Microbial footprints of tomato domestication

Sarango Flores, S.W.

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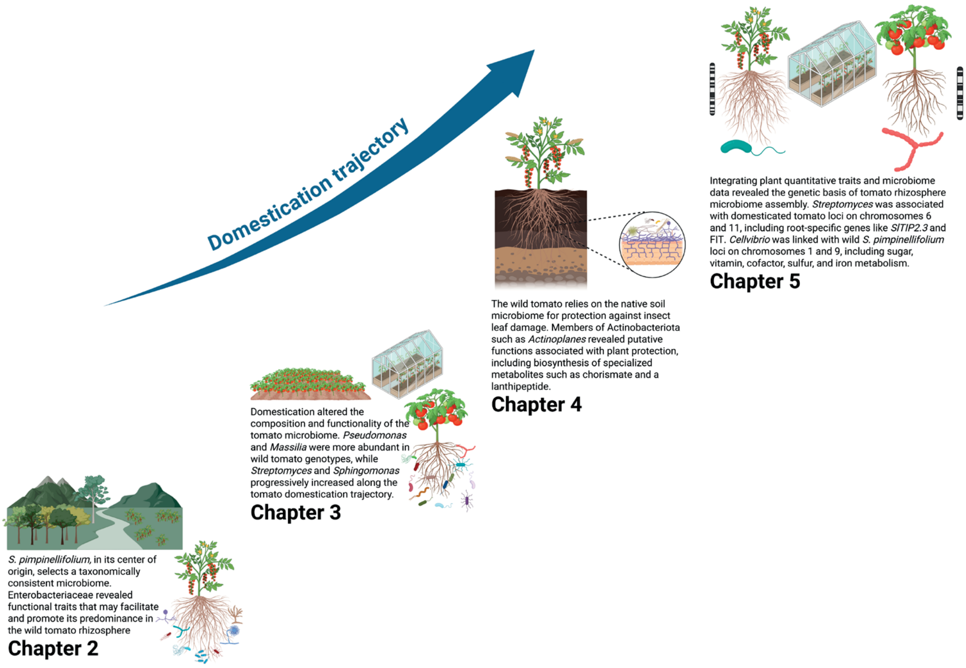
Chapter 6

General Discussion

Plant domestication is an evolutionary process driven by both intentional and unintentional human influences (Purugganan, 2022). Early domestication involved the conscious selection of desirable plant traits, such as larger fruit or more vigorous growth. At the same time, changes in cultivation practices and environmental conditions, also referred to as habitat domestication, exerted selective pressures similar to natural selection, shaping domesticated plants in unintended ways (Soldan et al., 2021). These combined forces, human intervention and the environmental context, have influenced both plant traits and their interactions with their surrounding ecosystem, including the microbiome (Pérez-Jaramillo et al., 2016; Mo et al., 2024). The plant-associated microbiome plays a key role in supporting the host plant via nutrient acquisition and stress tolerance (Santhanam et al., 2015; Pérez-Rodriguez et al., 2020; Adedayo et al., 2023). In current conventional agriculture, domesticated crops are highly dependent on fertilizers and pesticides, which can either replace or deplete microbial functions (Pérez-Jaramillo et al., 2018; Soldan et al., 2021). Moreover, the loss of genetic variation in domesticated cultivars is thought to have disrupted the effective communication with their microbiome, as supported by several studies (Chen et al., 2022; Huang et al., 2022; Nerva et al., 2022; Yue et al., 2023). Consequently, beneficial microbial associations may have been significantly affected during the long trajectory of plant domestication (Smulders et al., 2021; Oyserman et al., 2022). Deciphering how plant domestication impacted the assembly and functionality of the microbiome remains a serious challenge. Addressing this knowledge gap is essential for translating microbiome research into practical strategies for sustainable crop production in a world of increasing human population, climate change, and intensified (a)biotic stresses in degraded agroecosystems.

