Uncovering the Cannabis sativa methylome through Enzymatic Methyl-seq

Cannabis sativa is an industrial crop producing more economic value than the top five crops in California combined. In the medical field, cannabinoids are used to treat and alleviate symptoms for a wide range of diseases, including nausea in cancer patients undergoing chemotherapy. Sex determination in cannabis is an important economic factor. Pollination dramatically reduces cannabinoid expression in female plants. To express high levels of cannabinoids, female plants are grown in isolation of male pollen. However, female plants are capable of stress induced hermaphroditism that can lead to access of pollination and low cannabinoid yields.

S-methylthiourea (5mC) is an important epigenetic mechanism that regulates many cellular processes, including sex determination. To better understand the hermaphroditic process in cannabis, more 5mC surveying tools are needed to treat and alleviate symptoms for a wide range of diseases.

SAMPLE PREPARATION

- Jamaican Lion genomic DNA was extracted from female clones (leaf, seed, and unseeded flowers) and male sibling (flowers) plants.
- 50 ng of genomic DNA, spiked with control (unmethylated lambda DNA and GmC methylated pUC18) were sheared using the Covaris S2 instrument.
- Sheared DNA was then ligated to modified sequencing adaptors.

DATA ANALYSIS

- Reads were aligned to the Jamaican Lion reference genome (August 2018 assembly) and analyzed using the tools in the Illumina CLC Genomics Server.
- Four contigs from this assembly showed anomalous methylation patterns and were excluded.

METHODS

RESULTS

- EM-seq libraries were generated from female flower DNA, seed, and unseeded flower DNA. (A) EM-seq libraries are shown in the figures A-E: (A) Less PCR cycles are required (5 vs 6 cycles respectively) for EM-seq. (B) EM-seq libraries have larger library insert sizes than WGBS. Additionally, the EM-seq protocol has been optimized for both standard and high complexity samples. The common library standard for determining 5mC, whole genome bisulfite sequencing (WGBS), introduces DNA damage and GC-biased sequencing resulting in skewed methylation profiles. To further complicate matters, the cannabis genome is 68% AT-rich and 83% AT-rich after bisulfite conversion. Very few technologies currently address the complexity of plant methylation signals, and to date no methylome exists for cannabis.

Differential methylation analysis comparing female flower and seed flower (stamens) and the male flower (stamens). (A) % 5mC levels in the Cpg, CHG, and CHH contexts for all female EM-seq libraries. The female methylation levels for flower and seed flower were higher than the male flower indicating methylation patterns are potentially determined by sex. (B) Region 1 BLASTs to the sex-specific family of the Cannabis sativa genome. This region is linked to the positive regulation of seed production. The female flower is Cpg methylated in this region, suggesting the expression of the gene is turned on. The female seed flower is unmethylated at this CpG site. (C) Region 2 BLASTs to the THC acid synthase (THCA) gene of the Cannabis sativa genome and this gene is linked to the positive regulation of THC production. Interestingly, the female flower is CpG unmethylated in this region, suggesting the expression of the gene is turned off, while the male flower is methylated at this CpG site, suggesting the expression of the gene is turned off. Male flowers produce low scale less THC.

CONCLUSIONS

- EM-seq enables the first in depth analysis of the Cannabis sativa methylome and differential methylation identified genes involved in seed production and THC production.

REFERENCES