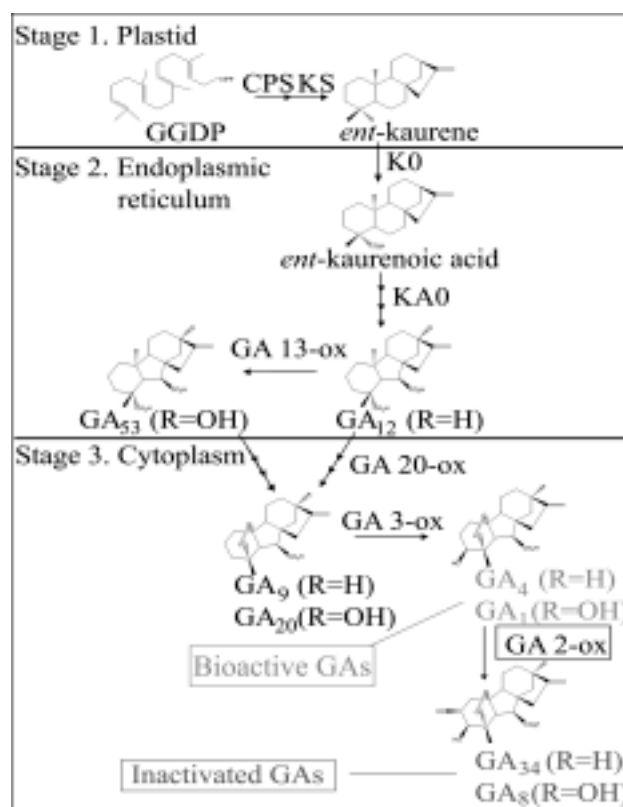


Figure 1. GA metabolic pathway. Enzymes in the cytoplasmic stage exert strong control over the final levels of bioactive gibberellins. Overexpression of enzymes from the ER or chloroplast stages does not affect the final level of bioactive GAs. Note that GA2ox affects both the levels of the bioactive GAs and their immediate precursors, thus providing strong control over GA influx.

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Figure 2. Wild type (WT, right) and *stumpy* mutant (left) plants after more than a year of growth in the greenhouse. The mutant plant is approximately four-fold shorter than WT. Diameter growth of *stumpy* is not significantly different from the WT plants. The mutants have a larger diameter at the apex, suggesting less taper. *Stumpy* also displayed profuse leaf and stem pubescent, most likely resulting from trichome outgrowth.

bioactive GAs² (Figure 1). These enzymes are encoded by small gene families. Thus, these genes are logical targets for biotechnological manipulation of GA levels.

Using activation tagging, we identified a dwarf transgenic hybrid poplar (*Populus tremula* x *P. alba*)³. The mutant, which we have dubbed *stumpy* because of its short, stout form, was approximately four-fold shorter than the corresponding wild type (WT) (Figure 2). The cause of the phenotype was a hyperactivated gene encoding GA 2-oxidase (GA2ox), the major gibberellin (GA) catabolic enzyme in plants. The vegetative characteristics of the *stumpy* mutant are similar to the phenotype of GA-deficient mutants that contain defective GA biosynthetic or hyper-activated catabolic genes. They show severely reduced stem elongation, decreased leaf size, and dark green foliage. Consistent with the expectation that the mutant phenotype was caused by perturbation of the GA metabolic pathway, we recovered a nearby genomic sequence and then isolated a corresponding cDNA copy of the gene that showed high homology to GA 2-oxidase genes from a number of plant species.



Figure 3. Phenotypic rescue of *stumpy* with GA₃ application to the shoot apex. A droplet of 10mM GA₃ was applied to the shoot apex of the plant to the left at four-day intervals for two weeks.

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Analysis of the GA content in the mutant indicates a several-fold decrease in the bioactive GAs (GA_1 and GA_4) and a several-fold increase in their main C-hydroxylated inactive catabolites (GA_8 and GA_{34}). We also detected a nearly two (1.8)-fold increase in GA_{29} , the catabolite of GA_{20} (the main GA_1 precursor). The changes in GA profile were consistent with the expected biochemical function of the GA2ox enzyme. Exogenous application of GA_3 , which is resistant to catabolism by GA2ox, rapidly restored normal development to the mutant (Figure 3), strongly supporting the hypothesis that the mutant phenotype is a result of the deficiency of the bioactive GAs, GA_1 and GA_4 .