My PhD thesis examined the central question if and how tomato domestication has left discernible imprints on the rhizosphere microbiome. By integrating a plethora of ecological, genetic and microbiological approaches, this thesis explores how plant and habitat domestication impacted the assembly and functionality of the tomato rhizosphere microbiome, from its closest ancestor in the center of origin region to cultivated tomatoes in a center of production (The Netherlands). Tomato was selected as the model plant for this study due to various relevant reasons: it is an economically important crop worldwide, has a relatively short life cycle, and offers extensive genetic resources, including seedbank accessions, commercial varieties, hybrids and recombinant inbred lines (RILs) derived from crosses between wild tomato species and their domesticated counterparts. Additionally, the inclusion of both native and agricultural soils from the tomato's center of origin in Southern Ecuador provided a unique opportunity to assess environmental influences on microbiome dynamics across multiple dimensions, including biogeography, tomato genotype, soil context and local/native plant-insect interactions (Figure 1). To accomplish this, we first characterized the rhizosphere microbiome of the wild tomato relative *Solanum pimpinellifolium* in its native habitat in the province of Loja, Ecuador (**Chapter 2**). As a follow-up, we examined the changes in taxonomic and functional traits of the rhizosphere microbiome associated with eight tomato genotypes, both wild and domesticated, grown in a controlled greenhouse setting with native and agricultural Ecuadorian soils —representing the center of origin— as well as a Dutch greenhouse soil —representing a center of tomato production (**Chapter 3**). Next, we investigated whether the native soil microbiome enhances tomato tolerance to the invasive insect pest *Prodiplosis longifila* (**Chapter 4**). Finally, we identified key plant and rhizobacterial genetic traits associated with the assembly of the tomato rhizosphere microbiome (**Chapter 5**).



S. pimpinellifolium, in its center of origin, selects a taxonomically consistent microbiome. Enterobacteriaceae revealed functional traits that may facilitate and promote its predominance in the wild tomato rhizosphere

Domestication altered the composition and functionality of the tomato microbiome. *Pseudomonas* and *Massilia* were more abundant in wild tomato genotypes, while *Streptomyces* and *Sphingomonas* progressively increased along the tomato domestication trajectory.

The wild tomato relies on the native soil microbiome for protection against insect leaf damage. Members of Actinobacteriota such as *Actinoplanes* revealed putative functions associated with plant protection, including biosynthesis of specialized metabolites such as chorismate and a lanthipeptide.

Integrating plant quantitative traits and microbiome data revealed the genetic basis of tomato rhizosphere microbiome assembly. *Streptomyces* was associated with domesticated tomato loci on chromosomes 6 and 11, including root-specific genes like *SlTIP2.3* and FIT. *Cellvibrio* was linked with wild *S. pimpinellifolium* loci on chromosomes 1 and 9, including sugar, vitamin, cofactor, sulfur, and iron metabolism.

Figure 1. Visual summary of the main findings of this thesis. Created with BioRender.com.

Assembly of wild tomato rhizosphere microbiome in the center of origin

Wild relatives of modern crops have served as a genetic source for past and present crop breeding (Bai & Lindhout, 2007; Grandillo et al., 2011; Gao et al., 2019; Mata-Nicolás et al., 2020; Wang et al., 2020). Despite the knowledge that members of the plant-associated microbiome play a fundamental role in plant growth and health, studies profiling indigenous microbiomes of wild tomato populations growing in their native habitats still are scarce (Cordero-Ramírez et al., 2012; Fuentes et al., 2020; Runge et al., 2022). The exploration of microbiomes of wild tomato relatives in their native habitats will allow us to understand the natural microbial interactions that could be of interest for the design of novel sustainable agriculture management practices (Wallenstein, 2017; Barajas et al., 2020). Our results (**Chapter 2**) revealed that *S. pimpinellifolium,* growing in its center of origin, selects a taxonomically consistent microbiome, despite a locally diverse physical, chemical, and soil microbiome background. These results suggest that the wild tomato ancestor in its native habitat drives a deterministic assembly of the rhizosphere bacteriome dominated by Enterobacteriaceae and *Rhizobium.* Further examination of the metagenome assembled genomes (MAGs) of Enterobacteriaceae revealed functional traits that may facilitate and promote its predominance in the tomato rhizosphere, such as motility, chemotaxis and antimicrobial compounds production. For instance, type IV secretion pili (Craig et al., 2019; Costa et al., 2023), iron acquisition via siderophore production (Ahmed & Holmström, 2014; Timofeeva et al., 2022), as well as stress resistance features for osmotic (Frossard et al., 2012; Herrou et al., 2017) and high-temperature conditions (Raivio & Silhavy, 2001; Dai et al., 2015), may facilitate direct interactions between bacteria and the host (Wallden et al., 2010; Zechner et al., 2012), and may increase competitiveness in interactions with neighboring rhizosphere microbes (Loper & Buyer, 1991; Loper & Henkels, 1999; Chepsergon & Moleleki, 2023).

