

Environmental and microbial drivers of nectar flows: the case study of sunflower *Helianthus annuus*

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Introduction

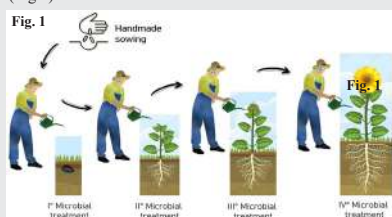
Among nectarous plants of interest for the sector, sunflowers (*Helianthus annuus*) are of particular interest: they are rich in nectar and pollen and can ensure substantial honey harvests for beekeepers. However, a drop in sunflower nectar secretions, and therefore, in honey production, has been complained by beekeepers since 2017 at least. This decline is mainly driven by heat waves and particularly intense dryness, but also other factors might affect plant performance: the decreased microbial diversity of soils in the agroecosystems, which does not allow the establishment of a beneficial "dialogue" between sunflowers and soil microbiome. Soil microorganisms are responsible for the solubilization of microelements useful for nectar secretion such as manganese and magnesium. Plant Growth Promoting Bacteria (PGPB) are soil-born microorganisms widely used as bio-fertilizers in agriculture to increase plant growth and resistance to environmental stress factors, also in microbial degraded soils.

The research aim: to determine the impact of PGPB fertilizers on: a) Soil microbial communities of the Sunflower rhizosphere. b) Gene expression of passive sugar transporters (SWEET) responsible for nectar secretions.

Methods

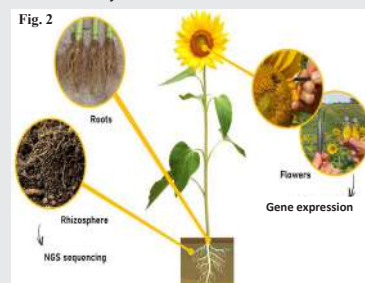
1. In field trial

A field trial involving a high oleic hybrid sunflower variety (LST907) and a non-hybrid variety (Peredovick) was established in 2023 and 2024. Both sunflowers varieties were divided into plots, and the rhizosphere was inoculated with different microbial inoculants of *Bacillus*, *Paenibacillus*, and *Lactobacillaceae*. Microbial treatments were performed once a month, till blooming (Fig.1).



2. Samples collection

Samples collection of flowers, rhizosphere, roots were collected from the different experimental conditions at blooming time. All samples were immediately stored in dry ice until DNA and RNA extraction, which was performed with commercial kits. Extracted RNA was immediately converted in cDNA with RevertAid First Strand cDNA Synthesis Kit



3. Samples analysis

NGS: rhizosphere microbiota and endophytes were analyzed at University of Bologna. Extracted DNA was amplified (V3-V4 regions of the 16S rRNA) and sequenced on Illumina Novaseq 4000 platform.

Gene Expression: BLAST (blastn suite) was used to identify the *Helianthus annuus* SWEET9 gene region on a de novo assembled whole genome sequencing of *H. annuus*, by aligning the SWEET9 gene's nucleotide sequence of *Brassica rapa*. Primer design was performed with PRIMER 3 software. We quantified the expression of SWEET genes in sunflower by qPCR in triplicates. Relative abundance was calculated using the $2^{-\Delta\Delta C_t}$ method: Ct values of SWEET genes were normalized to a stable reference gene β -tubulin and the results compared to all the experimental conditions described in "In field trial" section".

4. Statistical analysis

Datasets were analyzed with ANOVA when data were normal and homoscedastic, while GLM was applied for non-normal homoscedastic data.

Results

Some PGPB are drivers of sunflower rhizosphere microbial composition, impacting more than the variety

- ASVs showed that the rhizosphere of LST907 sunflower variety acquired a different microbiome when compared to the Peredovick variety (PCoA - Fig. 3), with 164 missing microbial genera (16.2%) in LST907.

- LST907 did not show root colonization by typical honeybee commensal microorganisms such as *Bombella*, *Commensalibacter*, *Bartonella*, and *Frischella* that actually were present in the Peredovick variety.

- *Lactobacillaceae* could colonize and drive the sunflower rhizosphere better

than other PGPB. *Lactobacillaceae* also showed a greater colonization of the Peredovick rhizosphere as showed by the weighted unfrac PCoA analysis ($p < 0.05$, Fig. 4a and 4b).

