

Rhizosphere Microbiome Diversity and Breeding Relevance in Cucurbit Crops from Tunisia

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The Cucurbitaceae family, including melons, watermelons, and pumpkins, represents a major group of dietary staples worldwide. Understanding their microbiomes has far-reaching implications for crop sustainability and quality. Root-associated microbiomes play a fundamental role in plant growth, nutrient acquisition, disease resistance, and overall crop performance (Sandrini et al. 2022). However, cucurbit breeding programs have traditionally focused on plant genetics alone, largely overlooking the contribution of rhizosphere microbiota to agronomic performance. Recent studies demonstrate that plant genotypes actively shape their associated microbiome communities and that these communities, in turn, influence yield and fruit quality (Sandrini et al. 2022; Aydi-Ben-Abdallah et al. 2021). This growing understanding opens new perspectives for holobiont-based breeding strategies that integrate both plant and microbiome traits to enhance productivity while supporting sustainable agriculture (Kusstatscher et al. 2021).

To explore the potential of microbiome-assisted selection in cucurbit breeding, three complementary studies conducted at CRRHAB-Tunisia characterized rhizosphere microbiome diversity in melon and snake melon (*Cucumis melo* L. and *C. melo* var. *flexuosus*), watermelon (*Citrullus lanatus*), and pumpkins (*Cucurbita* spp.). In total, 64 genotypes were assessed for key rhizosphere microbiome components, including total bacteria, actinomycetes, total fungi, *Pseudomonas* spp., *Trichoderma* spp., and *Aspergillus* spp., and their relationships with yield and fruit quality traits (Aydi-Ben-Abdallah et al. 2021; 2023; 2024).

In melon and snake melon, Aydi-Ben-Abdallah et al. (2021) showed that genotypes selectively recruit distinct rhizosphere microbiome communities. The microbiomes of most genotypes exhibited significantly higher bacterial and actinomycete populations compared with the control (a bulk soil sample taken prior to planting and not directly attached to plant roots) while total fungal populations remained similar. Twelve genotypes were characterized by enrichment of *Pseudomonas* spp., a group known for plant growth promotion and disease suppression. Superior agronomic performance, including increased plant height and fruit weight, was observed in specific melon genotypes (H0, H7, H11, H17, H52), while snake melon genotypes (H47 to H50) displayed the

highest yields. Overall, top-performing genotypes tended to harbor higher bacterial populations (especially *Pseudomonas* spp.), abundant actinomycetes, and elevated populations of *Trichoderma* spp., suggesting a strong association between microbiome composition and crop productivity.

Watermelon accessions also displayed genotype-dependent rhizosphere microbiome profiles (Aydi-Ben-Abdallah et al. 2023). Significant variation was detected in actinomycetes and *Trichoderma* spp. populations, and hierarchical analyses grouped accessions according to their microbiome composition. Notably, rhizosphere soil properties, particularly pH and electrical conductivity, varied among accessions, suggesting that plant genotype influences both soil characteristics and microbiome structure. The top-performing accessions (P1 and P8), which produced the highest fruit weight and sweetest taste, were characterized by the highest actinomycetes and *Aspergillus* spp. populations in their rhizosphere. These associations suggest a potential role of actinomycetes and *Aspergillus* spp. in enhancing watermelon yield and fruit quality. These findings support the use of associated microbiota as complementary selection criteria in watermelon breeding programs.

Similarly, pumpkin accessions across three species (*C. pepo*, *C. maxima*, and *C. moschata*) revealed strong genotype- and species-level effects on rhizosphere microbiome composition (Aydi-Ben-Abdallah et al. 2024). The top-performing *C. maxima* accessions (C5, C23, C14.2, C6.2) combined superior fruit weight and yield with the highest populations of bacteria, actinomycetes, *Trichoderma* spp., and *Aspergillus* spp., alongside the lowest total fungal populations in their rhizosphere. Positive correlations were observed between bacterial populations and fruit weight, and between *Trichoderma* spp. and yield, while total fungal populations were negatively associated with both traits. These patterns indicate that productive pumpkin genotypes maintain distinctive microbiome communities characterized by the highest number of beneficial microorganisms.

Overall, these studies demonstrate that cucurbit genotypes consistently shape their rhizosphere microbiome communities and that microbiome composition is closely associated with yield and fruit quality (Table 1). In particular, the enrichment of beneficial microbial groups—especially

actinomycetes, *Pseudomonas* spp., and *Trichoderma* spp.—in top-performing genotypes (Table 2) suggests a potential functional role of microbiome recruitment in enhancing nutrient availability, growth promotion, and disease suppression.