Our findings show how core taxa of the rhizosphere bacteriome, such as members of Enterobacteriaceae, can thrive in the harsh native environments where wild tomatoes grow naturally. Metagenomic analyses revealed bacterial traits that may contribute to adaptation to these environmental conditions. Furthermore, our observations highlight the importance of conducting local studies in regions from where commercial cultivars originated, as these are crucial for understanding plant-microbe interactions in an evolutionary context. Regarding the wild tomato's rhizosphere mycobiome, our results indicated a high proportion of unclassified fungi as well as fungi classified as *Fusarium* and *Aspergillus,* which are known either for their pathogenicity or for their beneficial effects on tomato plants via induced systemic resistance (ISR) (Fuchs et al., 1997; Veloso & Díaz, 2012) and plant growth promotion (Adedayo et al., 2023; Daigham et al., 2023). The presence of numerous unclassified fungal taxa in this native wild tomato habitat points to a largely unexplored soil mycobiome. Altogether, this underscores the necessity of identifying potentially valuable microbial candidates for functional validation assays, to assess whether traits beneficial in native ecosystems could support modern tomato cultivation in overcoming biotic and abiotic stresses.

Genotype and habitat domestication altered the composition and functionality of the tomato microbiome

Tomato domestication resulted in multiple phenotypic changes to satisfy human needs (Purugganan & Fuller, 2009). However, the transition from the original native habitats to present-day agricultural production systems not only impacted tomato traits but also unintentionally affected the assembly of their associated (beneficial) microbiome (Fernie & Yan, 2019; Mo et al., 2024). In **Chapter 3,** we tested eight tomato genotypes as representatives of different stages of the domestication trajectory, in combination with native and agricultural soils from the tomato's center of origin (Ecuador) and from one of the current centers of production (The Netherlands). Although our study involved a limited number of tomato genotypes (n = 8) and soil types (n = 5), it provided a first comprehensive screening of the putative impact of domestication on microbiome assembly. Moreover, it offers a basis for generating new hypotheses that can be tested in a broader range of tomato genotypes and soils. We observed differences in the compositions of the rhizosphere microbiome between wild and domesticated tomatoes. These differences were largely determined by the soil type in which the tomato genotypes were grown (native, agricultural, or greenhouse soils). Furthermore, our results suggest that variations in microbiome assembly emerged later in the tomato domestication process, as genotypes classified as wild and intermediate showed comparable rhizosphere microbiome compositions, but different from those of domesticated tomato genotypes.

On one hand, we found that wild tomatoes in native soils associated with specific bacterial genera, such as *Brevundimonas, Cellvibrio* and *Rhizobacter* (Proteobacteria) and *Chitinophaga, Dyadobacter, Fluviicola,* and *Ohtaekwangia* (Bacteroidota), which are known for various functional traits like motility, chemotaxis, and nutrient cycling. On the other hand, the rhizosphere microbiome of domesticated tomatoes grown in agricultural and greenhouse soils typically associated with Actinobacteria (*Actinoplanes, Nocardioides*), Cyanobacteria (*Nostoc, Sericytochromatia*), and Firmicutes (*Bacillus, Paenibacillus*), exhibiting functional traits exemplifying adaptation to modern agricultural management practices, such as the application of fertilizers. Results of our greenhouse experiment further showed that there was a microbial signature for wild and domesticated tomatoes across all soils: *Pseudomonas* and *Massilia* (Proteobacteria) were more abundant in wild-intermediate tomato genotypes, while *Streptomyces* (Actinobacteriota) and *Sphingomonas* (Proteobacteria) progressively increased along the tomato domestication trajectory. The same abundance pattern of *Streptomyces* in domesticated tomato *S. lycopersicum* was observed in the rhizosphere microbiome analysis of the recombinant inbred line (RIL) population of a cross between wild *S. pimpinellifolium* and domesticated *S. lycopersicum* (**Chapter 5**).

