Identifying genes involved in hydrocarbon biosynthesis within maize silks

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Abstract

The main purpose for this research project is to understand the genetic structure of hydrocarbon biosynthesis in maize silks. In this project we genotyped transposon lines to identify potential gene knockouts in genes of interest. These lines will then undergo surface lipid comparison to determine if the genes contribute to surface lipid biosynthesis.

Hypothesis

We hypothesize that one gene from a region of the maize genome impacts surface lipid biosynthesis because this region was previously correlated to differences in hydrocarbon abundances between the B73 and Mo17 maize lines.

Background

Hydrocarbons are a simple class of surface lipids composed of only carbon and hydrogen atoms. They are found in high levels in the cuticle of maize silks and act as a protective barrier. Different maize genotypes have different abundances of hydrocarbons which allows them to be compared to identify genetic differences that contribute to hydrocarbon accumulation. In the end, we hope genes identified in this research will be used to make biofuels, lubricants and other waxy materials.

Methods

In my project, I compared the genes from two maize lines. To do this I collected maize leaf tissue from transposon lines grown in the field for DNA extraction. I then performed PCR (polymerase chain reaction) for genotyping. The reaction was optimized to limit nonspecific amplification.

Genotyping Schematic:

We genotyped plants that were born from a transposon insertions (foreign DNA that might cause a loss of gene function). During PCR the target gene region was amplified in two separate reactions. The first only contained the GSPs which flank the potential insertion. When this happens only one product of a known size is amplified if there is a normal (WT) gene unless there is a transposon on both chromosomes in which case a much longer product if any is amplified. In the second reaction the GSPs will anneal along with MuPs which bind to the sides of the transposon if it is present and copy two smaller fragments and the larger one again if there is a WT gene.

Discussion/Results

We were able to successfully carry out genotyping. The gel below shows the two reactions for 12 different maize plants from the same self crossed parent. The gel has segregating genotypes which shows that the parent is heterozygous. From this gel we found four plants that were homozygous for the transposon (lines #6, 7, 9 and 11) and two homozygous WT plants (lines #3 and 12).

In this gel, we can see if the genes from the maize plants (numbered) have transposons on one or both chromosomes. The first lane corresponds to a reaction with only the GSPs while the second lane also has the MuP.

The end result of this project is to have both types of homozygous maize lines from the same parents. This enables us to examine the differences in hydrocarbon accumulation between the WT gene and the transposon gene so we can determine if the gene plays a role in hydrocarbon biosynthesis.

Acknowledgment

I would like to thank the CBiRC YES program for this opportunity and Adah Leshem the program director. I also appreciate the guidance from my mentor, Bri Vidrine, and the rest of the Nikolau group members on the fourth floor of BRL.

References


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Emerging Silk Hydrocarbon Content by Genotype

B73 accumulates significantly more hydrocarbons than Mo17 and the hybrid accumulates an intermediate level of hydrocarbons because this trait has a genetic basis.

The three possible genotypes are: homozygous wildtype (two regular genes without mu insertions WT/WT), homozygous mutant (mu insertions in both chromosomes mu/mu) or heterozygous (one chromosome with a mu insertion and one with the wildtype gene WT/mu).