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

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Characterization of sugar-response mutants. To identify the factors involved in regulating plant sugar responses, and to determine the effects of disrupting this type of regulation, we have isolated two groups of sugar-response mutants from *Arabidopsis thaliana*. 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 β -amylase production in response to sugar. Five reduced β -amylase (*ram*) mutants were identified using this screen. We also screened mutagenized *Arabidopsis* for plants that are insensitive to the sugar-mediated inhibition of early shoot development. Nineteen sugar-insensitive (*sis*) mutants were identified using this screen.

Efforts during the period funded by this grant focused on characterizing the *ram* and *sis* mutants and identifying the *RAM* and *SIS* genes. Northern, western and activity analyses on the *ram1* mutant indicate that it has wild-type levels of β -amylase mRNA but greatly reduced protein levels and almost undetectable enzymatic activity. The *ram1* locus was found to map to a position near the chromosomal location of two β -amylase encoding genes. Therefore, the *ram1* mutation is very likely to lie in one of those two genes. Sequencing of the β -amylase genes from the *ram1* mutant is currently underway to determine whether either of them does contain a mutation. If, as expected, the *ram1* mutation is in the gene encoding most of the β -amylase activity present in wild-type plants, the *ram1* mutant will be a very useful tool for testing the hypothesis that β -amylases function in phloem loading.

Characterization of the *sis* mutants revealed the existence of interactions between sugar and phytohormone response pathways. The *sis4* and *sis5* mutants were found to be allelic to the *aba2* (can't make abscisic acid) and *abi4* (can't respond to abscisic acid) mutants, respectively. Testing of other ABA-insensitive mutants revealed that only mutations in the *ABI4* gene result in a strong sugar-insensitive phenotype. The *sis1* mutant was found to be allelic to *ctr1* (constitutive response to ethylene), indicating a connection between sugar and ethylene responses. The phenotypes of several other *sis* mutants, including the *sis2* mutant, indicate a

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connection between sugar and gibberellin responses, as these mutants are able to germinate in the presence of paclobutrazol, an inhibitor of gibberellin biosynthesis.

Additional work on the response of wild-type plants to sugar revealed that exogenous sugar inhibits breakdown of seed storage lipids in germinating *Arabidopsis* seeds. This inhibition may reflect the existence of a previously uncharacterized mechanism to feedback regulate seed storage lipid breakdown and gluconeogenesis. In addition, exogenous sugar inhibits chloroplast development in very young seedlings. This finding provides the first evidence for an effect of sugar on organelle development in a higher plant. The *sis5-1* mutant was found to be insensitive to both of these responses.

Some of the above results are described in the following manuscript (copy attached):

Laby, R.J., Kincaid, M.S., Kim, D., To, J.P.C. Reiter, W.-D. and Gibson, S.I. (2000) The *Arabidopsis* sugar-insensitive mutants *sis4* and *sis5* are defective in abscisic acid synthesis and response. *Plant J.* (submitted)

In addition, a summary of some recent advances in the plant sugar-response field is provided in the following manuscript (copy attached):

Gibson, S.I. and Graham, I.A. (1999) Another player joins the complex field of sugar-regulated gene expression in plants. *Proc. Natl. Acad. Sci. USA* **96**, 4746-4748.

Use of "DNA chips" to identify sugar-regulated genes from *Arabidopsis*. Despite evidence suggesting that sugar-regulated genes are quite common in plants, systematic attempts to identify sugar-regulated genes from plants have been lacking. The identification of sugar-regulated genes should be extremely useful in determining which biochemical and developmental pathways are regulated, at least in part, by soluble sugar levels. Together with scientists from Monsanto, we used high density DNA microarray analysis to identify sugar-regulated genes. We screened 9,888 *Arabidopsis* Expressed Sequence Tags (ESTs) using probes derived from RNA isolated from the shoot systems of adult *Arabidopsis* plants fed either 3% glucose or 3% sorbitol (as an osmotic control). Approximately 10% of the 6,135 ESTs that were expressed at detectable levels are sugar-regulated (defined as exhibiting ≥ 2 fold difference in expression levels between glucose and sorbitol fed plants). These results *confirm our hypothesis that soluble sugar levels regulate the expression of a significant percentage of plant genes*. In addition, these experiments indicate that a number of genes encoding key components of developmental and biochemical pathways are sugar-regulated. Glucose-repressed genes include genes involved in ubiquitin metabolism, protein synthesis and fatty acid desaturation. Glucose-induced genes include genes involved in controlling flower formation, meristem function, cell shape and cell cycle progression. These results should be very useful in developing models for the molecular mechanisms by which soluble sugar levels affect plant development and metabolism.

Characterization of fixed carbon channeling to fumaric acid. 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*. In fact, depending on plant age and growth conditions, the amount of fixed carbon present in the form of fumaric acid can exceed that present as starch or soluble sugars. We

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have determined 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 other plant species, including agriculturally important species, such as soybean, also accumulate fumaric acid to high levels. Finally, we have determined that phloem sap from *Arabidopsis* and hollyhock contains high concentrations of fumaric acid, raising the intriguing possibility that significant amounts of fumaric acid are being transported from one part of the plant to the other through the phloem.

The above results are described in the following manuscript (copy attached):

Chia, D.W., Yoder, T.J., Reiter, W.-D. and Gibson, S.I. (2000) Fumaric acid: an overlooked form of fixed carbon in *Arabidopsis* and other plant species. *Planta* (submitted).