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Identification of cold stress-responsive microRNAs and mRNA in *Eucalyptus grandis*

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MicroRNAs (miRNAs) are a class of non-coding endogenous small RNAs that play a crucial role in plant growth, development, morphogenesis, signal transduction, and stress response. miRNAs negatively regulate gene expression at post-transcriptional level, causing translational repression or/and transcript degradation. The role of miRNAs in understanding gene expression reprogramming under different stress types has been pointed out. Low temperature is an important factor limiting the growth and distribution of plants. Under cold stress, plants attempt to maintain homeostasis and acquire cold acclimation, which involves extensive gene expression and metabolism reprogramming. Particularly, cold stress limits the distribution and productivity of *Eucalyptus* plantations, significantly affecting the survival and yield of seedlings. Despite the consensus on the relevance of miRNAs in regulating gene expression in plants and *Eucalyptus*'s huge importance for the forest industry worldwide, information on miRNAs in *Eucalyptus* is still very scarce. In this work, we studied the miRNA and mRNA response to cold stress in *E. grandis*, one of Uruguay's most widely planted *Eucalyptus* species. Since seedlings are more susceptible to chilling injury, we subjected 3 different genotypes of seedlings to a cold shock followed by a 12-hour recovery phase at 25°C, analyzing a total of 4 treatments (G1: control, G2: 12 hours at 4°C, G3: 24 hours at 4°C, and G4: 24 hours at 4°C + 12 hours at 25°C). Cold treatment was evaluated measuring the electrolyte leakage. The membrane's damage was verified at 12 hours at 4°C, increasing the parameter at 24 hours. The membrane was partially recovered after 12 hours at 25°C, decreasing the electrolyte leakage. Transcriptome analysis showed an increase in the expression of genes belonging to the AP2|ERF family, including the DREB1|CBF genes —a group of genes that control the activation of protection mechanisms under cold stress. Within down-regulated genes, GO terms related to photosynthesis were enriched. Several conserved and new microRNAs were identified, but only a minority changed their expression with cold treatment, among them, the miR828. This microRNA was down-regulated after the cold treatment, which, according to several studies, induces the expression of anthocyanins. Anthocyanin levels are known to increase under different conditions, including cold stress. These results provided data on gene silencing mechanisms in *E. grandis* and allowed characterizing the response to cold mediated by small RNAs. This data could be potentially used by selection and improvement programs seeking to select clones more tolerant to cold stress.

Keywords: cold stress, microRNA, mRNA