Validation of miRNAs involved in regulation of gene expression associated with dormancy and cold requirement in sweet cherry (*Prunus avium*)

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Abstract

Sweet cherry (*Prunus avium L.*) is a flowering plant belonging to Rosaceae family that is grown on temperate climates. In the early fall the temperature and daylight hours decrease promote the entry to the dormancy state, an evolutionary phenomenon relevant for survival during the adverse conditions on winter. Dormancy is divided into three stages, para-dormancy, endo-dormancy and eco-dormancy in which certain requirements must be completed in order to resume grown. The first requirement correspond to cold accumulation during winter (endo-dormancy) and then warm accumulation at the beginning of spring (eco-dormancy) to finally lead to flowering. There are several genome regions that are transcribed but do not encode for proteins, many of these transcripts produce sRNAs (small RNAs) which regulate biological processes by modifying gene expression. Micro RNAs (miRNAs) are a type of sRNA that generate direct mRNA cleavage, translational silencing or transcriptional silencing by DNA methylation after the recognition of they target sequences. In our laboratory, the analysis of sRNAs during dormancy was made in flower buds of *Prunus avium* cv. 'Bing' by high throughput sequencing. miRNAs with differential expression patterns were identified during bud dormancy in sweet cherry. The miRNA miR156 increases its expression towards eco-dormancy, and by using an in silico analysis it was observed that the *PavSPL13* gene that is related to flowering in *Arabidopsis* was recognized by miR156.

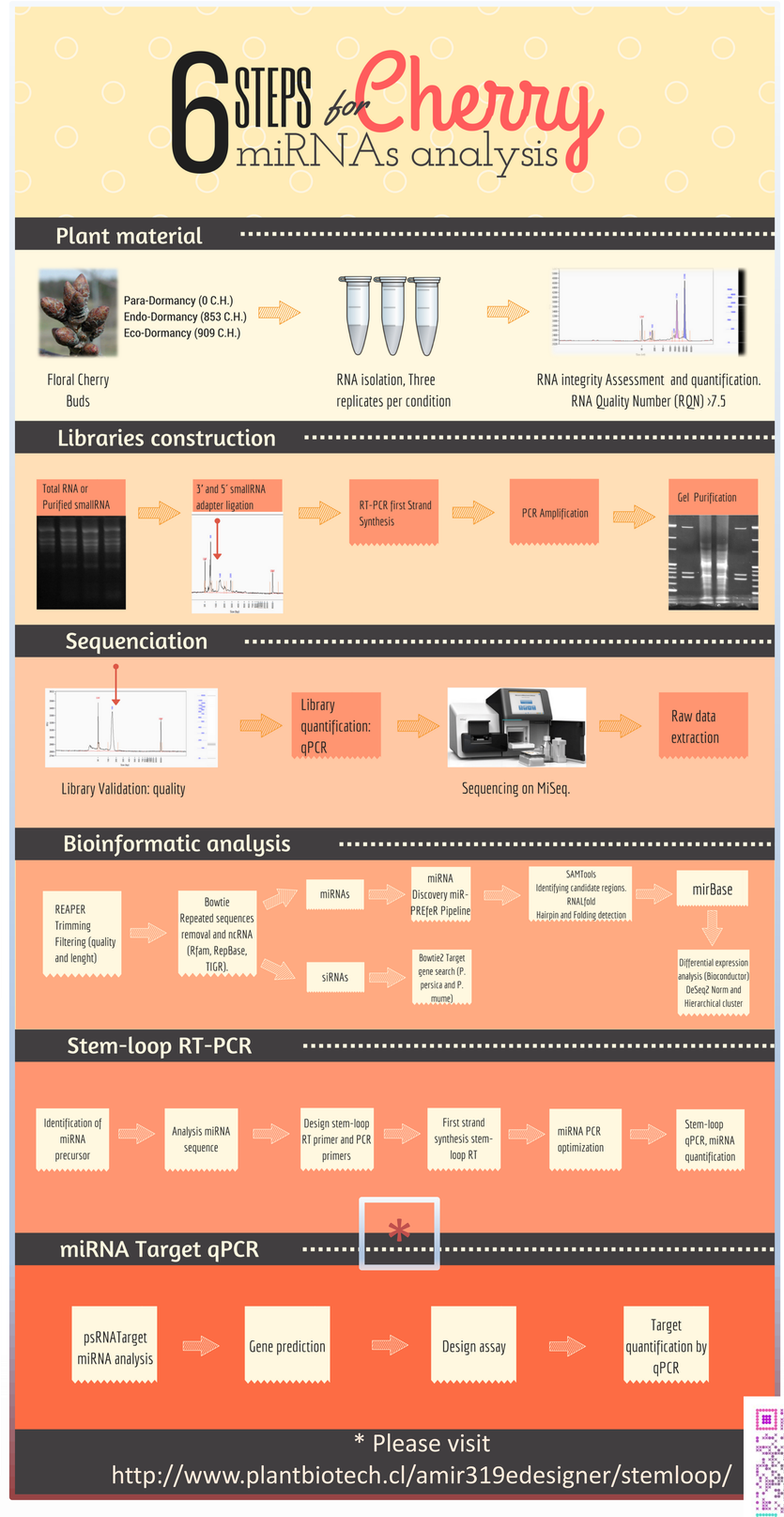
Plant material

Cultivar: 'Bing'

Complete chilling requirement : ≥ 50% of bud break in 14 days with forcing conditions.

