Title: "A Molecular-Genetic Approach to Studying Source-Sink Interactions in *Arabidopsis thaliana*"

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Final Report

Introduction. The ultimate goal of our research is to elucidate the molecular mechanisms by which the complex interactions between sources and sinks of fixed carbon are controlled in plants. As soluble sugar levels have been shown to play a vital role in a variety of source-sink interactions, a key aspect of our research is to determine the role of sugar-regulated gene expression in mediating source-sink interactions. In addition, as a critical aspect of source-sink interactions is the channeling of fixed carbon into different storage forms, we have pursued our finding that fumaric acid represents a significant form of storage carbon in *Arabidopsis thaliana* and other plant species. In the future, a better understanding of the mechanisms by which interactions between sources and sinks of fixed carbon are coordinated will be a pre-requisite to developing more rationale approaches to improving harvest indices in crop species.

Analysis of Storage Forms of Fixed Carbon in *Arabidopsis thaliana* and Other Plants. An important component of source-sink interactions is the channeling of fixed carbon into different storage forms. While most of the previous work in this area has focused on the relative importance of soluble sugars and of starch, we have pursued our finding that fumaric acid represents a major form of fixed carbon in *Arabidopsis thaliana*. During the past three years our work in this area had three major goals, all of

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which have currently been fulfilled. The first of these goals was to develop a faster and more reliable technique for measuring sugar levels in plant tissues. The second of these goals was to examine the roles of light intensity, soil nitrogen levels, photoperiod, developmental stage and ability to synthesize starch in determining fumaric acid levels. The final goal of this part of our work was to measure soluble sugar and fumaric acid levels in *Arabidopsis* phloem sap.

In our initial experiments we measured soluble sugar levels in plant tissues using a standard protocol that involves extracting the sugars into ethanol, drying down the ethanol, resuspending the sugars in water and then using a chemical assay to measure sugar concentrations. This procedure is time consuming to use for large numbers of samples due to the significant amounts of time needed to dry down the ethanol fractions and then completely resuspend the sugars. We therefore worked out a different method for measuring sugar levels that eliminates the need to dry down and then resuspend each sugar sample. This stream-lined protocol has allowed us to measure sugar concentrations in many more samples than was previously possible.

During the last three years, we have measured fumaric acid levels in literally thousands of plant tissue samples. The results of these experiments indicate that, in *Arabidopsis*, fumaric acid levels rise with plant age and with increasing light intensity. We have also determined that fumaric acid levels are far higher in tissues capable of carrying out photosynthesis than in non-photosynthetic tissues, and that fumaric acid levels are higher at the end of the day than at the end of the night. In addition, *Arabidopsis* plants that can not make starch accumulate relatively high levels of fumaric acid, indicating that, in these plants, some of the photosynthate that would normally be used to synthesize starch is instead being used to make fumaric acid. We also determined that some, but not all, other plant species accumulate fumaric acid to high levels. In particular, hollyhock accumulates fumaric acid at levels up to 10 mg/gram fresh weight. Also, at least one agriculturally important plant species, soybean, accumulates substantial quantities of fumaric acid (approximately 0.5 mg/gram fresh weight). Finally, we have determined that phloem sap from both *Arabidopsis* and hollyhock contains high concentrations of fumaric acid, raising the intriguing possibility that significant amounts of fumaric acid are being transported from what part of the plant to the other through the phloem.

Characterization of Mutants in Sugar-Regulated Gene Expression from *Arabidopsis*. To identify the factors involved in controlling sugar-regulated gene expression in plants, and to determine the effects of disrupting this type of regulation, we have isolated and characterized mutants of *Arabidopsis* that are defective in the sugar induced expression of the gene encoding beta-amylase. Prior to the start of the work funded by this grant, we screened several thousand EMS-mutagenized *Arabidopsis* plants for those that fail to regulate beta-amylase production in response to sugar. This

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initial mutant screen was done using a very quick, but crude, procedure that allowed us to qualitatively determine beta-amylase activity levels in large numbers of plants within a reasonable amount of time. As a result of this work we identified approximately 3 dozen putative mutants, which we call reduced β -amylase (*ram*) mutants.

During the past three years we have measured beta-amylase levels in progeny of the original putative mutants. The results from these experiments indicate that eight of the mutant lines contain heritable mutations that result in significant reductions in beta-amylase levels. To date, we have focused on five of these eight mutant lines. These five mutants have been assigned to four complementation groups. We have determined that all of these mutant lines exhibit wild type induction of chlorophyll and anthocyanin levels, indicating that the sugar-regulated genes involved in chlorophyll and anthocyanin biosynthesis are being correctly regulated. All five of these mutant lines also show a wild type response to sugar in an etiolation/hypocotyl length assay. These five mutants also show wild type interactions between sugar responses and responses to abscisic acid and to ethylene. In addition, high concentrations of exogenously supplied sugars inhibit early seedling development in the mutant lines, just as they do with wild type plant lines.

Analysis of Potential Plant Homologues of Yeast Genes Involved in Controlling Sugar-Regulated Gene Expression. While almost nothing is known about the molecular mechanism by which sugar-regulated gene expression is controlled in plants, a number of genes that are involved in this process in yeast have been identified. Although not within the scope of our original proposal, we initiated experiments to study potential plant homologues of some of these genes. Our decision to conduct these experiments was based on results from other laboratories that suggest that the mechanism by which sugar-regulated gene expression occurs in plants may be similar to the mechanism by which it occurs in yeast. Additional support for this possibility comes from random sequencing of plant cDNAs that has resulted in the identification of plant genes with significant sequence similarity to several yeast genes that are involved in sugar-regulated gene expression in yeast. To elucidate the role of these putative homologues in plants, we screened T-DNA tagged *Arabidopsis* lines for plants that contain insertions in one of these genes. Together with a colleague, Dr. Janet Braam, we prepared DNA from 14,000 independently transformed *Arabidopsis* lines, in pools of 100 lines each. We then used PCR to screen these DNA preparations for insertions in 5 different genes. To date, we have identified one insertion mutation. While this insertion appears, unfortunately, to lie downstream of the gene of interest, our success in detecting this insertion demonstrates the utility of this approach for future work aimed at obtaining mutations in genes of interest. In addition, we have made our DNA samples available to approximately 15 other research groups, some of whom have been able to use these samples to identify plant lines containing mutations in genes of interest.