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Novel winter-associated regulators of the circadian clock in poplar.

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Main text (1,000 word maximum: including references, acknowledgments, tables but excluding authors names and affiliations. Character Arial 12pt font).

Background

Winter dormancy is an adaptive mechanism that allows trees from temperate and cold regions to survive the harsh conditions of this season. Critical steps of this process are strongly influenced by environmental cues, mainly daylength and temperature. The mechanism that integrates these signals is the circadian clock. Despite the importance of the correct functioning of the clock for the healthy state of the plant [1], low temperatures cause the disruption of the circadian clock in trees, which consists in a transcriptional activation followed by an arrhythmic expression [2-5].
In this work we uncover winter-associated regulators of the circadian clock in poplar.

Methods

Firstly, we made a transcriptional fusion with the promoter of *LHY2*, a circadian clock gene, and the luciferase gene. This construct was used to generate transgenic poplars (717-1B4, INRA clone). With these events we characterized the expression of this promoter under different conditions of photoperiod and temperature. To this aim we have set up a circadian luminiscence assay registering luciferase activity from leaf discs with a luminometer. Then we carried out a Yeast One Hybrid (Y1H) screening with a library enriched in winter-associated factors and using this promoter as bait. Candidate regulators are tested *in vivo* using Golden Braid technology [6] and transient assays in poplar, by which we overexpressed and silenced the candidate genes.

Results and Conclusions

Here we present the characterization of the *Populus tremula x alba LHY2* promoter under three different photoperiod conditions. Our results indicate the selected promoter region contains the circadian elements as well as the luciferase activity shows the expected expression under both long and short days.
In the Y1H screening, we found several candidates that are classified either as transcription factors or chromatin remodelers. We will discuss the possible role of these proteins as regulators of the poplar circadian clock.

Competing interests

The author declares that they have no competing interests.

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