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# Optimizing root exudate collection to enhance metabolomic analysis of neighboring plant interactions

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#### **Abstract:**

Plant roots release significant amounts of root exudates into the surrounding soil, shaping rhizosphere dynamics and mediating plant-soil interactions. These exudates include high-molecular-weight compounds such as proteins and mucilage, as well as a diverse array of low-molecular-weight compounds like primary metabolites (e.g., amino acids sugars, carboxylates) and secondary metabolites (e.g., sorgoleone, flavonoids, coumarins). Understanding the composition and functions of these exudates is essential for elucidating mechanisms of plant-to-plant communication. However, the effects of environmental stressors, such as drought, on these interactions remain poorly understood, representing a critical knowledge gap for advancing crop resilience strategies.

In this study, we investigated root exudate secretion under controlled conditions, testing three sterilized substrates—river sand, glass beads, and epoxy-resin-coated sand. Additionally, we developed an innovative 3D-printed pot system that physically separates two root-growing environments while allowing unidirectional root communication. This setup enabled the evaluation of self and non-self interactions between the roots of *Solanum lycopersicum* L. and *Tagetes patula* L. Two irrigation methods were compared: nutrient solution recirculation and daily irrigation with discharge of percolating solution. To optimize exudate collection, we tested two eluents: distilled water and a methanol:water:formic acid mixture (50:49.9:0.1, v/v).

Our findings emphasize the importance of selecting appropriate substrates, eluents, and experimental setups for root exudate studies. These insights advance our understanding of crop resilience mechanisms, providing valuable tools for improving agricultural sustainability under climate change.

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**Keywords:** Crop resilience; drought; growing substrates; root exudates; secondary metabolites.

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