

# Genome-wide identification and expression analysis of *PIN* gene family during adventitious rooting of *Juglans regia* L. microcuttings

Mónica Marques<sup>1</sup>, Rita Pires<sup>1</sup>, Catarina Estevão<sup>1</sup>, Lénia Rodrigues<sup>1</sup>, Valeriya Ustymenko<sup>1</sup>, Catarina Campos<sup>1</sup>, Augusto Peixe<sup>2</sup>, Hélia Cardoso<sup>3</sup>

<sup>1</sup>MED—Mediterranean Institute for Agriculture, Environment and Development & CHANGE — Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

<sup>2</sup>MED—Mediterranean Institute for Agriculture, Environment and Development & CHANGE — Global Change and Sustainability Institute, Escola de Ciências e Tecnologia, Departamento de Fitotecnia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

<sup>3</sup>MED—Mediterranean Institute for Agriculture, Environment and Development & CHANGE — Global Change and Sustainability Institute, Escola de Ciências e Tecnologia, Departamento de Biologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Corresponding author: Hélia Cardoso, [hcardoso@uevora.pt](mailto:hcardoso@uevora.pt)

Walnut tree (*Juglans regia* L.) is one of the most widely spread crops worldwide, highly valued for its fruits. The propagation of *J.regia* cultivars is mostly done through grafting based on seed propagation or through *in vitro* microcuttings. However, even when used optimized *in vitro* protocols several walnut cultivars exhibit a recalcitrant behavior upon the stimulus of adventitious roots development (AR). The auxin indole-3-butyric acid (IBA) is the phytohormone most used in AR protocols. Genes involved in IBA metabolism, including auxin influx/efflux transporters, have been widely characterized in several plant species due to their involvement in AR formation (Velada et al., 2020).

PIN-Formed Auxin Efflux Carrier genes encode one of the most important group of polar auxin transporters in plants, being involved in many developmental processes (Cardoso et al., 2022). To further investigate the involvement of *PIN* in AR efficiency in walnut genotypes, an *in silico* characterization of this gene family was performed. *PIN* sequences retrieved from *Olea europaea* were used as queries in walnut tree genome databases (<https://plants.ensembl.org/index.html>), and the resulting sequences were used as secondary queries.

In total, fourteen *JrPIN* genes were identified, exhibiting a well-conserved exon-intron structure, with most members comprising six exons. To classify the identified *JrPIN* sequences, a phylogenetic tree was constructed using *PIN* sequences of nine Magnoliopsida species. Cluster analysis showed a distribution of the *JrPIN* members across 5 *PIN* subfamilies, with some members belonging to the canonical proteins, characterized by larger sequences comprising a central hydrophilic intercellular loop (HL) (named *JrPIN1a* to *JrPIN1f*, *JrPIN2a* to *JrPIN2b* and *JrPIN3a* to *JrPIN3b*), and 4 members grouped as non-canonical proteins (*JrPIN5* and *JrPIN8a* to *JrPIN8c*), characterized by shorter sequence lacking the HL. Details related with protein features and exon-intron sequence analysis will be presented.

## References:

*Plants* **2020**, *9*(2), 185; <https://doi.org/10.3390/plants9020185>  
*Biology* **2022**, *11*(7), 1040; <https://doi.org/10.3390/biology11071040>

**Acknowledgement:**

This work was financially supported by national funds through FCT (Foundation for Science and Technology) under the Project UIDB/05183/2020.