MAIZE EPIGENETICS

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HOW ENVIRONMENT AND GENOME INTERACT

BACKGROUND

Short RNA (sRNA) molecules have pivotal roles in regulating gene expression. The principal class of sRNA is the 24nt small interfering RNA (siRNA), which direct DNA methylation and promotes formation of condensed chromatin (heterochromatin) that is generally less accessible for transcription.

Maize has a highly repetitive genome; ~85% comprises transposable elements[1] (TEs) that can produce siRNA which direct methylation and prevent transposon activity. DNA methylation can therefore be considered a defense mechanism that protects genome integrity.

However, it is not known how TEs modulate expression of nearby genes in response to environmental stresses to form novel epigenetic variants (epialleles).

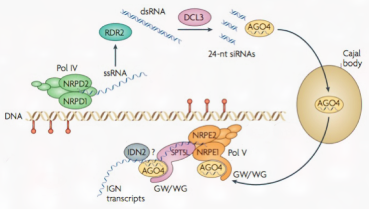

Fig.1[2] | A possible mechanism for RNA directed DNA methylation (RdDM). Sections of repetitive sequences, such as transposable elements, are transcribed by RNA polymerase IV (Pol IV). The single stranded RNA (ssRNA) is amplified into double stranded RNA (dsRNA) by RNA dependent RNA polymerase (RDR2) which is cleaved into 24nt siRNA by DICER-like 3 (DCL3). Argonaute 4 (AGO4) forms a complex including the siRNA. Through homology to the siRNA, RNA polymerase V (Pol V) is directed to discrete loci where an epigenetic modification, such as methylation, may be transferred.

METHODS

- Illumina sequencing to profile siRNA abundance in 3 tissues (Fig.2) using barcoded libraries
- Separation of reads based on barcode and quality checking using Perl
- Alignment of unique reads by Bowtie[3] using up to 3 mismatches and 50 multiple alignment positions for each read
- Blast unique reads against transposable element and miRNA databases
- Alignments, read abundance and significant Blast hits are stored in a MySQL database

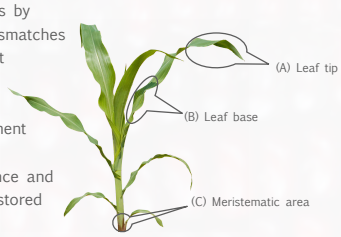

Fig.2 | Diagram showing the samples used in this analysis

AIM

To investigate how RNA-directed DNA methylation influences the formation and maintenance of environmental epialleles.

1: GENOME-WIDE siRNA PROFILES

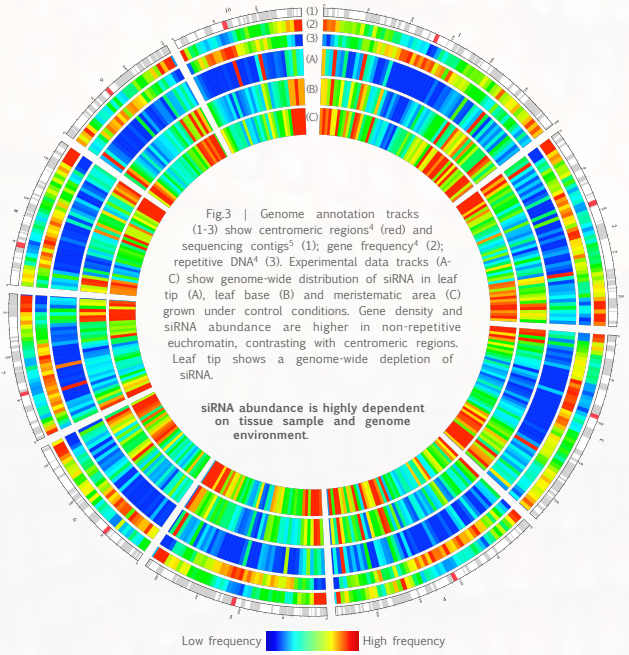

Fig.3 | Genome annotation tracks (1-3) show centromeric regions[4] (red) and sequencing contigs[5] (1); gene frequency[4] (2); repetitive DNA[4] (3). Experimental data tracks (A-C) show genome-wide distribution of siRNA in leaf tip (A), leaf base (B) and meristematic area (C) grown under control conditions. Gene density and siRNA abundance are higher in non-repetitive euchromatin, contrasting with centromeric regions. Leaf tip shows a genome-wide depletion of siRNA.

siRNA abundance is highly dependent on tissue sample and genome environment.

2: siRNA TARGETING GENES

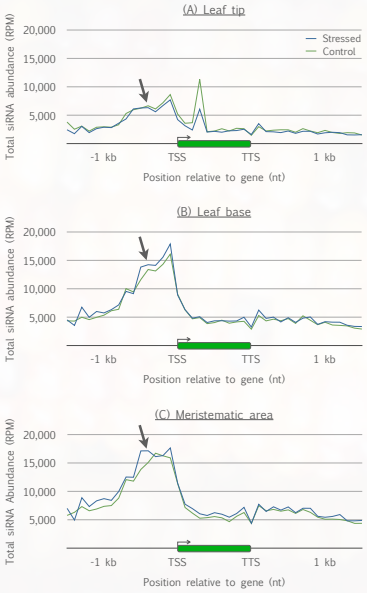

Fig.4 | Comparative profiles of siRNA alignments to genes. A peak of siRNA abundance is observed upstream of transcription start site (TSS) in all samples, but is reduced in leaf tip (A). Abundance in the gene body and beyond the transcription termination site (TTS) show less variation. Control and stressed samples show very similar profiles.

siRNA abundance peaks in the regulatory region of genes. Environmental stress does not cause a genome-wide response.

3: TE-DERIVED siRNA

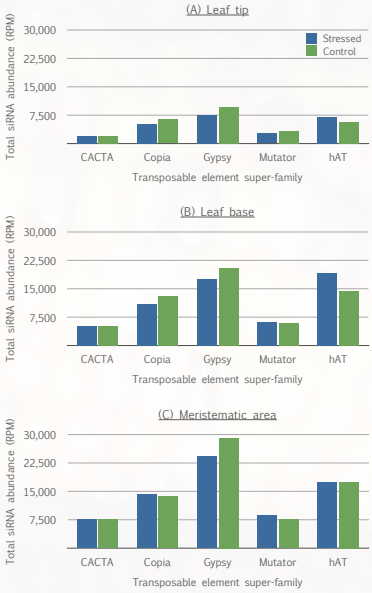

Fig.5 | Comparative abundance of siRNA that have significant homology to known maize transposons.

Transposable element derived siRNA are more abundant in meristematic area (in proximity to the stem cell niche).

SUMMARY

1. **Genome-wide responses to environmental stress are not widespread but occur at specific loci.**
2. **Leaf tissue has distinct sections. siRNA profile of tip is different to base and meristematic area.**
3. **Reduced abundance of siRNA in leaf tip suggests reduced RdDM, which in turn increases expression of TEs.**

CONCLUSION

siRNAs act as a specific intermediary signal between environment and genome

Acknowledgements

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