The Correlation of the GL1 Mutation and the Glabrous Phenotype in Various Arabidopsis Ecotypes

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Table 1: Ecotypes by Geography

Wild-type Col	Col	
gl1-1 mutant CS28175	Col-5 (gl1)	
CS1379	Mir-0	Italy
CS77236	Sarno-1	Italy
CS78858	Wu-0	Germany
CS77165	Pdl-0	Spain
CS77053	LP3413.41	USA
CS6002	PHW 230,002	Italy
CS78856	Wil-2	Lithuania
CS183	Ler	

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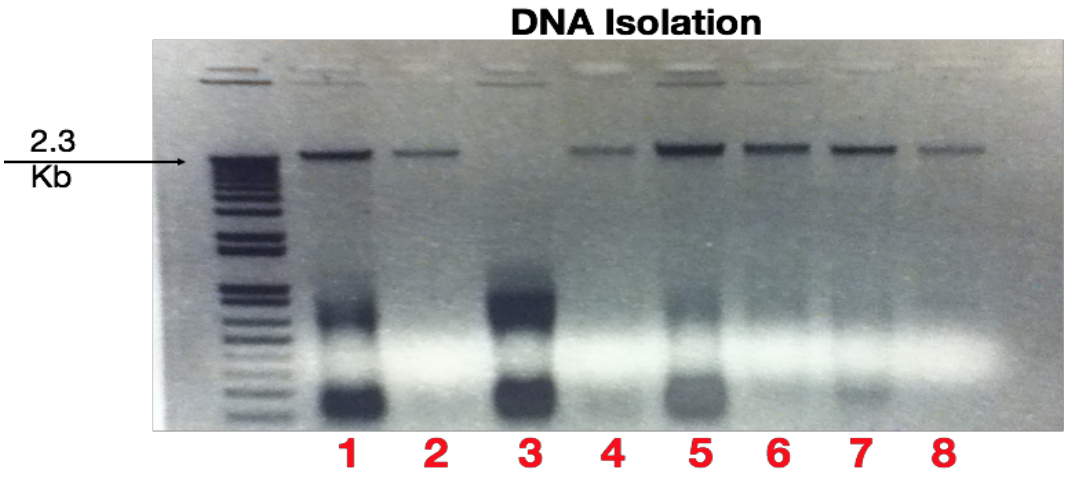

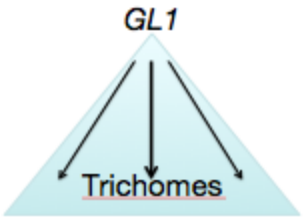

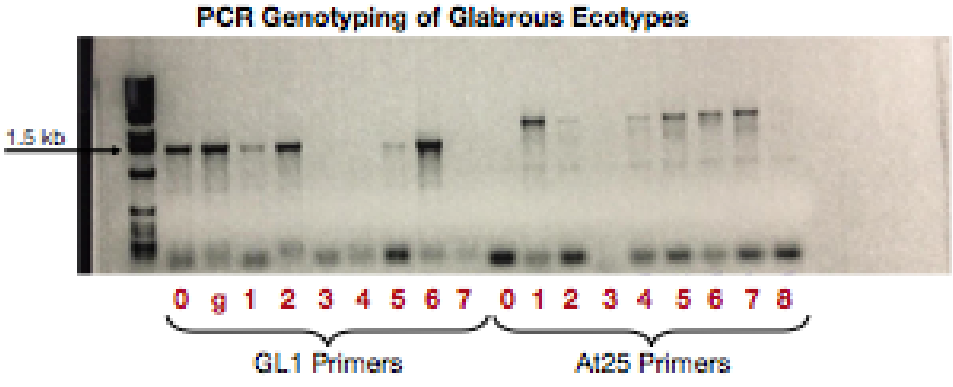

Figure 4: Glabrous Plants

Introduction:

There are over 300,000 species of plants that have been discovered, which provide most of the world's molecular oxygen, foodstuffs, and are precursors for drugs. However, plants are increasingly vulnerable to attack by pathogens and organisms that decrease the survivability of a species. In response to pathogenic and etymological attack, plants have employed various methods of defense, including physical and chemical barriers.

