An analytical pipeline for detection of differential DNA methylation from restriction reduced genomic representation: a pilot study in Eucalyptus.

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Abstract

Phenotypic plasticity, the ability to display a range of phenotypes as a function of variable environments, is a key feature in land plants. Nevertheless, knowledge of the extent and underlying mechanisms of phenotypic plasticity in response to abiotic stresses is still fragmentary. Besides genetic diversity, epigenetic variation is believed to contribute to tree phenotypic plasticity and adaptive potential. We set out a new approach to perform genome-wide differential methylation analysis by means of parallel construction of double digestion restriction libraries, namely PstI-MspI (methylation insensitive) and PstI-HpaII (methylation sensitive), followed by short-read NGS sequencing. For technical validation we evaluated the differences in methylation patterns in three tissues (xylem, juvenile and adult leaves) from clone BRASUZ1, the tree sequenced in the Eucalyptus grandis genome project. A new computational pipeline was created, using open source tools, to process this type of NGS data in a fully reproducible way. The final goal is to identify and annotate differentially methylated regions across samples with the assumption that the data follows a negative binomial distribution. The pipeline results for this experiment provided reproducible, genome-scale methylation measurements at 22,000 genomic sites consistent in all tissues and biological replicates. From this, 4,000 methylated sites were observed and the majority (64%) conserved among the analyzed tissues. On the other hand, approximately 6% of the sites were specific for each of the three tissues. Considering the genomic context, 58% (2,335) of the verified methylated sites fall within genes, whereas 570 (14%) are in transposons. Contrarily to the expected, the methylation profile in the genic space is favored and this experimental bias offers a cost effective alternative to contrast epigenetic states of a sizeable fraction of genes in plant genomes. At the end of the study, we expect to provide a flexible tool for easy execution of this approach to other species.

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