Future studies could further build on the microbiome results described here by integrating root exudation profiling and metatranscriptomics to better capture the dynamic nature and underlying mechanisms of selection in tomato microbiome assembly and functioning. To this end, biobanking wild tomato rhizosphere microbiomes is needed for designing targeted bioassays and to monitor the phenotypic effects of compatible host-microbiome combinations *in vivo*. Phenotypic assessment of plant traits such as growth, nutrient uptake and stress tolerance in response to isolated microbial genera can then provide valuable mechanistic insights.

The wild tomato relies on the soil microbiome for protection against insect leaf damage

Wild crop relatives and their native microbiomes have co-evolved for millions of years to withstand local (a)biotic stresses (Pérez-Jaramillo et al., 2016; Wallenstein, 2017; Barajas et al., 2020). However, plant domestication and environmental manipulation for crop cultivation may have caused the loss of beneficial interactions or an adequate communication between domesticated plants and the soil microbiome (Cordovez et al., 2019; O'Brien et al., 2021; Oyserman et al., 2021). In **Chapter 4**, we studied the impact of the native soil microbiome on leaf damage caused by the local sap-sucking insect *Prodiplosis longifila* on the wild tomato ancestor *S. pimpinellifolium* and the cultivated tomato *S. lycopersicum*. The results of our experiment conducted under greenhouse conditions showed that the wild tomato species relies on the soil microbiome for protection against the leaf insect, whereas the modern tomato did not. The results suggested a putative functional role of specific microbial taxa, such as Actinobacteriota and Cyanobacteria, in mediating defense responses against this endemic leaf insect. Specifically, *Actinoplanes* showed a reduced abundance in the rhizosphere of wild tomato plants grown in sterilized soils with higher levels of insect leaf damage aboveground. Our findings support the hypothesis that wild tomato relies on their native soil microbiome, presumably on members of Actinobacteriota such as *Actinoplanes,* to protect itself from leaf damage caused by *P. longifila*. The *Actinoplanes* MAG (metagenome-assembled genome) revealed putative functions associated with plant protection, including genes for motility, chemotaxis, membrane transport, and the biosynthesis of specialized metabolites such as chorismate and a lanthipeptide. To further validate and expand these findings, future experiments should involve isolation and reintroduction of *Actinoplanes* as a potentially beneficial microbial candidate into sterilized soils to directly test its ability to provide insect tolerance. In addition, testing a much broader range of tomato genotypes would help to determine if microbiome-mediated insect tolerance is a conserved trait or genotype-specific. It also remains to be investigated whether the protective effect of the native microbiome extends to other tomato insect species. If members of Actinobacteriota can confer broader insect tolerance, this could open new avenues for sustainable pest management practices that harness native microbiomes. Although extrapolating these findings to field conditions presents challenges, such as managing environmental variability and soil heterogeneity, our study highlights the potential of native rhizosphere microbial genera as a complementary strategy for pest control in modern agricultural systems.

Integrating plant quantitative traits and microbiome data revealed the genetic basis of tomato rhizosphere microbiome assembly