Many of these defense mechanisms have a direct effect on the herbivore by negatively affecting its physiology (e.g. through toxins or anti-nutritional compounds) or by interfering with its behavior (e.g. through repelling or deterring compounds) (Poecke-2007). Trichomes are fine outgrowths of plants and certain protists, commonly seen as plant hairs. Recent research has suggested the possibility of glandular trichomes as a physical barrier against pathogens, and the ability to produce insect-repellent chemical secretions has been observed in Arabidopsis thaliana (Bruner-2009). However, in some ecotypes of Arabidopsis, trichomes are not present. The mechanisms that allow formation of such trichomes have not been researched and will be examined in this study.

Arabidopsis is a genus in the family Brassicaceae. They are small flowering plants related to cabbage and mustard. Arabidopsis was the first plant to have its genome sequenced, and due to the simplicity of its genetic structure, Arabidopsis became an ideal plant for the modeling of plant mechanisms. Some of the glabrous ecotypes of Arabidopsis have the hairless phenotype because of the *gl1-1* mutation. The mutation of the GL1 gene consists of a 6.5 kbp deletion, provides the phenotypic response of glabrous leaves, or without trichomes. Previous research have found the *gl1-1* gene to be responsible for some role in the initiation of trichomes; however,

phenotypic effects (glabrous) from the deletion of the gene have not been observed.

Arabidopsis ecotypes consist of phenotypic as well as genetic differences. The analysis of the mutation in various ecotypes provides a broader scientific scope to the research; as well as crucial information needed for future studies. Research will consist of investigation in the following ecotypes: Mir 0, LP3413.41, Wil-2, Lithuania, Pd1-0, PHW 230,002, Wu-0, and Sarno-1. Although, Arabidopsis is not of major agronomic significance, it offers important advantages for basic research in genetics and molecular biology. For example, the previously mentioned Brassicaceae family consists of agricultural significant plants such as cauliflower, broccoli, and cabbage; known as the cruciferous vegetables. The presence of a GL1 gene has been observed in plants of the Brassicaceae family, and further research of Arabidopsis directly links to implications in agriculture.

The GL1 gene is a MYB type gene, and the study of the specific gene can allow implications to the entire MYB family of genes. In humans, the MYB gene encodes for a MYB proto-oncogene protein that regulates haematopoiesis, the formation of blood cellular components (Morrison et al.) and in tumorigenesis. The MYB gene has also been studied in cotton fiber development; the promoter of a cotton fiber gene, *RD22-like1* (*RDL1*), confers with trichome-specific expression in Arabidopsis (Wang et al.).

In the determining the formation of trichomes, the analysis of the *gl1-1* gene will be used to provide further insight into two major areas of future scientific study: theoretical analysis and empirical investigation. Theoretically, the mechanisms for the formation will provide further insight into the mechanisms of trichome formation in different ecotypes of Arabidopsis, as well as different species of plants. Empirically, the isolation of the *gl1-1* will provide additional

research into the genomic structure of Arabidopsis and the phenotypic responses. If the glabrous phenotype is observed without having a gl1-1 mutation, the glabrous phenotype is not due to the GL1, glabrous gene.

Methodology:

Greenhouse Screen of Arabidopsis ecotypes:

In order to find specific ecotypes that were glabrous, a greenhouse screen was performed using visible observations of all the ecotypes. A checklist was used to record the data into two categories: glabrous and trichomes. Samples of leaves of the glabrous ecotypes were kept frozen for further analysis.

DNA Isolation:

Scissors were disinfected with EtOH before cutting each sample, and leaves were cut approximately ¼ inch in diameter and placed in a clean 1.5 mL tube. 400 ml of Edward's extraction buffer was added to each tube. Leaves were grinded by twisting a blue pestle against the inner surface of the tube and until the extract became a light green color. The color green meant that chlorophyll was released and cellular and intracellular membranes had been broken. A detergent in the extraction buffer, similar to the one we use for shampoos or dishwashing detergents dissolves lipids and helps break cellular membranes. The EDTA in the extraction buffer preserves the DNA and keeps it from degrading. The Tris in the extraction buffer keeps the pH at 7.5, creating a stable environment for the DNA. NaCl is important for precipitation because it interrupts the hydrogen bonds between water and DNA molecules (begins the separation of DNA from the solution). Then 600 uL of extraction buffer was added to each tube. Mix by vortexing for 5 seconds. Boil for 3 minutes in a heating block ~90C. Heating is helpful to further break down cell walls and cellular membranes. Centrifuge for 2 minutes (14.8 rpm, 20C) to remove the cell debris, which will form a pellet. Centrifuging will separate the different substances in the solution. The heavier cell debris (composed of broken cell walls and cell

