Genetic and Molecular Controls on Carbon Sequestration – Implications for Terrestrial Ecosystems

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Introduction

Carbon sequestration in terrestrial vegetation and soils is a poorly understood process, but ultimately represents a summation of biological activities including the initial incorporation of atmospheric CO_2 into plant carbohydrates through photosynthesis, carbon partitioning to chemical forms of organic matter resistant to microbial decomposition and carbon allocation to recalcitrant pools of biomass located either above-ground in leaves and woody stems or below-ground in roots. While a key objective in carbon management research is to enhance the natural capacity of plants and soils to sequester carbon, substantial gains in improving the sequestration potential of terrestrial ecosystems will require major scientific advancements in understanding the fundamental biological processes that control the initial uptake, ultimate chemical forms, and subsequent carbon transfer and turnover in plants and soils. The challenge, however, lies not in simply describing rates of photosynthesis and/or patterns of carbon allocation and partitioning, but rather in understanding the genetic and environmental mechanisms that control these processes in biological systems and how photosynthesis, allocation, and partitioning potentially impact carbon sequestration in terrestrial ecosystems.

Objectives

In the broad context, we are exploring the genetic and environmental mechanisms that control whole-plant allocation of biomass above- and below-ground, and the cellular partitioning of photosynthate to long-lived chemical pools of carbon. Specifically, we seek to discover the genetic mechanisms controlling the quantity and quality of photosynthate allocation into secondary cell walls in stems, branches, leaves, tap roots, coarse roots and fine roots of woody plants as a means of understanding the biological processes that underlie carbon sequestration in terrestrial ecosystems (Figure 1). That is, does carbon allocation to these various tissues and their chemical composition influence either the absolute amount or longevity of plant and soil-based pools of organic matter? If so, how many genes influence above- and below-ground

carbon allocation, and the chemical composition (e.g., lignin, cellulose, hemicellulose) of these tissues? Are these genes differentially expressed under conditions of altered soil moisture and/or nutrition? Do genes controlling cell wall chemistry operate independently above- and below-ground, or is the genetic control of these two processes tightly coupled? And finally, do master regulatory genes exist for these traits and, if so, do they lend themselves to genetic manipulation?

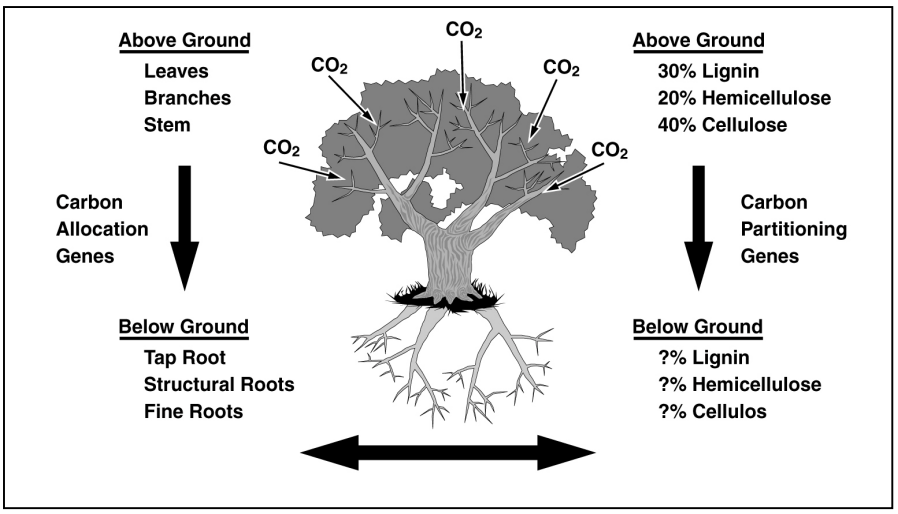

Figure 1. Schematic representation of the direct genetic control of carbon allocation and carbon partitioning in woody plants.

Specifically, our inclusive objectives are:

- to collect biomass samples from two three-generation segregating F_2 hybrid poplar families for identifying phenotypic differences in carbon allocation above- and below-ground,
- to determine the chemical composition (e.g., lignin, hemicellulose and cellulose) of selected tissues from progeny in each F_2 family using pyrolysis molecular beam mass spectrometry (pyMBMS),
- to develop co-linear genetic maps for each hybrid poplar family that will subsequently be used, along with phenotypic data on carbon allocation and partitioning, in a Quantitative Trait Loci (QTL) analysis to identify genes for carbon sequestration potential.

