

Characterization of sugar-response in Arabidopsis mutants to engineer plants for higher soydiesel and soy protein production  
by Xin Li (2008)

**Table of Contents**

Abstract.....	1
Introduction.....	2
Materials .....	3
Methods .....	3
Analysis .....	5
Results.....	5
Discussion.....	6
Conclusion .....	6
Acknowledgments .....	7
Sources Cited .....	8
Appendix: Genotypes of six of 58 mutants and wild-type tested; results of t-test .....	9

## Abstract

To derive more soy protein and cheaper renewable fuels from soybean plants, scientists are working to develop genetic lines of soybeans that will direct more photosynthesized nutrients (sugar) to harvested portions of the plant. To do this, scientists must understand the way in which sugars regulate metabolism and development because this process is still largely unknown. The purpose of my project was to model the soybean plant using *Arabidopsis thaliana* in order to identify genes that are regulated by sugars to better understand sugar signaling in plants. I measured Arabidopsis primary root length and anthocyanin levels of 58 insertion-induced mutants and wild-type that were grown in 6% glucose and sucrose. Results showed that, when grown in 6% glucose, average root length of one Arabidopsis mutant was significantly shorter than wild-type ( $p=0.025$ ), suggesting the mutant is hypersensitive to glucose. When grown in 6% sucrose, average anthocyanin level of seedlings of the same mutant was significantly higher than that of wild-type ( $p=0.0006$ ). The disabled gene in that mutant is at1g06230. This gene contains a bromodomain protein, which may play a role in sugar regulation. The next step is to substantiate that at1g06230 is a sugar-related gene by growing this mutant in sorbitol.

## Introduction

Soybeans are renewable sources of soydiesel and soy protein. Scientists are working to develop genetic lines of soybeans that will direct more photosynthesized nutrients (sugar) to harvested portions of the plant in order to derive more soy protein and cheaper renewable fuels from these plants (1). To do this, scientists must understand the way in which sugars regulate metabolism and development; however, this process is still largely unknown (2). The purpose of my project was to model the soybean plant using *Arabidopsis thaliana* in order to identify genes that are regulated by sugars to better understand sugar signaling in plants.

I used *Arabidopsis* because it is a fast-growing, five-chromosome plant with a mapped genome, and genetic mutant *Arabidopsis* seeds are readily available commercially. Additionally, *Arabidopsis* is a good model of soybeans because both *Arabidopsis* and soybeans are oilseed plants (1).

*Arabidopsis* mutants have been developed through insertion-induced mutation, where transfer DNA is inserted into the *Arabidopsis* genome so that given genes lose function (3). Growth and utilization of nutrients of mutants can be compared to wild-type *Arabidopsis*, which are plants with an unaltered genome. My goal was to identify *Arabidopsis* mutants with disabled sugar-related genes. Identifying mutants with disabled sugar-related genes is the first step in determining genes that are responsible for sugar signaling.

I grew *Arabidopsis* seeds in glucose in order to determine the effects of glucose on primary root development of mutants because according to a study by Zhou et al. (2000), glucose effects growth development in *Arabidopsis* (4). I also grew *Arabidopsis*

seeds in sucrose to determine the effects of sucrose on anthocyanin levels in mutants based on a study by Teng et al. (2005) that said sucrose is “the most effective inducer of anthocyanin biosynthesis in Arabidopsis” (5), and a study by Hiratsu et al. (2003) that showed increased sucrose amount in plants increases anthocyanin levels (6).

There are two types of sugar-related Arabidopsis mutants: hypersensitive and insensitive. While all Arabidopsis mutants are missing a gene, only hypersensitive and insensitive mutants are missing sugar-related genes (3). A study by Gibson (2005) showed that, when grown in high concentrations of sugars, mutants that are hypersensitive to sugar grow shorter root lengths and have higher anthocyanin levels than wild-type Arabidopsis. Conversely, mutants that are insensitive to sugar grow longer root lengths and have lower anthocyanin levels than wild-type Arabidopsis (2). Based on this work, I looked for mutants with root lengths and anthocyanin levels that were different from the wild-type when grown in sucrose and glucose.

