Exploring Unexpected Gene Expression in a Corn Glossy Mutation

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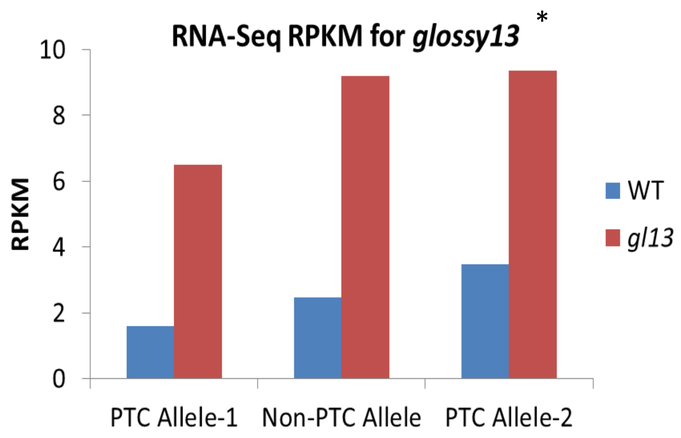
What is a glossy mutation?

Glossy is a phenotype in maize that is caused by a mutation in one of the twenty identified genes involved in the wax biosynthetic pathway. This mutation leads to the cuticle of the leaves having more wax accumulation than normal. This results in water beading up in droplets on the leaf, which is how a mutated plant is identified. The glossy mutation is intriguing because leaf cuticles with more wax accumulation can help protect the leaf, reduce water loss through the leaf, and protect the plant from heat. These could all be important traits, especially in drought-prone areas.



Introduction:

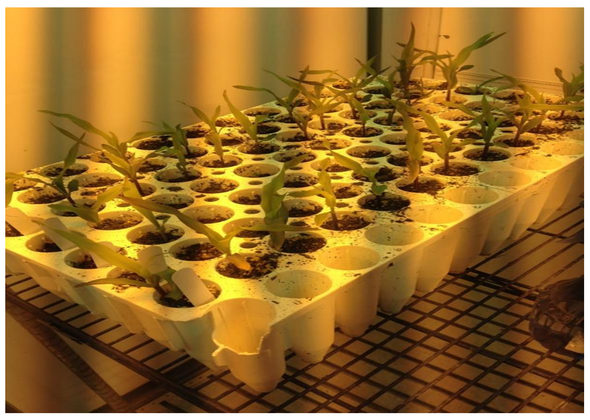
The glossy mutation studied is caused by a Premature Termination Codon (PTC) allele. This allele causes translation to terminate before it is complete which results in degradation of the remaining mRNA. For this reason, the mutant allele with the PTC is expected to have lower levels of mRNA than the wild type allele. However, this is not the case and there are higher levels of mRNA in the PTC-containing mutant. My goal in this project was to look at heterozygous glossy mutants and determine the allele-specific expression; more specifically, is the mutant allele more highly expressed than the wild type allele in a heterozygous plant? For this experiment, *glossy*13 and *glossy*3 were used. G*lossy*3 was used as a control because it showed higher levels of mRNA in the wild type versus the mutant, which is expected. *Glossy*13 was used as a comparison to *glossy*3. Both contain PTC alleles.

Discussion:

Looking at allele-specific expression for heterozygous plants could help determine what caused the higher expression of the mutant allele in the homozygous mutant plant. If both *glossy*3 and *glossy*13 had the same expression in their heterozygote plants, then maybe the lack of the functional glossy13 generates a feedback circle that tries to increase expression of the gene, resulting in more mutant mRNA than the cell can degrade. On the other hand, if the *glossy*13 mutant allele has higher levels of mRNA than the wild type, while the wild type of the glossy3 has higher levels of mRNA than the mutant, this could mean that there is expression regulation specific to the mutant allele.

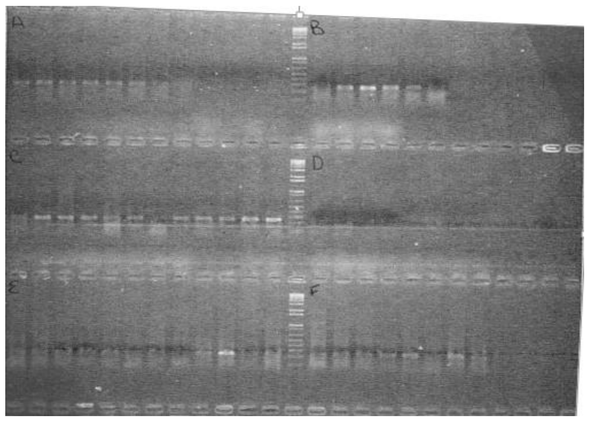
However, this project did not reach the intended end goal. This was possibly due to potential problems with primer pairs. Dimethyl sulfoxide and magnesium chloride were both added in hopes of improving the reactions. Magnesium chloride worked, while dimethyl sulfoxide did not. Magnesium chloride is a cofactor for Taq polymerase and PCR would not work without the magnesium chloride. Increasing the level of magnesium chloride can improve the reaction, but increasing it too much can cause the reaction to be inhibited. It is important to optimize the amount of magnesium chloride for each set of primers. Dimethyl sulfoxide makes DNA denature more easily and can improve the binding of polymerase to the DNA.

Method:

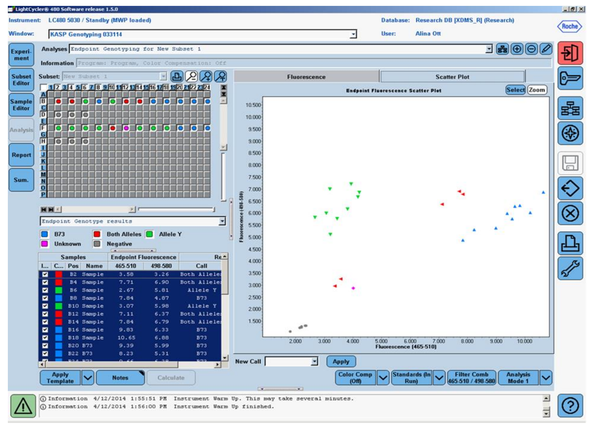


1.) Plant seeds from segregating packet to collect DNA.

2.) Phenotype seedlings to determine if they show the glossy trait.



3.) Run PCR reaction and gel on glossy DNA to check DNA quality and ensure amplification of primers.



4.) Run LightCycler Reaction to show whether samples are homozygous or heterozygous for either trait.

Conclusions:

1.) We were able to improve our results by improving the reaction, but our genotyping results were still unexpected.

2.) A new method, like Sanger sequencing, should be used because the genotyping method used in this experiment did not give expected results.

Acknowledgements:

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References:

* Li Li, Iowa State University