Development of Reference Epigenomes and Transcriptomes in Switchgrass

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Epigenetic mechanisms are one of the most important regulatory processes in plant development and physiological response to environmental signals. The majority of the plant epigenetic studies have been conducted in Arabidopsis, rice and maize and there is a need to expand these studies to other plants, including switchgrass (Panica virgatum L.). Switchgrass is an economically important, warm-season, and widely adapted C4 perennial grass. Switchgrass shows tremendous promise as a candidate crop for the U.S. biofuel industry. High biomass yield potential under marginal environments, sequestering large amounts of atmospheric carbon, and sustainable productivity across a wide geographical range, made switchgrass, a dedicated herbaceous bio-energy crop for cellulosic biofuel production. Until now, most research in switchgrass has been focused on the improvement of biomass yield and yield related traits. Epigenomics research, especially in the area of histone-DNA interactions as related to stress have not been studied. In this study we developed a protocol for switchgrass chromatin immunoprecipitation (CHIP) followed by next generation sequencing (CHIP-seq) in two switchgrass genotypes AP13 and VS16 for understanding global binding locations of two histone marks, H3K9me2 and H4K12ac, and the relationship of histone binding and gene expression. These epigenomes and transcriptomes will then be used for understanding the effects of abiotic and biotic stresses on the epigenome as well to discover genotype-specific epigenomic variation.

RESULTS

Table 1. Summary of (A) CHIP-seq and (B) RNA-seq statistics

Table 2. Significantly enriched genes in AP13 with FDR value of <0.05 from RNA-Seq

Table 3. Cellulose synthase genes in (A) AP13 and (B) VS16 with FDR value of <0.05

Table 4. (A) Genes involved in fatty acid biosynthesis in VS16 (RNA-seq) and (B) C4 pathway genes in AP13 and VS16 for H3K9me2 and H4K12ac modifications

Fig. 2. Overview of Switchgrass Chromatin Immunoprecipitation (CHIP-seq Protocol)

Fig. 3. AgriGO analysis of genes common to RNA-seq and CHIP-seq (A) RNA-seq and H4K12-associated genes and (B) RNA-seq and H3K9me2-associated genes

DISCUSSION

- CHIP-seq and RNA-seq experiments were performed on leaf tissues of switchgrass genotypes AP13 and VS16.
- ~75% of CHIP-seq reads mapped to the switchgrass reference genome. The percentage of mapped reads in RNA-Seq are higher than CHIP-seq (Table 1).
- Identified significantly enriched genes (log, fold change >8) in RNA-Seq analysis of AP13. Disease resistance related transcripts appear to be highly expressed (Table 2).
- Cellulose synthesis genes seem to be significantly enriched in both AP13 and VS16 (Table 3).
- More than 10 significantly enriched fatty acid biosynthesis genes were identified in VS16 RNA-seq experiment. Among them, 3-ketocyl-Coa synthase 6, Enoyl-CoA hydratase and Cinnamyl alcohol dehydrogenase genes are highly expressed (Table 4A).
- A number of C4 photosynthesis pathway genes were seen to be associated with H3K9me2 and H4K12ac marks (Table 4B).
- The majority of unigenes were involved in metabolic pathways followed by genes in biosynthesis of secondary metabolites and microbial metabolism in diverse environments (Table 5B).
- AgriGO analysis of highly significant and common genes from RNA-Seq/Chip-seq shows that the majority of genes are involved in cellular and metabolic processes (Fig. 3).

REFERENCES

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