IDENTIFICATION OF GENES ASSOCIATED TO COLD ACCLIMATION IN *Eucalyptus nitens* BY ANALYSIS OF GENE EXPRESSION *in silico*.

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Introduction

Eucalyptus nitens Maiden is a fast growing species used principally for pulpwood and solid-wood production. *E. nitens* is preferred over other *Eucalyptus* species at high elevation, due to its cold tolerance. Several studies have shown that the frost tolerance in *Eucalyptus* spp. has a genetic control. Advances in RNA-Seq techniques give the promise of enabling a rapid and reliable way to identify candidate genes responding to this kind of environmental stress. Transcriptomic analysis and functional annotation have demonstrate to be a powerfull tool to understand the cold acclimation mechanisms (Liu et al. 2014). Additionally, could provide a useful technical resource for efforts in the molecular breeding of frost tolerance in *Eucalyptus* spp. This study presents the identification of differentially expressed genes (DEGs) involved in the cold acclimation process of *E. nitens* by RNA-seq approach using an *in silico* analysis.

Methods

12 expression libraries containing reads sequences by Ion-torrent platform were pre-processed and mapped against the *Eucalyptus grandis* genome (Gaete, 2017) were used for this analysis. DEGs were identified comparing a non-acclimation condition (NA) and three low temperature treatments; plants exposed to chilling temperatures (CABF), freezing temperatures (CAAF) and to a de-acclimation treatment (DA), generating six comparisons: NA/CABF, NA/CAAF, NA/DA, CABF/CAAF, CABF/DA and NA/DA. To identify exclusive DEGs two different statistical models were used: DEGseq and edgeR by using the following parameters: *p*-value ≤ 0.05, False discovery rate (FDR) ≤ 0.05 and Fold-change (FC) ≥ 1.5 to up-regulated genes and FC ≤ -1.5 to down-regulated genes.
The agriGO database was used for the Gene Ontology analysis (GO) of DEGs. GO Terms were represented by functional categories; biological process (BP), cellular component (CC) and molecular function (MF), filtered using Fisher test method ≤ 0.01.