## **Materials**

I used 58 insertion-induced Arabidopsis mutants. The genotypes of six of 58 mutants are shown in Appendix A.

## **Methods**

*Seed Surface Sterilization:* About 100 seeds of each mutant and wild-type were put into separate microtubes. Tubes were labeled, and 800  $\mu\text{L}$  of 50% commercial bleach were added. The microtubes were vortexed for ten minutes, centrifuged, the bleach layer was removed, and 800  $\mu\text{L}$  of  $\text{dH}_2\text{O}$  were added. The seeds were rinsed with  $\text{dH}_2\text{O}$  three times before 500  $\mu\text{L}$  of 15% agar solution were added to keep seeds suspended in the

microtubes. The microtubes were covered with aluminum foil and stored at 4 °C for three to four days for stratification—a process that results in seed germination at approximately the same time.

*Root Growth and Measurement:* After stratification, half of the seeds for each mutant and wild-type were pipetted onto 1% glucose media in individual labeled Petri dishes and allowed to germinate for 24 to 40 hours at 23 °C in constant light. After germination, forceps were used to transfer about 15% of randomly chosen seedlings of each mutant and wild-type onto 6%-glucose media across the top of sectioned square Petri dishes. The Petri dishes were labeled and wrapped with aluminum foil so that light could enter only from the top edge. The dishes were placed sideways with the top edge up so that roots would grow along the media instead of into the media. After 11 days, root lengths of the *Arabidopsis* seedlings were measured.

*Anthocyanin extraction:* The other half of the seeds for each mutant and wild-type were spread using a plate spinner onto 6% sucrose media in individual labeled Petri dishes. The seeds were grown at 23 °C in constant light for eight days. Seedling roots (which do not contain anthocyanin) were removed and leaves were weighed. Leaves were put into labeled microtubes, 450 µL of 1.5 M HCl:95% ethanol (15v:85v) were added, and the microtubes were vortexed at 4 °C for 20-48 hours. The samples were centrifuged for five minutes at 1350 rpm, and the ethanol in each microtube were transferred to another set of tubes. Finally, 450 µL of chloroform were added to each tube containing ethanol, which were then centrifuged for five minutes at 1350 rpm. The absorbency of the ethanol layer that contained anthocyanin was read at OD530 and OD657.

## Analysis

Data were analyzed to find the anthocyanin levels, using the equation:

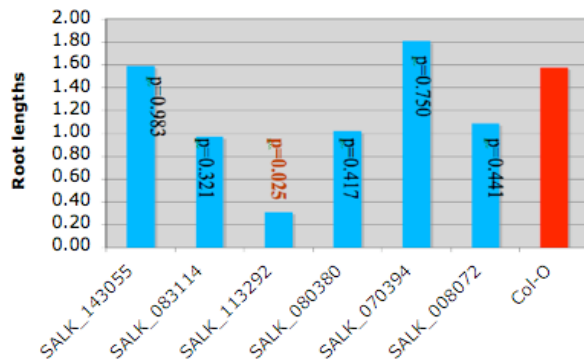
$$\text{Anthocyanin quantity (unit/gram)} = (\text{OD530} - 0.25 \times \text{OD657}) \times 1.2 \text{ mL/mass}$$

T-tests were done to compare root lengths and anthocyanin levels of mutants to wild-types. Significance level was set at  $p < 0.05$ .

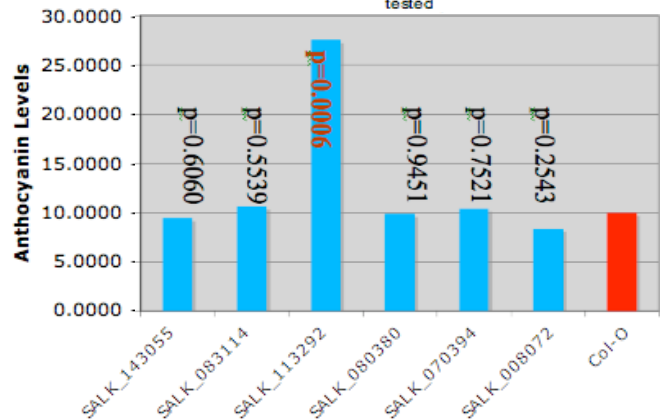
## Results

Of the 58 different Arabidopsis mutants tested, there were no significant differences in anthocyanin levels and root lengths between the wild-type and 57 of the mutants compared to the wild-type. Figure 1 shows that the root length of SALK\_113292 had a significantly shorter root length than wild-type ( $p=0.025$ ). Figure 2 shows that anthocyanin level of SALK\_113292 was significantly different from the wild-type ( $p=0.0006$ ). (Results for the mutants shown in Figures 1 and 2 except for SALK\_113292 are representative of all other non-sugar 57 Arabidopsis mutants tested.)

