Small RNA sequencing and DNA methylation analysis in floral bud reveal that RNA directed DNA Methylation (RdDM) participates during cold accumulation and dormancy release in sweet cherry (*Prunus avium* L.)

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RNA directed DNA methylation (RdDM) is a plant epigenetic mechanism that involves several proteins and small/long non-coding RNAs. This process can generate *de novo* DNA methylation in every cytosine context (CG, CHG, CHH) and occurs mostly on transposons and repetitive elements. Usually, epigenetic modifications can be directly affected by environmental conditions like prolonged exposition to cold temperatures required for dormancy release and flowering in spring. It is known that in some members of the *Rosaceae* family, MADS-box genes involved in bud dormancy are negatively and epigenetically regulated by cold temperatures. In this work we analyzed RdDM participation during bud dormancy and cold accumulation through small RNA deep sequencing and bisulfite sequencing of two sweet cherry MADS-box genes (*PavMADS1* and *PavMADS2*). For small interference RNA analysis, adapters were removed from total reads and the range of size selected was between 18 and 24 nt long. Filtered reads were mapped with perfect match using Bowtie and *PavMADS1/2* sequences were used as reference. For *MADS1* first intron we observe siRNAs only before bud break, coincident with DNA methylation in this locus in all cytosine contexts. On its second large intron, the presence of complementary siRNA in every condition was also related with the maintenance of DNA methylation and the presence of a repetitive element. On the other hand, for *MADS2* no complementary siRNA were present and the methylation mechanism changes to CG methylation. Another RdDM component analyzed was the *DRM2* methyltransferases. Three putative *PavDRM2* were found in *Prunus avium* genome and their RNA level increased together with cold accumulation and bud break. Additionally, *MADS2* relative RNA level was higher than *MADS1* during dormancy, concomitant with the absence of siRNAs and methylations for the first gene. All together these results suggest that RdDM could be involved in dormancy regulation, producing a decrease in the RNA level of target genes.

Funding: FONDEF G09I1008, INNOVA Chile/CORFO 13CTI21520-SP05