Results

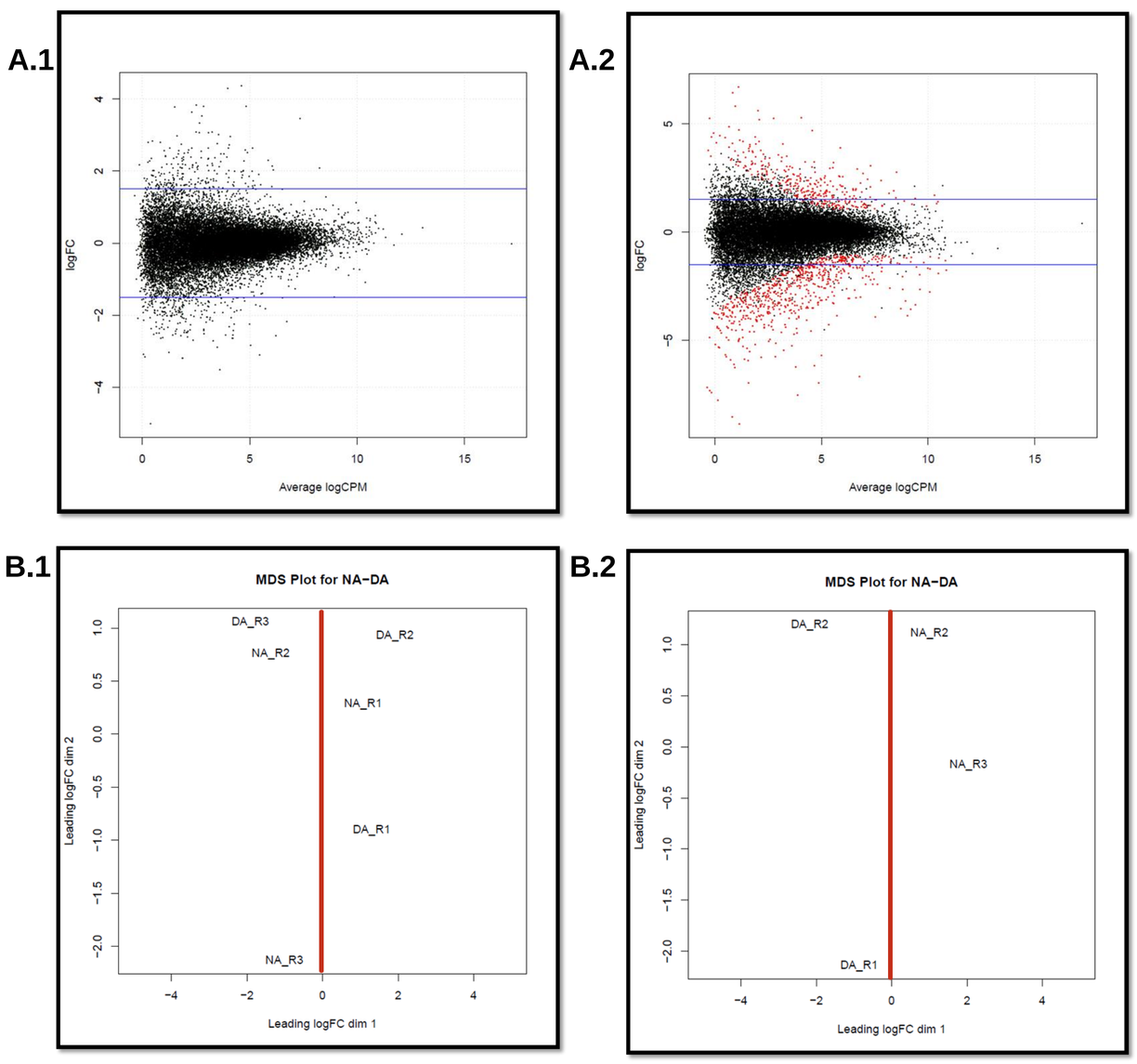

Fig. 1 A.- Amount of differential expression genes detected by edgeR in NA-DA comparison.
B.- MDS plot of replicates for NA-DA comparison.
1. Not considering biological variability. 2. Considering biological variability.