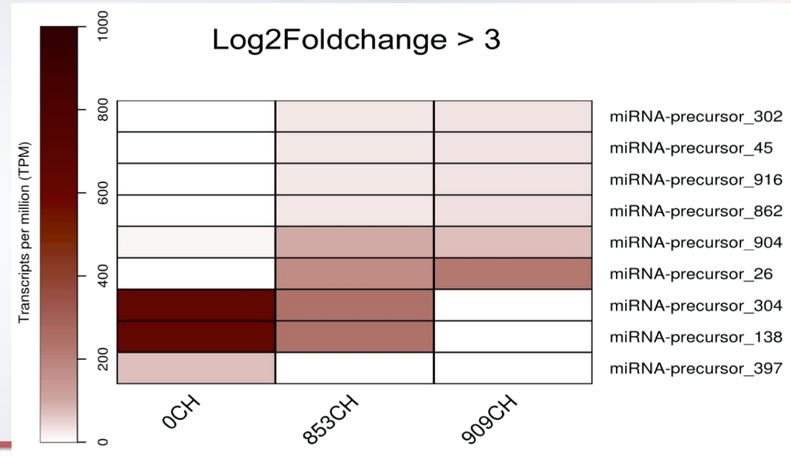
Chilling Hours	693	786	796	853	909	989	1008
Bud break	0%	0%	12.21%	14%	57.68%	83.35%	100%

Plant material. (A) Trials were carried out using sweet cherry flower buds grown at INIA-Rayentué experimental center, O'Higgins region of Chile (34 ° 19 '17 "S, 70 ° 50' 4.2" W). As the trees accumulated cold in the field, cuttings were collected and placed in a greenhouse at 25 ° C and long day photoperiod (16 h/ 8 h). Dormancy output (% of bud break) was established and cold requirement was determined following the cold hours accumulation model (hours < 7.2 ° C; CH-chilling hours). The accumulation of cold defined for endo dormancy (853 CH) and eco-dormancy (909 CH) in 'Bing' are shown (red boxes)

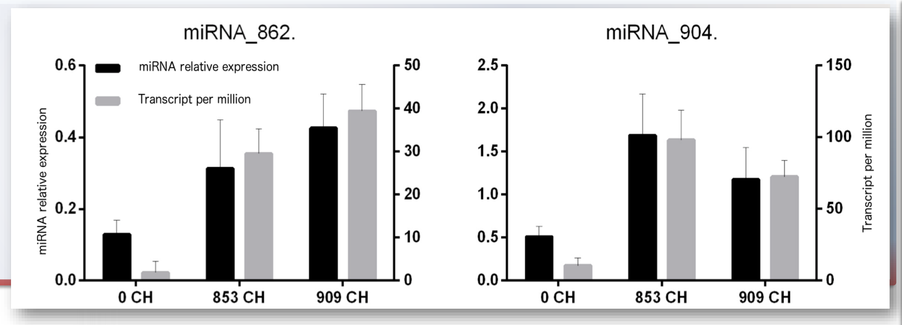


Results

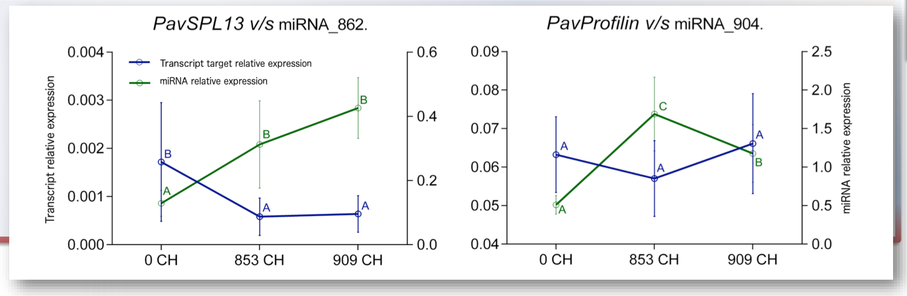
Hierarchical cluster of putative miRNA precursors in *Prunus avium* buds at different CHs. The NGS analysis included nine sequencing libraries for three different stages of cold accumulation. The short sequences obtained were treated according to the bioinformatic pipeline described above, identifying new miRNA precursors.



Expression patterns of two sweet cherry miRNAS affected by chilling accumulation using Stem-loop PCR and NGS. Several of the candidate precursors derived from NGS experiments were experimentally validated using dedicated qPCR.



Comparative expression analysis between miRNAs and their candidate targets. Dormant bud total RNA samples were subjected to qPCR for mRNA level analyses.



Conclusions

- Sweet cherry miRNA precursors, showing differential pattern accumulation in floral buds under cold accumulation, were proposed from NGS.
- NGS results were validated by stem loop PCR for relevant miRNAs.
- Correlation between specific miRNAs and their target genes was achieved.

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