Epigenetic Reprogramming During Phase Change in Maize

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The Big Question

Do epigenetic changes, such as small RNA abundance or DNA methylation, really occur during vegetative phase change in maize (*Zea mays*)?

Introduction

The maize plant goes through **three** main **developmental stages**, which are **juvenile, adult and reproductive** growth. The juvenile-to-adult growth transition, known as **vegetative phase change**, makes the plant reproductively competent. Phase change is an important step, which calls for **protection against "unwanted" heritable change**. Given that **85% of the maize genome is composed of transposable elements** (TEs), the importance of **TE regulation and silencing** cannot be over emphasised.

Aims and Objectives

To **validate the occurrence of epigenetic reprogramming** during vegetative phase change, by:

1. Identifying **differential gene expression** between juvenile and adult tissue.
2. Identifying **epigenetic modifications** during phase change.
3. Identifying **differences in growth patterns** between wild-type plants and mutants.

Materials and Methods

B73 wild-type plants and **mutants** of the *required to maintain repression6 (rmr6)* gene were used. Plants were grown under greenhouse conditions, with 17hr days and temperatures that ranged between 22°C and 28°C. Samples for **DGE and siRNA library construction** were collected at vegetative growth stage 6 from the **6th leaf** of B73 wt seedlings, which **is a mosaic of juvenile (tip) and adult (base) tissue**.

Results

1. From the ∼ 30 million Illumina reads, **529 features were differentially expressed**, 301 of which were annotated. 282 genes were up regulated in the base and mostly involved in catalytic activity and binding.

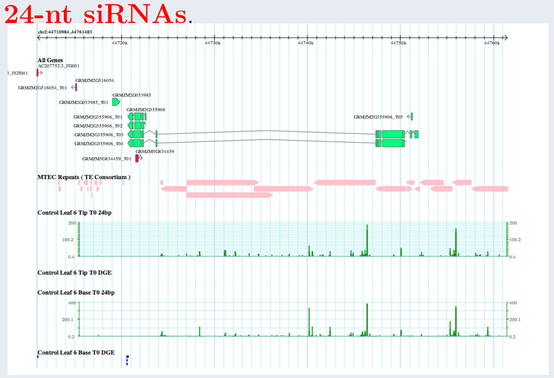
Summary of differentially expressed genes

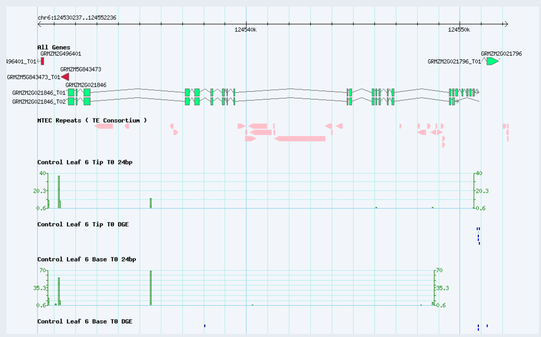
	number	GO	α	*p-value*
DE	529			
Annotated	301			
Up-regulated	282	lipid metabolism	0.05	0.000015
		polysaccharide metabolism	0.05	0.00037
		hydrolase activity	0.05	0.00066
Down-regulated	38	hydrolase activity	0.1	0.022

Gene regulation and 24-nt siRNA abundance

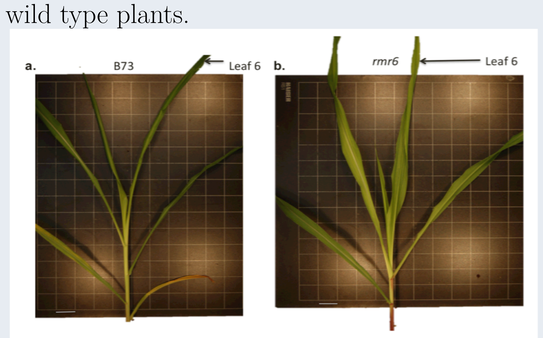
ID	Expression Tip	Expression Base	siRNAs Tip	siRNAs Base
GRMZM2G121942	up	down	high	low
GRMZM2G021846	up	down	low	high
GRMZM2G310947	up	down	low	high
GRMZM2G403797	down	up	low	high
GRMZM2G446213	down	up	high	low
GRMZM2G372870	up	down	high	low
GRMZM2G106574	down	up	low	high
GRMZM2G045392	down	up	low	high
GRMZM2G303118	down	up	low	high
GRMZM2G475139	down	up	low	high
GRMZM2G346168	down	up	low	high
GRMZM2G355906	down	up	low	high
GRMZM2G171045	down	up	low	high
GRMZM2G367411	down	up	low	high

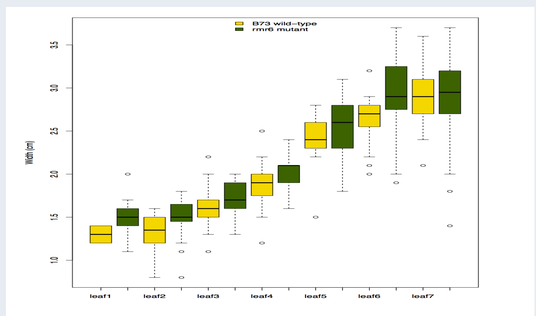
2. 14 genes had 24-nt siRNAs within their coding regions. (a) Intragenic 24-nt **siRNAs** were **co expressed with 11** of the host genes. (b) **3** host genes were **not co expressed with intragenic 24-nt siRNAs**.





3. Mutant plants exhibited **irregular growth patterns**. Lengths of the leaves were **not significantly different** (p-value = 0.20). **Mutant leaves were wider** (p-value = 0.0016) than wild type plants.





Conclusions

1. **Epigenetic reprogramming does occur** during phase change as evidenced by differences in siRNA abundance between adult and juvenile tissue.
2. Co-expression of 24-nt siRNAs and host genes suggests that the **siRNAS are only produced on transcription of their host genes**. This may be because they are produced by the introns in response to the transcription of nearby transposable elements.
3. Where siRNA abundance is not co-expressed with the host gene, this is indicative of a **self regulatory mechanism** whereby the gene produces siRNAs, that in-turn repress its transcription once their abundance has reached a certain level.
4. *Rmr6* mutants exhibited **irregular growth patterns due to a drop in 24-nt siRNAs** that initiate and maintain DNA silencing.

Further Work and Outlook

The relationship between transposon proximity to introns, siRNA abundance and gene expression needs to be fully explored. Using siRNA abundance as a proxy for DNA methylation does not enable the visualisation of methylation changes, thus methylation patterns exhibited by genes with intragenic siRNAs need to be analysed.

References

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