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Probing the hormonal activity of fractionated humic molecular components in tomato auxin mutants

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Auxin-like action of humic substances on plants lateral root induction has been since long recognized, though uncertainty remains on the bioactivity process. Since both apparently small and large molecular-size humic substances similarly affect lateral root emergence, the auxin-like effects appear rather related to structural composition of humic matter. In this work we progressively reduced the complexity of vermicompost humic matter (HV) by a Humeomics approach based on a mild sequential removal of humic components. A stepwise fractionation sequentially extracted unbound or free components, weakly-, and strongly-bound molecules from the supramolecular HV structure. Auxin-like response of residues from each step was tested using tomato auxin low sensitive dgt mutant and transgenic DR5::GUS plants. Typical bell shape curve was observed using HV and their promotion of lateral root emergence was comparable to that of an exogenous auxin treatment. When strongly-bound components were removed by breaking ester and ether bonds from complex humic structures, humic residues lost their activity on lateral root emergence, while this was retained after removal of only free or weakly-bound molecules. These findings confirm that auxin-like activity in humic matter is associated to complex hydrophobic structures, whose simplification by hydrolysis releases auxin-like molecules. Although other phenomena may contribute to the physiological response of humic substances, such as the increase of nutrient absorption, the lateral root emergence is to be ascribed to action of auxin-like molecules incorporated in humic hydrophobic domains.