The rapid reversion to normal growth by exogenous application of GA that is resistant to the action of the enzyme provides a potential method for control of transgenic plants overexpressing *GA 2-oxidase* genes for horticultural purposes. For example, the rate of growth during commercial propagation could be greatly increased by GA application, allowing rapid nursery production. Once GA application ceases after transplanting, the slow growth and dwarf form would resume. Landscape managers might also choose to speed early growth via GA application, thereby allowing growth rate to attenuate only after plants reach a desired size. It can also be used by owners of the plants to achieve a customized form and size.

Trees of short stature can provide substantial benefits for urban forestry and wood products industries. Due to their large size, trees require intensive pruning to avoid damage to homes and power lines. Tree maintenance costs comprise a significant proportion of electrical utility budgets. Utility companies in U.S. spend \$1.5 billion per year trimming trees and controlling brush, including herbicide and growth retardant treatments⁴. Despite these expenditures, trees are the largest single cause of power outages⁵. Pruning and tree removal are two of the highest street-tree maintenance costs; approximately 58% of urban tree-care budgets are allocated to tree trimming, removal, and disposal⁶.

When trees are intensively cultured as wood fiber crops, they are likely to benefit from substantial alterations in form and structure that might be achieved by modification of GA metabolism, but are difficult to achieve via conventional breeding⁷. Domesticated trees may be substantially shorter and stouter, which could reduce the amount of low-quality “reaction wood” that forms in response to bending and leaning. They might also have a higher harvest index, show improved harvesting/handling efficiencies, and have greater unit-area fiber yields. Resistance to water movement, and thus onset of water stress, might be reduced by a shorter distance from soil to photosynthetic surfaces. Crowns could be engineered to be narrow, allowing for a greater number of stems per unit area. Wood properties may also be modified via GA alteration to increase product value, such as by increasing fiber length to yield stronger paper products⁸.

This work appears to be the first case of successful forward genetics—where a gene has been isolated based on a mutant phenotype—in a tree. Activation tagging, which produces dominant phenotypes, is particularly suitable for identifying genes in trees because of their long juvenile periods and high genetic loads, which make inbreeding to expose loss-of-function (recessive) alleles difficult. Because of the many developmental differences between annual and perennial plants—including vegetative dormancy, delayed onset of flowering, extended periods of secondary (woody) growth, and gradual vegetative maturation—activation tagging and other forward genetic approaches may uncover many more types of regulatory genes useful for tree biotechnology. Gene tagging approaches have become even more powerful in poplars of late; its newly published genome sequence (<http://genome.jgi-psf.org/poplar0/poplar0.home.html>) greatly speeds the identification of genes.

References

1. Webster T. (2002) Dwarfing rootstocks: past, present and future. *Compact Fruit Tree* 35: 67-72.
2. Hedden P. and Phillips AL. (2000) Manipulation of hormone biosynthetic genes in transgenic plants. *Current Opinion in Biotechnology* 11: 130-137.

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3. Busov VB *et al.* 2003. Activation tagging of a dominant gibberellin catabolism gene (*GA 2-oxidase*) from poplar that regulates tree stature. *Plant Physiology* 132: 1283-1291. <http://www.plantphysiol.org/cgi/content/abstract/132/3/1283?ct>
4. EPRI. (1995) The right tree for the right-of-way. *Technical Report-TB-105029*.
5. Simpson P and Bossuyt R. (1996) Tree caused electric outages. *Journal of Arboriculture* 22: 117-121.
6. Nowak DJ. (1990) Street tree pruning and removal needs. *Journal of Arboriculture* 16: 309-315.
7. Bradshaw HD and Strauss SH. (2001) Breeding strategies for the 21st century: Domestication of poplar. In D. I. Dickman, J. G. Isebrands, J. E. Eckenwalder, and J. Richardson, editors, *Poplar Culture in North America, Part B*, 383-394. NRC Research Press, Ottawa.
8. Eriksson ME *et al.* (2000) Increased gibberellin biosynthesis in transgenic trees promotes growth, biomass production and xylem fiber length. *Nature Biotechnology* 18: 784-788.

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