Rosmarinic acid phytotoxicity in Arabidopsis roots is associated with mitochondrial dysfunction and metabolomic changes connected with the production of Reactive Oxygen Species.

Fabrizio Araniti*†[1], Aitana Costas-Gil[2], Carla Díaz-Tielas[2], Manuel J Reigosa[2], Adela Sanchez-Moreiras[2], and Maria Rosa Abenavoli‡[1]

[1]Dipartimento di AGRARIA, Università Mediterranea di Reggio Calabria – Feo di Vito, I-89124 Reggio Calabria, Italy

[2]Department of Plant Biology and Soil Science; Faculty of Biology; University of Vigo – Lagoas-Marcosende, Vigo, Spain

Abstract

The phytotoxic potential of rosmarinic acid (RA), a caffeic acid ester largely found in aromatic species (Boraginaceae and Lamiaceae families), was evaluated *in-vitro* on *Arabidopsis thaliana* (L.) Heynh through a metabolomic (GC-MS) and microscopic approach (TEM and confocal microscopy).

In-vitro bioassays pointed out that rosmarinic acid was able to affect root growth and morphology and detailed microscope analysis evidenced a strong reduction of root meristem accompanied by an alteration of its organization and ultrastructure. In particular, a high vacuolization and alteration of mitochondria structure and function was observed. RA-treatment (175 μM) caused an increase of mitochondria number and a strong reduction of mitochondrial transmembrane potential ($\Delta\Psi$m). These data suggested an energy deficit, which was confirmed by the metabolomic data were a strong alteration of the TCA cycle and amino acids metabolism (alanine, aspartate, glutamate, ß-alanine, glycine, serine, threonine etc.) was highlighted. In addition, root meristem of treated seedlings where characterized by a high accumulation of H2O2 and O2– accompanied by a reduction of catalase and superoxide dismutase activity, which suggest that seedlings exposed to RA are experiencing oxidative stress and are unable to cope with it. Finally, the trypan blue staining showed a high number of root death cells which perfectly correlate with the high vacuolization observed.

Based on these results, we proposed that the alteration of mitochondrial membrane potential accompanied by the inhibition of cell respiration, probably due the increase of reactive oxygen species, which consequently causes cell death, could be the mechanism by which rosmarinic acid induces its strong phytotoxicity on *Arabidopsis* seedlings.

*This research was supported by the Italian Ministry of Education, University and Research (MIUR), project SIR-2014 cod. RBSI14L9CE (MEDANAT).

*Speaker

†Corresponding author: fabrizio.araniti@unirc.it

‡Corresponding author: mrabenvaoli@unirc.it

Keywords: Rosmarinic acid, plant cell death, metabolomic, TCA cycle, mitochondria