Volatile organic compounds mediate plant–bacteria interactions and influence root development in *Brassica juncea* (L.) Czern.

Brassica juncea (Indian mustard) is a metal-accumulator plant used to remediate contaminated soils through phytoremediation. A promising strategy to enhance its efficiency is the exploitation of bacteria that release volatile organic compounds (VOCs), or the direct application of selected VOCs to prime plants for stress tolerance and growth. However, the mechanisms underlying VOC-mediated plant–bacteria interactions are still poorly understood.

This study investigates the interaction between Indian mustard and selected strains of plant growth-promoting bacteria (PGPBs) to identify VOCs that may promote plant growth under both non-stress and metal-stressed conditions.

A rectangular steel pot was filled with autoclaved sand. *B. juncea* seeds were sown at one end, while a consortium of selected PGPBs was inoculated in the centre, 10 cm from the seeds. VOCs were collected over time using perforated Teflon tubes connected to a vial containing an SPME fibre and subsequently identified by GC–MS analysis. At the end of the experiment, VOCs were also detected in *B. juncea* roots. In parallel, bacterial strains were identified in seeds and roots to assess the establishment of symbiotic relationships.

Identified VOCs were classified into two groups: compounds involved in plant–microorganism interactions and compounds associated with plant development. Among the latter, 3-octanone, dimethyl disulfide, and tetradecanal were selected as VOCs potentially released by *Bacillus* and *Microbacterium* strains detected as root endophytes. Preliminary tests showed that these compounds modified seedling root system architecture and generally promoted their root growth. Further experiments will evaluate their effects on Indian mustard plants under Cd stress.

- **Plant–bacteria interactions mediated by volatiles shape root growth in Brassica juncea**
- *Brassica juncea* (Indian mustard) is widely recognized for its capacity to accumulate heavy metals and is therefore employed in phytoremediation strategies for contaminated soils. One emerging approach to improve its performance involves the use of plant growth-promoting bacteria (PGPB) that emit volatile organic compounds (VOCs), as well as the direct application of selected volatiles to enhance plant growth and stress resilience. Despite increasing interest, the biological mechanisms driving VOC-mediated plant–microbe interactions remain largely unresolved.
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- In this work, we explored the interaction between Indian mustard and selected PGPB strains, with the aim of identifying bacterial VOCs capable of modulating plant growth under both optimal and metal stress conditions. Plants were grown in a rectangular steel container filled with sterilized sand. *B. juncea* seeds were sown at one end, while a consortium of selected plant growth-promoting bacteria (PGPBs) was inoculated at the center of the pot. A distance of 10 cm separated the inoculated area from the plant growth area. Teflon tubing was installed to explore the soil and collect emitted VOCs into glass vials from three zones in the pot: the plant area (PA), the inoculated area (IA), and the area opposite the plants containing only sandy soil (SA). Volatile compounds emitted by roots and bacteria were identified over time. VOCs in the vials were trapped by head-space solid phase microextraction (HS-SPME) and analyzed by gas chromatography-mass spectrometry (GC-MS).
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- Seeds were placed at one end, while a consortium of bacterial strains was inoculated centrally, at a distance of 10 cm. VOCs emitted during the experiment were sampled over time using perforated Teflon tubing connected to a vial containing an SPME fibre, and subsequently analyzed by GC–MS.
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- At the conclusion of the experiment, VOCs were also detected in root tissues.
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- In addition, bacterial colonization of seeds and roots was assessed via high-throughput 16S rRNA gene Illumina sequencing to verify the establishment of plant–bacteria associations.
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- The identified VOCs were grouped into compounds related to plant–microbe communication and those associated with plant developmental processes. Among these, 3-octanone, dimethyl disulfide, and tetradecanal were selected as candidate molecules likely produced by *Bacillus* and *Microbacterium* strains isolated as root endophytes. Preliminary assays indicated that these compounds alter root system architecture and tend to stimulate root growth in seedlings. Ongoing experiments are aimed at evaluating their effects on plant performance under cadmium-induced stress.