membranes) will form a pellet a the bottom of the tube, which you do not want. You want the liquid (the supernatant). Transfer 350 uL of each supernatant to a fresh 1.5 mL tube. This supernatant contains the DNA. Add 400 uL isopropanol, and mix by inverting the tube several times. The isopropanol will finish precipitating the DNA (separating out the DNA from the rest of the solution. The alcohol rapidly precipitates nucleic acids, leaving you with a pellet of DNA, rather after centrifuging. Incubate at room temperature for 3 minutes. Doing so increases precipitation efficiency. Centrifuge for 5 minutes to precipitate the DNA. DNA will be in the pellet. Carefully pour off supernatant. Add 500 uL 70% EtOH to each tube. Centrifuge for 5 min. Washing with ethanol removes remaining salts and SDS allowing you to have a more purified DNA template at the end of isolation. Carefully pour off supernatant, air dry the pellets (~20-25 min). DNA will be in the pellet. Once pellets are dry, dissolve them in Tris (100 uL). Rotate in cold room for 2 days.

PCR: Thaw all the reagents for PCR on ice. Vortex to mix to remove concentration gradient and then spin down briefly. Set up 50 µl PCR reaction in a thin-wall PCR tube on ice by the following recipe: 5 µl 10X Taq buffer solution containing Mg2+. 1 µl 10 mM dNTP stock; 1 µl Forward primer (50 uM); 1 µl Reverse primer (50 uM); 2 µl Template (up to 100 ng/µl) sterile or filtered H2O 39.5 µl sterile or filtered H2O; 0.5 µl Taq polymerase (5 units/µl). Program PCR cycler as following and start: Initial denaturation: Then 30 cycles of: Extension: When the temperature of PCR cycler reaches 94°C, put PCR reaction tube in and continue the program. 94°C for 3 minutes, 94°C for 30 seconds, 55°C for 45 seconds, 72°C for 60 seconds (about 1 kb/minute), 72°C for 7 minutes. Analyze PCR fragments on a agarose or polyacrylamide gel.

Results:

Greenhouse Screen:

After looking at over 400 plants of the Arabidopsis specie plants were identified on whether they had trichomes or were glabrous. Out of the three greenhouses scanned only 8 out of 400 plants had the glabrous phenotype. The names of the 8 plants were: *Mir-0, Sarno-1, Wu-0, Pdl-0, LP3413.41, PHW, Wil-2,* and *Lithuania.*

DNA Isolation of Leaf Samples:

To extract the DNA from each of the plants one leaf was taken from each sample. After being ground up and froze by liquid nitrogen some of the plants were ground more green than others. The more green the plants the more DNA concentration they would have in the experiments. The samples were then run on a gel with a 23Kb genome. However, some of the plants had RNA contamination, seen through the lower bands, causing the PCR not to work. Thus, to fix this problem the DNA isolation process had to be repeated adding RNase into the reaction. This would break down the RNA and give clean, non-contaminated DNA. The second time through, all of the plants had clean DNA and the PCRs worked adequately.

PCR and Gel Electrophoresis:

Due to the fact that the *GL1-1* mutation consists of a 6.5 Kb deletion including the *GL1* gene (the primers were coded to amplify the *GL1* gene), the expected results for the agarose gel electrophoresis were a 1.5 Kb band if the *GL1-1* mutation was not present and lack of a band if the *GL1-1* mutation was present. For samples *Wu-0* and *Pdl-0* there was no band present. Thus we can conclude that these samples contained the *GL1-1* mutation.