Approach

Central to the above objectives is the premise that there is an explicit link between carbon allocation, the chemical composition of plant tissues and the potential for soil carbon sequestration in terrestrial ecosystems. Thus, identifying and manipulating genes that control tissue chemistry (i.e., carbon partitioning) and biomass accumulation (i.e., carbon allocation) will provide options that currently do not exist for future carbon sequestration/management efforts. Furthermore, the complexity of carbon sequestration in terrestrial ecosystems makes it doubtful that natural systems can be used to identify detailed genetic and possibly even the environmental factors that regulate the magnitude of carbon sequestration. Therefore, model systems must be selected as ecosystem surrogates in which to study these processes and to ensure that genetic

conditions are sufficiently defined so as to identify genetic factors and environmental processes of important to carbon management.

Members of the *Populus* genus are well-suited for genetic and environmental studies of carbon sequestration as they are among some of the fast-growing trees in the world. They have worldwide importance and are the primary short-rotation woody crop (SRWC) species in North American, with over 40 million acres of excess, idle or surplus agricultural land suitable for SRWC production (Graham 1994). Poplar plantation are clonally propagated, thus allowing rapid deployment of genetic gains made in growth or performance traits. Furthermore, more is known about *Populus* physiology, biochemistry, agronomics, and genomics than any other woody crop species (Stettler *et al.* 1996). The genetic resources (i.e., genetic stocks, genetic maps, and BAC libraries) exist that uniquely provide molecular access to the mechanisms controlling carbon allocation and partitioning. Worldwide, there are more than 30 species of *Populus* that are generally inter-fertile, resulting in a wide range of genetic variability that segregates for nearly all plant growth traits, including crown form and branching patterns (Wu *et al.* 1998). The genome size of *Populus* is relatively small compared to other tree species (550Mb, roughly similar to rice, Bradshaw and Stettler 1995) and genetic transformation systems are routinely available for the insertion of genes controlling traits of interest. Because of these many practical advantages, *Populus* is generally regarded as a model system for nearly all other woody plant species, which together comprise the largest fraction of the living terrestrial carbon reservoir (Dixon *et al.* 1994).

Project Description

Task 1. Hybrid Poplar Pedigrees. Hybrid poplar, as discussed earlier, has a number of unique features that make them model organisms for a range of genetic studies. One of the more important ones for the purposes of this project is transgressive segregation or the expression of extreme phenotypes (e.g., allocation and cell wall chemistry) within an F_2 progeny population that generally exceed the range of phenotypes observed in either the parental or grandparental generations. Transgressive segregation reveals a more extensive set of morphological forms that are otherwise masked in the original parental species. In combination with QTL analysis, transgressive segregation provides a means of identifying genes that control growth and differentiation in general, and carbon allocation and partitioning in particular.

Task 2. Carbon Allocation and Plant Harvest. From hybrid poplar pedigrees, we will collect whole tree biomass samples to identify clonal differences in carbon allocation above- and below-ground. The above-ground portion of the plant will be severed at ground level and separated into stems, branches and leaves. The below-ground portion of each tree will be carefully excavated using a combination of manual and mechanized techniques. Coarse roots and fine roots will be separated from the tap root. Fresh weights of all plant tissues will be determined. Dry weight-to-fresh weight ratios will be used to calculate the total dry mass of each component. Carbon allocation to leaves, stems, branches, coarse roots, fine roots and tap roots will then be estimated as a fraction of total dry mass for each tree.

Task 3. Carbon Partitioning. A rapid analytical method (pyMBMS) will be used to determine cell wall composition for above- and below-ground samples collected from each pedigree. Cell

wall composition will be determined using a combination of pyMBMS and Projection to Latent Structures modeling (Tuskan *et al*. 1999). Genotypes of known extreme phenotypes will be selected and used as external calibration standards during the pyMBMS analysis. Principal components from the correlation matrix on normalized mass spectral data for each external calibration standard will be correlated with known cell wall composition. Projection of latent structure models will be developed for each cell wall component and used to characterize carbon partitioning for each tested genotype.

Task 4. SSR Identification. Microsatellite DNA or simple sequence repeats (SSR) have been used to construct high-density genomic maps in humans and mice and for QTL mapping in cattle and swine. High information content, due to the presence of multiple alleles, genome-wide distribution and the ability to integrate with other types of molecular markers, makes SSR particularly useful in map construction (Brown *et al.* 1996). We will develop SSR primers in *Populus* by creating a random genomic library of DNA enriched for SSR sequences. At least 25-50 polymorphic SSR loci containing multiple alleles will be developed for use in the mapping population.

