**Role of RAV genes in tree seasonal dormancy**

Tamara Hernández-Verdeja1, Alicia Moreno-Cortes1, Judith Lucia GomezPorras1, Chris Dervinis 3,4,5, José Manuel Franco-Zorrilla2, Matias Kirst3,4,5, Isabel Allona1

1 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 (tamara.hverdeja@upm.es; alicia.moreno@upm.es; jl.gomez@upm.es; isabel.allona@upm.es)
2 Centro Nacional de Biotecnología, Spanish National Research Council (CSIC), Madrid, Spain. (jmfranco@cnb.csic.es)
3 Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, USA (cderv@ufl.edu; mkirst@ufl.edu)
4 School of Forest Resources and Conservation, University of Florida, Gainesville, USA (cderv@ufl.edu; mkirst@ufl.edu)
5 Genetics Institute, University of Florida, Gainesville, USA. (cderv@ufl.edu; mkirst@ufl.edu)
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**Background**

Plants from temperate regions adapt to changing environmental conditions along the year. Trees have evolved mechanisms that allow them to monitor and anticipate the seasons, by sensing photoperiod and temperature changes, in order to modulate their growth and development. Trees cycle between growth and winter dormancy states. Dormancy is first initiated by shortening of photoperiod, and is characterized by growth cessation, bud development at the apex, and cold acclimation [1, 2]. In a second step, as a result of a drop in temperature, trees reach a state of endodormancy, the inability of resume growth in response to inductive conditions. Chilling requirement, exposure to low temperatures, needs to be fulfilled in order to release from endodormancy and gain the ability to resume growth in response to good conditions [1, 2].
The molecular and signalling networks that regulate dormancy in perennials are poorly understood. Several studies had shown similarities between the shortday (SD) mediated molecular pathways that control the transition to flowering in Arabidopsis and dormancy establishment in trees [3]. Accordingly, it has been described that Poplar orthologs of Arabidopsis FT and CO are implicated in SD induced growth cessation and bud set [3]. We had previously shown that CsRAV1, a chestnut homolog of Arabidopsis TEM1 and TEM2 [4], induced sylleptic branching in poplar [5]. In this work we characterize the role of chestnut and poplar RAV genes in winter dormancy.

**Methods**

For the annual gene expression analyses stems were collected from Castanea sativa or Populus alba adult trees in Madrid, Spain. Total RNA isolation and

quantitative RT-PCR analysis were performed as described previously [5]. Hybrid poplar Populus tremula x P. alba INRA clone 717 1B4 was used to generate the transgenic lines described in [5]. Poplar transgenic lines were screened using the customized arrays designed at the University of Florida [6]. Growth conditions for dormancy induction and release were performed essentially as described in [7]. Arabidopsis (Col-0) were used to generate transgenic lines as described in [8]. Arabidopsis developmental phenotypes were analyzed as described in [9].

**Results and Conclusions**

In order to determine the implication of CsRAV1, PtaRAV1, and PtaRAV2 in the regulation of winter dormancy we have characterized their expression along the year. The results showed that all three genes were induced in early winter and maintained high expression levels until early spring. These data suggested that CsRAV1, PatRAV1 and PtaRAV2 were involved in the regulation of winter dormancy in trees. To test this hypothesis we have used over-expressing CsRAV1 (3xHA:CsRAV OX), and knock-down PtaRAV1 and PtaRAV2 (PtaRAV1&2 KD) transgenic poplars [4]. The results for growth cessation, bud set and bud burst of the transgenic lines will be discussed.
To gain insight on the molecular function of tree RAV genes we screened in silico the promoter region of the homologous FT gene in Populus trichocarpa for the RAV1/TEM1 DNA recognition sites described in Arabidopsis (the bipartite sequence CAACA and CACCTG [10]), as it has been reported that Arabidopsis TEM1 binds to the FT promoter [4]. The search revealed that the RAV1 motif was not conserved, pointing to a functional divergence of RAV family members. To check this possibility, we generated transgenic Arabidopsis plants overexpressing CsRAV1 and looked for the developmental phenotypes described for Arabidopsis TEM1 and TEM2 over-expressors [4]. All the Arabidopsis CsRAV1 over-expressing lines showed WT phenotypes for all the analyzed traits, suggesting that CsRAV1 and Arabidopsis TEM1 and 2 have functionally diverged.
In conclusion, our study reveals a possible function of RAV transcriptional regulators in the control of winter dormancy in trees.

**Competing interests**

The author declares that they have no competing interests.

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