From a plant breeding perspective, the results highlight the potential value of integrating rhizosphere microbiome profiling into breeding pipelines as an early-screening tool to identify high-performing genotypes alongside traditional phenotypic assessments. Genotypes associated with the recruiting of beneficial microbial communities could be prioritized as parents in crossing schemes, supporting the development of holobiont-based selection strategies that consider the plant and its associated microbiome as an integrated biological system.

Beyond breeding applications, such microbiome-associated traits may also contribute to more sustainable production systems by reducing dependence on external inputs. Genotypes enriched in beneficial microbiota could potentially require lower applications of fertilizers and fungicides, an advantage that is particularly relevant for cucurbit cultivation in semi-arid environments such as Tunisia, where soil fertility constraints and input-use efficiency are critical challenges.

However, the microbiome–performance relationships reported across these studies remain correlative. Although consistent across species and traits, the underlying causal mechanisms have yet to be confirmed through controlled inoculation experiments and mechanistic studies. Future research should therefore validate these associations under diverse environmental conditions and further elucidate the functional roles of the key microbial taxa underlying the observed plant benefits.

Acknowledgements

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Table 1. Summary of rhizosphere microbiome composition in cucurbit crops (melon, watermelon, and pumpkin) grown at the Sahline Experimental Station of CRRHAB, Tunisia. (from Aydi-Ben-Abdallah et al. 2021; 2023; 2024)

Crop	BACTERIAL POPULATIONS			FUNGAL POPULATIONS				
	Total Bacteria	Actinomycetes	<i>Pseudomonas</i> spp.	Total Fungi	<i>Trichoderma</i> spp.	<i>Aspergillus</i> spp.	<i>Penicillium</i> spp.	<i>Fusarium</i> spp.
Melon/Snake melon (<i>C. melo</i>)	High in most; Highest in top-yielding accessions	High in most; Abundant in superior accessions	Elevated in top accessions	Comparable to control	Elevated in top snake-melon accessions	Elevated in top-yielding snake melon accessions	Present in some top accessions	Absent in most; Absent/low in best accessions
Watermelon (<i>C. lanatus</i>)	Variable among accessions	Highest in top-yielding accessions	-	Elevated in top accessions	Elevated in some accessions	Highest in top accessions	-	Variable, no significant difference
Pumpkin (<i>Cucurbita</i> spp.)	Highest in top accessions; Positive correlation with fruit weight	Highest in top-accessions	-	Lowest in top accessions; negative correlation with fruit weight and yield	Highest in top accessions; positive correlation with yield	Highest in top accessions	-	-

Table 2. The rhizosphere microbial community structure in cucurbit accessions grown at Sahline Experimental Station of CRRHAB, Tunisia. The table presents culturable populations present in the rhizosphere of cucurbit accessions (mean across samples) compared to populations present in bulk (control) soil samples taken prior to planting. Accessions were classified as “top-performing” based on fruit weight, yield, and microbiome profile. Watermelon values are mean log CFU g⁻¹ fresh soil. Pumpkins values are mean CFU g⁻¹ fresh soil. *Pseudomonas* spp. and *Penicillium* spp. were assessed in melon only.