Task 5. Genetic Mapping/QTLs. Co-linear genetic maps will be developed for each sampled family using the available 600 RFLP, STS, and RAPD markers, along with the SSR markers developed above. Phenotypic data on carbon allocation and partitioning will be combined with the genetic maps as part of the QTL analyses. Phenotypic data for traits with severely skewed distributions will be transformed to improve normality. If evidence for more than one QTL peak is found, the trait will be analyzed by composite interval mapping

Results

Cuttings from hybrid poplar family 331 were planted in April 2000 and one-year-old seedlings harvested the following October. Plants were severed at ground level and the above-ground portion of the plant separated into stems, branches (if there were any) and leaves. Standard excavation techniques were used to completely remove the below-ground portion of each plant and it too was separated into coarse roots and fine roots.

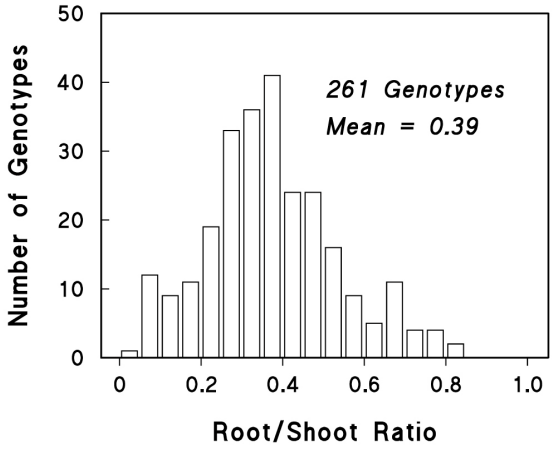

Figure 2. Ratio of total below-ground biomass to total above-ground biomass as an indication of plant allocation to roots versus shoots.

Considerable variation in the absolute amount of biomass allocated to above- and below-ground structures was observed (data not shown). Variation was also observed in the relative allocation of biomass to roots and shoots, as evidenced by the ratio of total below-ground biomass to total above-ground biomass (Figure 2). For the 261 individuals sampled and analyzed to date, the average root/shoot ratio was 0.39 with an upper estimate that approached 0.85. Saplings the clustered about the average root/shoot ratio allocated approximately 72% of total biomass above-ground and only 28% below-ground. The relative allocation of biomass for saplings at the upper range of root/shoot ratio was 59% to shoots and 41% to roots. Only about 10-12% of total below-ground biomass was in fine roots, with the remaining 88-90% in coarse roots. For the shoot portion of the plant, 42% of total biomass was in leaves, 24% in branches, and 34% in stems. Initial pyMBMS results for cell wall chemistry associated with stems indicates that lignin content varies from 21.7 to 27.0% and averages 24.4%. Chemical analyses of leaves and fine roots remains to be completed.

Application

Differences in whole-plant carbon allocation and cellular partitioning of chemical constituents will have consequences for ecosystem carbon sequestration. Selection for allocation to long-lived plant parts (stem *vs.* leaves) is expected, for example, to increase carbon sequestration in plant biomass. Selection for allocation below-ground *vs.* above-ground, even to plant parts with comparable intrinsic turnover times (e.g., fine-roots *vs.* leaves), might be expected to increase ecosystem carbon sequestration if the below-ground physical environment is less conducive to decomposition and rates of decay are retarded. Allocation of biomass to deeper roots will likely increase soil carbon sequestration when the deeper soil environment is less favorable for decomposition (e.g., cooler). Furthermore, differences in carbon partitioning may have consequences for carbon sequestration in biomass if there are significant differences in respiratory costs for constructing cell walls and growing plant parts with different relative carbon fractions. Differences in partitioning which lead to differences in litter chemistry (e.g., lignin/nitrogen ratio) are expected to have consequences for soil carbon sequestration. All things considered, chemical partitioning which leads to litter more resistance to microbial attack (e.g., more lignin) will increase soil carbon sequestration.

Future Activities

Replicate samples of leaves and fine roots will be analyzed in the next few months for chemical composition by pyMBMS and variability among individuals for lignin and cellulose concentration will be determined. Along with information on biomass allocation, these data will be subjected to QTL analysis. For this to occur, however, SSR primers in *Populus* will have to further developed from a random genomic library of DNA enriched for SSR sequences. Phenotypic data on carbon allocation and chemical composition will be combined with the genetic map as part of the QTL analyses.

Acknowledgements

Research on SSR development and mapping was sponsored by the Laboratory Directed Research and Development Program of Oak Ridge National Laboratory (ORNL), managed by UT-

Battelle, LLC for the U. S. Department of Energy under Contract No. DE-AC05-00OR22725. Research on carbon allocation and partitioning in leaves and fine roots was sponsored by DOE, Office of Biological and Environmental Science.

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