Discussion and Conclusion:

The purpose of this study was to determine the correlation between the GL1 mutation and the ecotypes of Arabidopsis. According to the agarose gel electrophoresis results, ecotypes samples 3 and 4 did not contain the 1.5 kb band. Thus, we can conclude that the 6.5 kb deletion was responsible for the absence of the *GL1* gene in the samples Wu-0 and Pdl-0. However, in the other samples, the glabrous phenotype was observed without having a *GL1* mutation. Thus, the ecotypes not including samples 3 and 4 proved the hypothesis. In these cases, the glabrous phenotype were most likely a result of another mutation, such as a mutation on the *GL3* gene. In fact, there are other mutations known to play a role in trichome formation. However, because the *GL1* gene is a master regulator, it was the logical first step.

Future studies include research into the various genes that also affect trichome development. Examples of additional genes include a base pair substitution of the *GL3* gene, for possibly the *gl3-1* mutation. A future application of the research in trichomes could be promoting greater trichome density in plants by the use of additional *GL1* genes. In many cases of genetic modification of plants, adverse effects have occurred to the plant; however, natural pesticide resistance would be greatly beneficial to the agriculture and textile industry. The model plant used in this study is related to cotton, which happens to have cotton fibers that are trichomes. Further comparison of the two plants reveal that cotton also shares the *GL1* gene, allowing for comparative research in cotton. An interesting fact noticed was that the samples 3 and 4, which did not have the mutation were from geographically similar regions of Germany and Spain (fig. 1). A possible explanation of such a phenomenon could be that the Arabidopsis ecotypes in Germany and Spain are able to resist pathogens by other means. For example, the habitat of the

German and Spanish Arabidopsis is the wetlands, which may contain less pathogens. Also, the presence of the a mutation could be a result of natural selection allowing greater survivability of more favorable traits. Then, the GL1 mutation might have been selected in other regions as more favorable traits.

Implications include improvements in the agriculture and the textile industry. However, because Arabidopsis is a model plant, the genome can be analysed and the trichome differentiation pathway can be investigated. Cotton, which also shared the GL1 gene is an important plant for human usage. Thus, research in the trichomes of Arabidopsis illuminates the future research into various other fields.

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Bruner, Laura, and Erich Grotewold. "The Study of Arabidopsis thaliana Trichomes for Source of Chemical Defense Against Pathogens" ["Defense Against Pathogens from Arabidopsis Trichomes"]. Department of Plant Pathology: 1-17. Print. This journal article is written by Laura Bruner and was advised by Dr. Erich Grotewold from the university of the Ohio State University. In this article the roles of trichomes in Arabidopsis were determined in the defense against pathogens, both chemically and physically. We will use the information in this article to determine the use of trichomes and plants and to be able to determine both if a plant has trichomes as well as the gene that affects the creation of trichomes.

Burdge, Graham C., et al. "Dietary protein restriction of pregnant rats in the F0 generation induces altered methylation of hepatic gene promoters in the adult male offspring in the F1 and F2 generations" ["Correlation between parental and offspring generations"]. British Journal of Nutrition (2006): n. pag. Print. This journal article is written by 6 PhD's in the University of Southampton UK, in the departments of Developmental Origins of Health and Disease Division and Development and Cell Biology. In this article, the researchers demonstrate the relationship between the diet of the F0 generation and how this affects the F1 and F2 generations. This article provides background information necessary for us about genetics and the relationship between the parental generation and future generations.

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State College, Ames, Iowa (1943): n. pag. Print. This article is written by Lanoy N. Hazel a PhD and professor at Iowa State College (University). In this article he utilizes phenotypical similarities between generations to provide patterns and equations for figuring out the corresponding generation of each organism. This article will be able to assist our research as we search for a way to find the generation of each plant.

Houle, David. "Comparing evolvability and variability of quantitative traits." Genetics Society of America (1991): n. pag. Print. This journal was written by David Houle a Ph. D. professor at State University of New York at Stony Brook. In this article the author demonstrates how the environment can affect the genetics and phenotypes of the organisms. He was able to calculate the fitness of a population using various variables and developing multiple equations. This article will be used in our background knowledge because it will assist in helping us figure our the generations of each plant and if they were affected by the environment.

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