Accession		Bacterial Populations			Fungal Populations					Yield / Fruit quality
		Total Bacteria	Actinomycetes	<i>Pseudomonas</i> spp.	Total Fungi	<i>Trichoderma</i> spp.	<i>Aspergillus</i> spp.	<i>Penicillium</i> spp.	<i>Fusarium</i> spp.	
Melon & Snake Melon (<i>Cucumis melo</i>) - Aydi-Ben-Abdallah et al. 2021 [n=45 melon + 7 snake melon genotypes]										
Bulk soil (control)	1.70 ×10 ⁸ CFU g ⁻¹	0.66 ×10 ⁴ CFU g ⁻¹	0	21 ×10 ⁴ CFU g ⁻¹	0	11 ×10 ⁴ CFU g ⁻¹	0	2.3 ×10 ⁴ CFU g ⁻¹	—	
Top melon genotypes: H0, H7, H11, H17, H52 Top snake melon genotypes: H47–H50	59–68% higher than bulk soil	28.5–92.6% higher than bulk soil	~3× bulk soil (enriched in 12 genotypes)	Similar to bulk soil	3–23× higher than bulk soil	36.8–53.8% higher than bulk soil (H49, H50)	Present in selected genotypes	Absent in most	Highest fruit weight, plant height & yield per plant positive correlation fruit weight with <i>Pseudomonas</i> & actinomycetes	
Other genotypes (lower performers)	Similar to bulk soil	Moderate increase vs. bulk soil	Low or absent	Similar to bulk soil	Low or absent	Low	Variable	Variable	Lower fruit weight & yield	
Watermelon (<i>Citrullus lanatus</i>) - Aydi-Ben-Abdallah et al. 2023 [n=7 accessions evaluated]										
Bulk soil (control)	6.15 (log CFU g ⁻¹)	3.26 (log CFU g ⁻¹)	—	3.53 (log CFU g ⁻¹)	0.76 (log CFU g ⁻¹)	1.60 (log CFU g ⁻¹)	—	0.89 (log CFU g ⁻¹)	—	
Top watermelon accessions: P1 & P8	5.49–5.81 (log CFU g ⁻¹) Variable vs. bulk soil	3.66–3.71 (log CFU g ⁻¹) Highest among top accessions	—	3.52–3.99 (log CFU g ⁻¹) Low to intermediate	0.40–1.53 (log CFU g ⁻¹)	2.05–3.09 (log CFU g ⁻¹) Highest among top accessions	—	0.60–0.65 (log CFU g ⁻¹)	9.5–10 kg fruit weight / 9.46–9.8 °Brix (sweetest) positive correlation with actinomycetes & <i>Aspergillus</i> spp.	
Remaining accessions (P2, P6, P14, P15, P16)	5.47–5.89 (log CFU g ⁻¹) Variable	3.37–3.60 (log CFU g ⁻¹) Lower than top accessions	—	3.50–4.02 (log CFU g ⁻¹) Variable	0.67–2.94 (log CFU g ⁻¹) Variable	1.78–2.35 (log CFU g ⁻¹) Lower than top accessions	—	0.23–1.04 (log CFU g ⁻¹) Variable	2.35–5.17 kg fruit weight ; 2.56–8.5 °Brix	

Accession		Bacterial Populations			Fungal Populations					Yield / Fruit quality
		Total Bacteria	Actinomycetes	<i>Pseudomonas</i> spp.	Total Fungi	<i>Trichoderma</i> spp.	<i>Aspergillus</i> spp.	<i>Penicillium</i> spp.	<i>Fusarium</i> spp.	
Pumpkin (<i>Cucurbita</i> spp.) - Aydi-Ben-Abdallah et al. 2024 [n=12 accessions, 3 species]										
Bulk soil (control)		2.99 ×10 ⁷ CFU g ⁻¹	0.95 ×10 ⁴ CFU g ⁻¹	—	1.62 ×10 ⁴ CFU g ⁻¹	1.12 ×10 ³ CFU g ⁻¹	0.12 ×10 ³ CFU g ⁻¹	—	0.18 ×10 ³ CFU g ⁻¹	—
Top <i>C. maxima</i> accessions: C5, C23, C14.2, C6.2		1.62–3.35 ×10 ⁸ CFU g ⁻¹ Highest among top accessions; positive correlation with fruit weight	1.13–1.68 ×10 ⁵ CFU g ⁻¹ Highest among top accessions	—	0.93–1.32 ×10 ⁵ CFU g ⁻¹ Lowest total fungi	1.75–2.08 ×10 ⁴ CFU g ⁻¹ Highest among top accessions positive correlation with yield	2.75–3.75 ×10 ⁴ CFU g ⁻¹ Highest among top accessions	—	0.07–1.50 ×10 ⁴ CFU g ⁻¹ No significant difference	4.18–8.48 kg fruit weight and 5.31–6.21 kg plant ⁻¹ yield negative correlation total fungi with fruit weight & yield
Remaining accessions (<i>C. pepo</i> & <i>C. moschata</i> + lower <i>C. maxima</i>)		1.48–2.66 ×10 ⁸ CFU g ⁻¹ Lower or comparable	0.74–1.08 ×10 ⁵ CFU g ⁻¹ Lower than top accessions	—	1.20–1.69 ×10 ⁵ CFU g ⁻¹ Higher total fungi	0.33–1.58 ×10 ⁴ CFU g ⁻¹ Lower Trichoderma spp.	1.92–3.33 ×10 ⁴ CFU g ⁻¹ Variable	—	0.07–1.66 ×10 ⁴ CFU g ⁻¹ Variable	2.53–3.9 kg fruit weight and 0.91–3.96 kg plant ⁻¹ yield