How to evaluate the hormone-like activity of different protein hydrolysates using phenomics data obtained from laboratory bioassays

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Plant derived-protein hydrolysates are an important chategory of biostimulants based on a mixture of peptides and amino acids with a positive effect on plant growth and development, as well as on the tolerance to environmental stresses. They contain bioactive compounds with hormone-like activity and precursors of phytohormone biosynthesis, and therefore they can affect plant metabolism and different physiological processes. The availability of laboratory-based bioassays for the determination of hormone-like activity is a substantial aspect for evaluating the efficacy of different protein hydrolysates on vegetable crops. Bioassays must be rapid, repeatable, effective, and must also provide physiological and/or morphological responses linked to specific hormone activity.

Specific laboratory bioassays have been identified and optimized to investigate several hormone-like activity of vegetal-derived protein hydrolysates on root development (auxin- like activity), hypocotyl elongation (gibberellin-like activity), cotyledon expansion and chlorophyll degradation (cytokinin-like activity).

In this study, to investigate auxin-like activity of vegetal-derived protein hydrolysates we conducted *Root Test of Tomato Cuttings* based on the key role of auxin molecules promoting formation of adventitious roots on the stem. For gibberellin-like activity we performed *Lettuce Hypocotyl Elongation Test* in which gibberellins express their contribute to hypocotyl elongation. Concerning cytokinin-like activity we used both *Cucumber Cotyledon Expansion Test*, to analyze the involvement of cytokinins in regulation of cell division and plant organ development, and Cucumber Cotyledon Chlorophyll Degradation Test showing the key role of cytokinins to inhibit foliar senescence. Phenomics data have been integrated with metabolomic analysis confirming as protein hydrolysates interfere with signaling and response pathways thereby representing an important player in cell-to-cell communication networks.

Increasing doses of indolacetic acid, gibberellic acid and kinetin (known growth regulators) were also applied to tomato cuttings, lettuce hypocotyls and cucumber cotyledons, respectively, and the obtained results were directly proportional to the hormone dose, confirming the effectiveness of bioassays in determining hormonal activity.

Keywords: biostimulants, tomato cutting, cotyledon expansion, chlorophyll.