Strigolactones as a signal of *"cry for help"* in water-saving species: Conclusions drawn from a field experiment

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Authors: **Lorena Ruiz de Larrinaga**[1], Francisco San Miguel Oti[2], Unai Artetxe[1], Juan M. García[3], Estefanía Berrio[3], Juan Antonio López Raez[3], Iker Aranjuelo[4], Jorge Curiel Yuste[2,5], Raquel Esteban[1]

Affiliations

(1) Department of Plant Biology and Ecology, University of Basque Country (UPV/EHU), B/Sarriena s/n, 48940, Leioa, Bizkaia, Spain

(2) BC3-Basque Centre for Climate Change, Scientific Campus of the University of the Basque Country, B/Sarriena s/n, 48940, Leioa, Bizkaia, Spain

(3) Dept. of Soil and Plant Microbiology, Estación Experimental del Zaidín (EEZ-CSIC), Granada, Spain

(4) Instituto de Agrobiotecnología (IdAB), Consejo Superior de Investigaciones Científicas (CSIC)-Gobierno de Navarra, Avenida Pamplona 123, 31192, Mutilva, Spain

(5) IKERBASQUE – Basque Foundation for Science, Plaza Euskadi 5, E-48009, Bilbao, Bizkaia, Spain

Abstract *(Between 300 and 400 words)*

Strigolactones (SLs) are carotenoid-derived plant hormones that play a crucial role in plant adaptation to abiotic stresses. By promoting symbiosis with beneficial soil microorganisms, these hormones enhance nutrient and water uptake, as well as stress resilience. Therefore, the analysis of SLs under water deficit scenarios could be a promising strategy for anticipating drought-induced health transitions in trees, reducing tree mortality, and minimizing forest decline. In this study, the expression of several SL biosynthesis genes (D27, CCD8, and MAX 1) were analyzed. To simulate drought scenarios at the tree level throughout the growing season, rain exclusion umbrellas were installed near Vitoria-Gasteiz (Basque Country, Spain) from March to October 2023. This experiment involved a total of eight trees of a water-saving species (*Pinus pinea* L.), with four trees subjected to drought simulation and the other four serving as controls (no drought simulation). This study aimed first to establish a successful protocol for extracting high-quality RNA and second to analyze the effect of drought on the expression of SL biosynthesis genes using quantitative RT-PCR (qPCR). To analyze the impact of drought at the leaf level, the physiological status of each tree, including carotenoid composition and the functioning of the photosynthetic apparatus, was monitored during the spring and summer of 2023. At the root level, non-structural carbohydrates and root architecture were measured at the end of the growing season. Afterward, four different RNA extraction methodologies were used for root samples: (i.) "RNeasy Plant Mini Kit" (Qiagen, Hilden, Germany), (ii.) Trizol method, (iii.) CTAB method and (iv.) a novel modified CTAB method. Our results showed that only the novel modified CTAB method allowed for obtaining high-quality RNA to conduct molecular subsequent analyses. Moreover, our observations highlighted relevant differences in the responsiveness of the physiological apparatus of *Pinus nigra* L. individuals. Besides,

an inhibition trend for the expression of the three SL genes under drought stress was observed. Overall, although this work will provide a crucial understanding of plant physiology under intense drought periods, providing valuable insights into tree health loss, further research is required to understand why trees reduce SL production under drought stress and whether this is related to the recruitment of beneficial soil microorganisms.

Keywords (Between 3 and 5): Strigolactones, drought experiment, roots, RNA extractions
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