Natural Biostimulants Strengthen Tomato Response to Nitrogen Shortage

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Improving nitrogen (N) use efficiency in crops is a major challenge in modern agriculture. Farmers often apply large quantities of N fertilizers to maintain yields, but much of this N is lost from the soil, contributing to greenhouse gas emissions, water contamination, and economic inefficiency. Finding sustainable solutions that sustain crops' growth with less N is therefore critical for both food production and environmental protection and is directly aligned with current European agricultural sustainability goals.

This study investigated whether plant-derived biostimulants, specifically a *Malvaceae*-based protein hydrolysate (C) and one of its molecular fractions (F2), can help tomato plants cope with limited N availability. Protein hydrolysates are natural products obtained from plant proteins and are increasingly used in agriculture as biostimulants to improve plant growth, stress tolerance, and nutrient uptake. However, their biological mechanisms and the specific role of different molecular fractions are not well understood, representing a key knowledge gap for their optimized agronomic application.

Tomato plants were grown either under optimal or reduced N supply and periodically sprayed with the unfractionated biostimulant (C), its medium-weight peptide fraction (F2), or with water (control). To understand how these treatments influenced the plants' functioning, the study combined transcriptomics (RNA-seq) and metabolomics, allowing the authors to observe changes in both genes' modulation and metabolic compounds and offering a comprehensive systems-level view particularly valuable for applied crop sciences.

Under optimal N conditions, the C biostimulant modulated genes involved in photosynthesis, stress response, and hormone signalling, suggesting a general strengthening of plant metabolism. Both C and F2 influenced genes linked with plant hormones such as auxins and cytokinins, and with the circadian rhythm, a system that helps plants coordinate growth and stress responses.

Under low N availability, the effects were even more relevant for sustainable agriculture. The biostimulant C activated genes related to N transport, light response, and stress protection, indicating an enhanced capacity to adapt to nutrient shortage. Metabolomic analysis showed that C increased the production of fatty acids, amino acids, and phenolic compounds, all associated with stress tolerance. In contrast, the F2 fraction mostly affected hormonal pathways and had a milder metabolic impact.

When integrating molecular datasets, it was found that C triggered a broader reprogramming of N-related processes, whereas F2 primarily influenced signalling pathways. These findings suggest that the full *Malvaceae*-derived hydrolysate is more effective than its isolated fraction in helping tomato plants use N more efficiently and adapt to low-fertility environments. Although fractionation can facilitate the selection and concentration of peptide groups with specific metabolic targets, this process may also inadvertently remove other bioactive peptide

components, thereby limiting the breadth of molecular pathways that can be modulated. This methodological aspect should therefore be considered when evaluating the comparative performance of whole versus fractionated biostimulants.
Overall, this study demonstrates that plant-based protein hydrolysates can enhance crop resilience while reducing dependency on synthetic fertilizers. Their ability to support tomato growth under N-poor conditions has clear implications for sustainable agriculture, including the possibility of adjusting fertilizer application rates to lower inputs, lower environmental impacts, and improved resource efficiency. In this perspective, the results support strategies consistent with current EU directives promoting reduced-input and environmentally responsible agriculture. Although these results are highly promising, further research is needed to clarify how different biostimulant fractions interact with plant metabolism, identifying key molecular targets and the mechanisms underlying enhanced resilience. Such knowledge is essential for optimizing their effective use in crop management strategies and will be crucial to guide future improvements in their formulation and agronomic deployment, ensuring maximum efficiency within more sustainable farming systems.

Keywords:
Solanum lycopersicum L., Protein hydrolysates, Biostimulant fractionation, RNA-seq, Untargeted metabolomic, Nitrogen deficiency