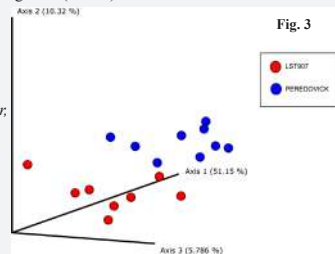


Fig 4a -LST

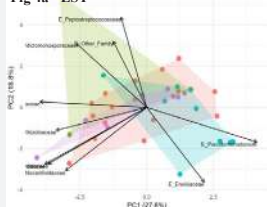
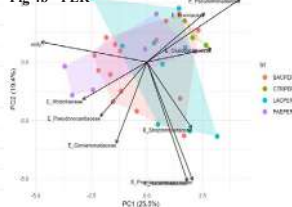


Fig 4b - PER

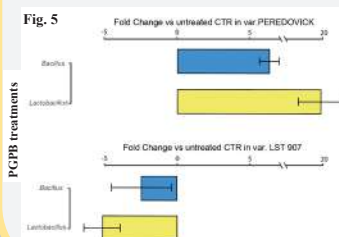


- *Bacillus* and *Paenibacillus* PGPB treatments, on the contrary, did not significantly alter the Sunflower's rhizosphere.

Results

Gene expression of sugar transporter *HeSWEET9*

The results clearly show a varietal effect, meaning that the two genotypes respond differently to the treatments. The application of PGPB strains led to an increase in the expression of the *HeSWEET9* gene in the Peredovick variety, where both the



microbial treatments increased the gene expression of 7.36 and 19.78 folds in Peredovick plants treated with *Bacillus* and *Lactobacillus* respectively, when compared to untreated Peredovick sunflowers (CTR).

The application of the same PGPBs led to an opposite result on the LST 907 variety (Fig 5).

Conclusions

Overall, this research highlights the impact of biobased microbial fertilizers (PGPB) on the rhizosphere microbial community and, consequently, on the regulation of sugar transporter gene expression in sunflower plants. Such modulation may influence the intensity and dynamics of nectar flow, providing potential benefits for pollinators and honeybees. In this way, PGPB treatments can affect not only plant physiology but also the trophic potential and ecological role of nectar-producing crops such as sunflowers.



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In the last decade, beekeepers operating in southern European regions have experienced consistent problems in honey production. Among nectarous plants of interest for the sector, sunflowers (*Helianthus annuus*) are of particular interest: they are rich in nectar and pollen and can ensure substantial honey harvests for beekeepers. However, a drop in sunflower nectar secretions, and therefore, in honey production, has been complained by beekeepers since 2017 at least. This decline is mainly driven by heat waves and particularly intense dryness, but also other factors might affect plant performance: the decreased microbial diversity of soils in the agroecosystems, which does not allow the establishment of a beneficial "dialogue" between sunflowers and soil microbiome. Soil microorganisms are responsible for the solubilization of microelements useful for nectar secretion such as manganese and magnesium. Plant Growth Promoting Bacteria (PGPB) are soil-born microorganisms widely used as bio-fertilizers in agriculture to increase plant growth and resistance to environmental stress factors. However, it is currently unknown how PGPB can modify the plant physiological processes leading to nectar secretion, thus influencing the plant-pollinators network. To shed light on these aspects, a trial involving hybrid and non-hybrid sunflower varieties was established in Italy. Sunflowers were sown in soil plots, which were inoculated with different PGPB such as *Bacillus* and *Lactobacillaceae*. About 200 flowers were collected from the different experimental plots and analyzed for the gene expression of SWEET passive sugar transporters (RT-qPCR). Simultaneously, sunflower root samples were collected, and the epiphytic microbial community was sequenced on Illumina Hi-Seq. Results showed that the sunflower variety mainly drove the microbial colonization of the rhizosphere. Some of the tested PGPB significantly altered the expression of SWEET passive sugar transporters genes. These results represent a first step in understanding the role of PGPB on sunflower attractiveness to pollinators, giving an insight into how climate change may affect ecological niches that are apparently not correlated with honeybees' lives but actually may play a pivotal role in honeybees' performances. This research was carried out in the European Union Next-Generation Framework (PNRR – mission 4, component 2, investment 1.4 – D.D. 1032 17/06/2022, CN00000022).