New insights on winter-associated regulators of the circadian clock in poplar.

José M. Ramos-Sánchez, Alicia Moreno-Cortés, Daniel Conde, Tamara Hernández-Verdeja, Mariano Perales, Isabel Allona

Centro de Biotecnología y Genómica de Plantas (CBGP, UPM-INIA). Departamento de Biotecnología-Biología Vegetal. Universidad Politécnica de Madrid. Campus de Montegancedo, 28223-Pozuelo de Alarcón (Madrid), España

jm.ramos@upm.es

Winter dormancy is an adaptive mechanism that allows trees from temperate and cold regions to survive the harsh conditions of this season. This process is strongly influenced by daylength and temperature, which are signals that are integrated by the circadian clock. Low temperatures cause the disruption of the circadian clock in trees, which consists in a transcriptional activation followed by an arrhythmic expression. In this work we uncover winter-associated regulators of the circadian clock in poplar. We generated transgenic poplars that express a transcriptional fusion between the promoter of a circadian clock gene and the luciferase gene. Setting up a circadian luminiscence assay by which we register the luciferase activity from leaf discs with a luminometer, we have characterized the expression of this promoter under different photoperiods. Then, we carried out a Yeast One Hybrid (Y1H) screening with a library enriched in winter-associated factors using this promoter as bait. Candidate regulators are tested *in vivo* by transient assays in poplar, overexpressing and silencing the candidate genes. 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 discuss the putative role of these proteins as regulators of the poplar circadian clock.

Funding

This work was funded by the Spanish Ministerio de Ciencia e Innovación AGL2011-22625/FOR and by the European KBBE Tree for Joules PIM2010PKB-00702. JM.R-S. is recipient of a predoctoral fellowship from the Spanish Ministerio de Educación, Cultura y Deporte (FPU12/01648). A.M-C. was partly supported by the JC postdoctoral program from the Universidad Politécnica de Madrid (JC/03/2010). M.P is recipient of Ramon y Cajal fellowship from the Spanish Ministerio de Economía y Competitividad (RYC-2012-10194).

10