**Figure 1.** Root lengths after 11 days: Results for the mutants shown in Figures 1 and 2 except for SALK\_113292 are representative of all other 57 Arabidopsis mutants tested



**Figure 2.** Anthocyanin levels of Arabidopsis Seedlings after 11 days: Results for the mutants shown in Figures 1 and 2 except for SALK\_113292 are representative of all other 57 Arabidopsis mutants tested



## **Discussion**

My goal to identify an Arabidopsis mutant without a sugar-related gene was successful. Average root length of SALK\_113292 was significantly shorter than wild-type ( $p=0.025$ ) (Figure 1), suggesting SALK\_113292 is hypersensitive to glucose. When grown in 6% sucrose media, average anthocyanin level of seedlings of SALK\_113292 was significantly higher than that of wild-type ( $p=0.0006$ ) (Figure 2). Because mutants with higher levels of anthocyanin are hypersensitive to sucrose, results support that SALK\_113292 is hypersensitive to sucrose.

The genotype of SALK\_113292 is SALK\_113292, which lacks gene at1g06230 (Appendix A). According to the database at “The Arabidopsis Information Resource,” gene at1g06230 is encoded with a DNA-binding bromodomain protein; therefore, I can conclude that bromodomain protein may regulate sugar responses (7).

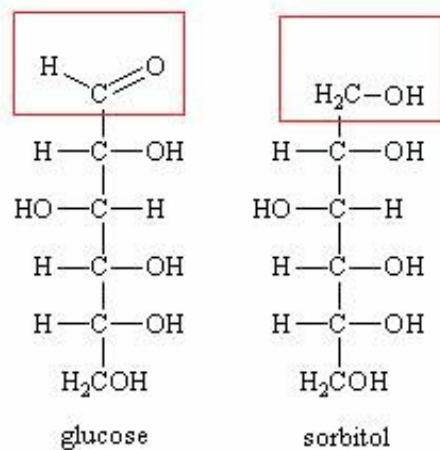
## **Conclusion**

The next steps of my research are to test the root lengths and anthocyanin levels of SALK\_113292 after it has been grown in low concentration of glucose and sucrose. This will substantiate that the mutant indeed carries a disabled sugar-related gene. This is necessary because the gene may be a growth-regulating gene. If growth in low concentration sugar shows the same significant short root length and high anthocyanin level as the wild-type, the disabled gene in SALK\_113292 is not a sugar-related gene.

Another step in my study will be to test for osmotic stress, which can be mistaken for sugar-response. Osmotic stress is caused by the differences in concentration between two sides of a cell membrane. Osmotic stress retards growth similar to that of sugar-response in plants. However, osmotic stress does not regulate plant growth like sugar (8).

A study by Gibson (2000) showed that osmotic responses can be eliminated by testing mutants in sorbitol since sorbitol has a structure similar to glucose (Figure 3) (2). If SALK\_113292 is missing a sugar-regulated gene, the mutant will respond only to sugar and not sorbitol. However, if the response is triggered by osmotic stress, then the mutant will respond to both sorbitol and sugar. If the gene is sugar-related, then my next step will be to determine the gene expression of the gene at at1g06230.

*Figure 3. Difference Between the Structures of Sorbitol and Glucose (9)*



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**Appendix: Genotypes of six of 58 mutants and wild-type tested; results of t-test**

Genotype	Gene location
SALK_143055	at1g70000
SALK_083114	at1g09970
SALK_113292	at1g06230
SALK_080380	at1g79000
SALK_070394	at1g72940
SALK_008072	at1g7240
Col-O	