Microbiomes play a crucial role in plant growth and health and hold great promise for crop breeding (Oyserman et al., 2018; Carrión, 2019; Finkel, 2020). Amplicon sequencing-based studies have previously shown that microbiome assembly by plants has a significant genetic basis (Pérez-Jaramillo et al., 2018; Favela et al., 2021). Moreover, with the latest technological advances, it is now feasible to treat microbiomes as "quantitative traits" of selection (Pereira et al., 1993; Bergelson et al., 2019; Deng, 2021). This fundamental knowledge paves the way for connecting microbial genes and pathways with specific genetic regions (QTLs) in the tomato genome. In **Chapter 5**, we analyzed the rhizosphere microbiome of an extensive RIL population (N = ± 100) derived from a cross between the modern tomato *S. lycopersicum* and the wild tomato *S. pimpinellifolium* (Voorrips et al., 2000), allowing us to identify potential plant and reciprocal rhizobacterial traits underlying microbiome assembly. We found the domesticated tomato was associated with increased overall microbiome abundance compared to its wild relative. Specifically, bacterial genera such as *Altererythrobacter, Asticcacaulis, Devosia, Novosphingobium, Sphingomonas,* and *Streptomyces* were selectively enriched by the domesticated tomato, whereas *Cellvibrio, Sphingopyxis, Sphingobium* were preferentially enriched in the wild relative tomato rhizosphere. Our results showed that the abundance of *Streptomyces* was strongly associated with genetic regions of the domesticated tomato located on chromosome 6 and 11. These regions included root-specific tomato genes such as aquaporin (SlTIP2.3) and the iron transcriptional regulator FIT. On the other hand, the abundance of *Cellvibrio* was linked with genetic regions on chromosomes 1 and 9 of the wild *S. pimpinellifolium,* harboring genes involved in sugar catabolism (arabinofuranosidase, fructose, cellulase/esterase, malate, mannonate, xyloglucan and xylulose), vitamin and cofactor metabolism as well as sulfur and iron metabolism. On the microbial side, these analyses revealed traits associated with various pathways, including plant polysaccharide degradation, trehalose metabolism, polyamine metabolism, acetolactate synthase activity, as well as vitamin and iron metabolism. Although the functional importance of these plant and microbial traits in assembly and functioning need to be experimentally validated, these findings pinpointed, for the first time, potential targets for microbiome-based plant breeding, also referred to as *M* breeding (Cernava, 2024). Experimental validation will include manipulating microbial communities in controlled assays to test the specific influence of candidate QTLs on microbiome composition and functionality. For instance, the putative role of aquaporin and FIT in *Streptomyces* tomato root colonization can be investigated using transgenic lines of domesticated tomato with site-directed mutations of specific genes. In the same way, experimental validation of genes linked to sugar and vitamin metabolism in *S. pimpinellifolium* could provide insights on how these pathways drive *Cellvibrio* recruitment, offering valuable targets for enhancing microbial resilience or productivity in domesticated tomatoes. By validating these findings also in field trials, we can move one step closer to harnessing the potential of the rhizosphere microbiome to improve tomato cultivation by optimizing yield stability under changing environmental conditions.

Concluding remarks and future outlook

This thesis highlighted the intricate and reciprocal effects of plant and habitat domestication on tomato rhizosphere microbiome assembly. While domestication has shaped beneficial phenotypes in crops, it has unintentionally altered their interactions with microbes. As a result, key plant-microbe associations may have been depleted or lost, which has implications for the adaptability and resilience of modern cultivars to changing environmental conditions. Our results revealed that domestication has profoundly influenced the taxonomic and functional diversity of the tomato's rhizosphere microbiome. The obtained results underscore the important role that microbial communities may play in the adaptation and resilience of plants like tomato, specifically when comparing native habitats to modern agricultural conditions.

The concept of microbiome rewilding (Raaijmakers & Kiers, 2022) presents a promising road map for restoring plant-microbe interactions by reinstating key plant-microbe partnerships that may have been lost during domestication. This approach follows a four-step roadmap: 1) grow wild relatives and domesticated crops in native and agricultural soils to identify key changes in the ancestral microbiome; 2) transplant the ancestral microbiome onto domesticated crops in agricultural soils to validate their beneficial impact on specific phenotypes, particularly tolerance to biotic and/or abiotic stresses; 3) identify plant genetic loci associated with the recruitment and functioning of beneficial ancestral microbiome members; and 4) identify key molecules in the root or shoot exudates that are associated with the recruitment or activation of the beneficial ancestral microbiome members.

