

Effect of biostimulants on salt-stressed lettuce plants: a multi-omic perspective

Sonia Monterisi¹, Leilei Zhang², Pascual Garcia-Perez², Monica Yorlady Alzate Zuluaga¹, Michele Ciriello³, Christophe El-Nakhel³, Valentina Buffagni^{2,4}, Mariateresa Cardarelli⁴, Giuseppe Colla⁴, Youssef Rouphael³, Stefano Cesco^{1,*}, Luigi Lucini², Youry Pii¹

¹ Faculty of Agricultural, Environmental and Food Sciences, Free University of Bozen/Bolzano, 39100 Bolzano, Italy.

² Department for Sustainable Food Process, Università Cattolica del Sacro Cuore, Piacenza, Italy.

³ Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy.

⁴ Department of Agriculture and Forest Sciences, University of Tuscia, 01100 Viterbo, Italy.

*Corresponding author: stefano.cesco@unibz.it

Keywords *Lactuca sativa* L., RNA-seq, Untargeted metabolomics, Biostimulant fractionation, Abiotic stress

The increasing soil salinisation is a major challenge for agriculture, as it irreversibly reduces crop yields. Many studies focus on this issue, expecting it to worsen mainly due to climate change. By 2050, half of the world's arable land is projected to be unusable due to high salinity. Plants are highly susceptible to salinity, which affects their growth and productivity. Consequently, over time, plants have developed defense mechanisms and strategies to adapt to salt stress. Key responses include phytohormone modulation, osmoprotectant production, and antioxidant enzymes.

In severe salinity, the plants' endogenous responses may not be enough to support growth. External interventions, like the application of plant biostimulants (PBs), could provide an effective and sustainable solution. Among PBs, protein hydrolysates (PHs) are particularly promising; formed by a mix of polypeptides, oligopeptides, and amino acids, their composition varies based on the protein source, thus determining their effects on the plant. A recent study demonstrated that PHs from *Graminaceae* induce diverse effects in lettuce (*Lactuca sativa* L.) under saline stress, although the contribution of individual components remains unclear.

The present study investigated the molecular effects of PHs derived from *Graminaceae* (P) and their light fraction F3 (<1 kDa) on lettuce under saline stress conditions using a combined transcriptomic and metabolomic approach. Lettuce plants were exposed to two levels of salinity, 0 mM and 30 mM NaCl. Biostimulant treatments were foliarly applied once a week, starting at 10 days after transplanting (DAT); control plants received distilled water. Molecular analyses were performed at commercial maturity (44 DAT).

The results confirmed that the efficacy and mode of action of PHs strongly depend on their composition and plants' physiological status. Both treatments, P and F3, significantly modulate the transcriptome and metabolome of lettuce under saline stress, albeit through distinct mechanisms. For instance, P treatment resulted in the up-regulation of genes involved in biosynthesis and remodelling of the cell wall, cellulose hydrolysis, organic acid biosynthesis, osmoprotectant production, and antioxidants. On the other hand, F3 treatment showed a positive modulation in response to ethylene and genes involved in the *MAPK* signalling pathway, highlighting a regulation of salinity response mechanisms. Interestingly, P up-regulates 17 genes encoding different nucleosome components, which could influence the degree of chromatin condensation, thus modulating its accessibility to transcription factors. Interestingly, the epigenetic mode of action has never been suggested before for PHs in plants. The multi-omic integration of transcriptomics and metabolomics revealed that, under stress conditions, both P and F3 trigger a significant response by phytohormones, including ethylene, abscisic acid, auxin, and brassinosteroids. The latter, in particular, play an important role in plants in counteracting the negative effects of salinity by promoting cell division and elongation.

In conclusion, *Graminaceae*-derived PHs derived can significantly modulate the molecular, biochemical, and physiological responses of lettuce under saline stress. Although P and F3 activate different mechanisms, both treatments provide benefits in counteracting severe salinity conditions. These findings underscore the importance of further investigating the bioactivity of the molecular components of biostimulants to fully maximise their potential in agricultural practices.

Reference.

Monterisi S, Zhang L, Garcia-Perez P, Alzate Zuluaga MY, Ciriello M, El-Nakhel C, Buffagni V, Cardarelli M, Colla G, Roupheal Y, Cesco S, Lucini L, Pii Y (2024) Integrated multi-omic approach reveals the effect of a *Graminaceae*-derived biostimulant and its lighter fraction on salt-stressed lettuce plants. *Sci Rep* 14: 10710