Results

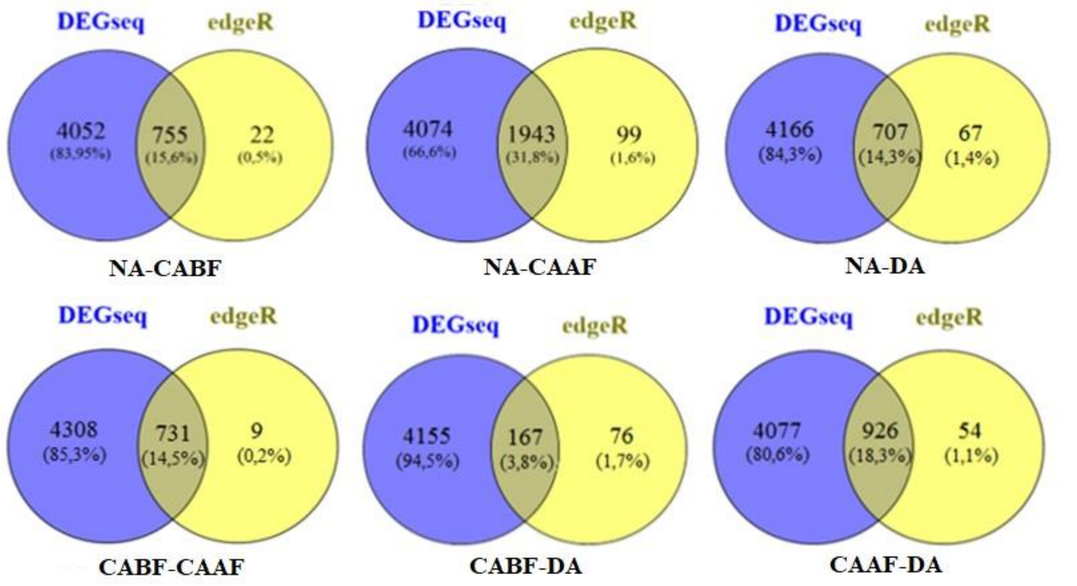

Fig. 2 Venn diagrams of DEGs detected by DEGseq and edgeR, considering biological variability of the sample, for all 6 possible comparisons.

Results

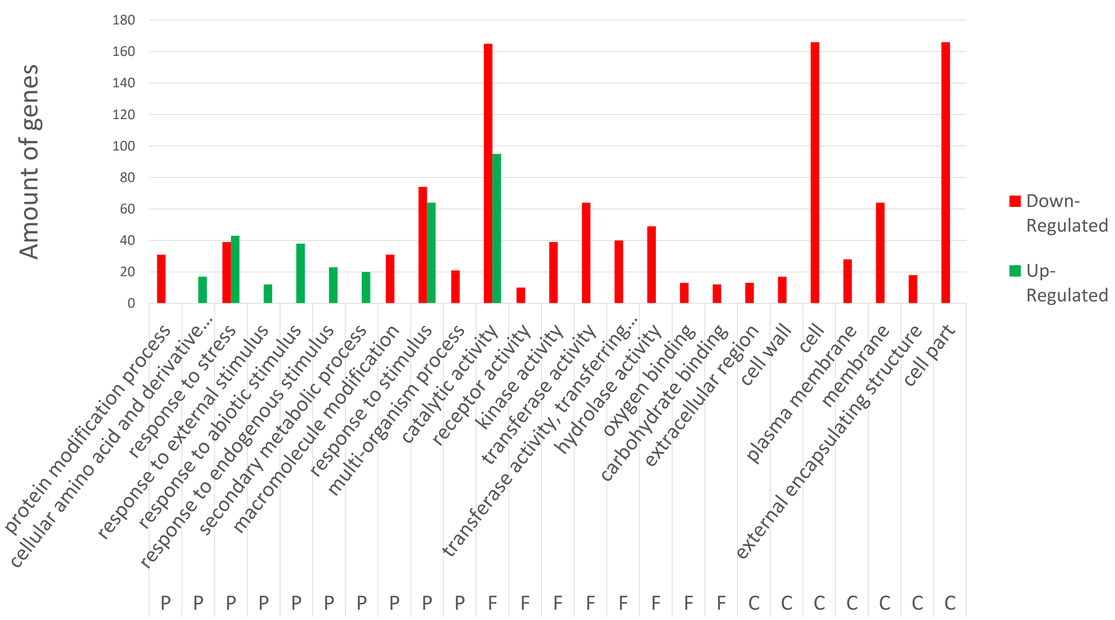

Fig. 3 GO assigment of all DEGs in the NA-CABF comparison.

Discussion and Conclusion

The results suggested that it is better to use the package edgeR for the analysis of differential expression (Guo et al. 2013), because it detects a smaller amount of false positives (Fig 2). Besides, edgeR offered the opportunity to analyze the biological variation among replications, this analysis allow us to identify high biological variability in comparison NA-DA (Fig 1).
The NA-CAAF comparison was the one with the highest numbers of DEGs detected, while CABF-DA showed the lowest one.
Analyzing the transcriptome profiling of DEGs by combining protein function clustering and enriched GO terms analysis, we identified clusters of genes responding to stress, stimulus, external stimulus, abiotic stimulus and endogenous stimulus (Fig 3) in all six comparison. In these categories we found genes involved in cold acclimation such as dehydrins, CBF transcription factor and LEA proteins.

References

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