In this thesis, we took the first steps of this roadmap by identifying key microbial taxa in the native habitat of wild tomato, as well as the differences in microbiome assembly between wild and domesticated tomato genotypes. These comparisons highlighted how the process of domestication has shaped the tomato microbiomes from their natural center of origin to modern production sites. Specifically, we explored microbial associations in both native and agricultural soils, including a contrasting soil from a greenhouse-intensive tomato production system, located far from the tomato's center of origin. By doing so, we reflected on the journey of domestication, going from wild habitats to present-day breeding strategies, and aimed at understanding and harnessing the microbial functions that influence plant traits. Additionally, we explored the protective function of the native soil microbiome in insect resistance of wild tomato. We also identified specific tomato loci associated with microbiome assembly, providing a foundation for future *M* breeding efforts to reinstate beneficial ancestral microbiome members into modern crops.

We demonstrated that the wild tomato ancestor *Solanum pimpinellifolium* recruits a consistent and functionally specialized rhizosphere microbiome in its center of origin. The host filtering effect observed under natural conditions in Ecuador emphasized that wild tomato selectively enriches for microorganisms linked to motility, siderophore production and stress tolerance. These traits likely evolved to help the plant tolerate the harsh and unpredictable conditions of its native habitat. It is possible that these microbial associations contribute to the plant's ability to persist through extreme environmental stress, such as prolonged droughts, supporting the ecological role of regional plant-microbe co-evolution. However, this remains a hypothesis for further investigation, as the microbiome's role in supporting plant survival under such extreme conditions was not directly tested. Furthermore, our results revealed that plant domestication has significantly altered the tomato microbiome, with the influence of soil habitat amplifying these effects. We found that microbiome divergence became especially pronounced in later stages of domestication. We observed selection for microbial genera adapted to extensively managed soils and agronomic inputs in domesticated tomatoes, while the wild tomato genotypes were associated with microbial features related to stress resilience and nutrient cycling.

At the functional level, we explored the protective role of the native soil microbiome in enhancing insect resistance in wild tomato. Notably, we identified members of the genus *Actinoplanes,* which were depleted in sterilized soil but correlated with reduced leaf damage in soils containing a native microbiome. These bacteria, which exhibited traits related to motility, membrane transport, and secondary metabolism, suggesting a critical role in mediating plant resistance to insect attack. Its presumed role in plant defense makes *Actinoplanes* a potential candidate for enhancing plant resilience. Similarly, we identified *Streptomyces* and *Cellvibrio* as candidates to further investigate the metabolic cross-talk in the tomato rhizosphere.

The results presented in my PhD thesis raise important questions concerning the functional implications of microbiome shifts throughout the domestication trajectory, particularly in the context of the challenges faced in modern agriculture. Central questions include: i) what key plant and microbial traits were lost during domestication, and can they be restored?; ii) how can microbiomes be harnessed to reduce dependence on chemical agricultural inputs such as fertilizers and pesticides?; iii) how can beneficial microbes be effectively integrated into crop breeding and agricultural management practices?; and, iv) how can our knowledge of the genetic basis of microbiome assembly be used in *M* breeding strategies to develop climate-resilient crops? Addressing these questions will require robust functional validation under diverse environmental conditions. Future research will focus on targeted greenhouse assays to test the effects of key microbial taxa, including *Streptomyces, Bacillus, Sphingomonas,* Cyanobacteria, *Actinoplanes, Cellvibrio, Enterobacter, Pseudomonas, Rhizobium,* and *Massilia,* on drought tolerance, nutrient use efficiency and biotic stress resilience. Additionally, high-throughput phenotyping and metabolomic profiling needs to be employed to track colonization dynamics by and host physiological responses to these key microbial genera.

In summary, the work described in this thesis contributes to broader discussions on the future of sustainable agriculture, offering insights into the role of the rhizosphere microbiome in shaping crop resilience. By integrating microbiome-compatible traits into breeding programs, we have the opportunity to restore critical microbial functions that may have been lost during domestication, while simultaneously optimizing crop yield, uniformity in plant growth or fruit quality, and resilience to (a)biotic stresses. With its rich history of domestication and intricate mechanisms of plant-microbe interactions, the tomato system serves an ideal model for advancing these efforts. As we face the challenges of climate change, resource scarcity, and the growing need for global food security, this research paves the way for innovative strategies in crop breeding that leverage the power of microbial partnerships. By identifying key genetic loci and microbiome traits that drive plant performance, we can adopt a promising strategy to expand our agricultural toolkit and meet the needs of future generations.