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Engineering the Crop Microbiota Through Host Genetics

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Abstract

The microbiota populating the plant–soil continuum defines an untapped resource for sustainable crop production. The host plant is a driver for the taxonomic composition and function of these microbial communities. In this review, we illustrate how the host genetic determinants of the microbiota have been shaped by plant domestication and crop diversification. We discuss how the heritable component of microbiota recruitment may represent, at least partially, a selection for microbial functions underpinning the growth, development, and health of their host plants and how the magnitude of this heritability is influenced by the environment. We illustrate how host–microbiota interactions can be treated as an external quantitative trait and review recent studies associating crop genetics with microbiota-based quantitative traits. We also explore the results of reductionist approaches, including synthetic microbial communities, to establish causal relationships between microbiota and plant phenotypes. Lastly, we propose strategies to integrate microbiota manipulation into crop selection programs. Although a detailed understanding of when and how heritability for microbiota composition can be deployed for breeding purposes is still lacking, we argue that advances in crop genomics are likely to accelerate wider applications of plant–microbiota interactions in agriculture.

1. INTRODUCTION

Plant–microbe relationships have been a hallmark of plant evolution since plants colonized the terrestrial environment (26). It is now widely accepted that, akin to their animal counterparts, plant organs represent microhabitats for distinct microbial communities (33). These plant-associated microbial assemblages, collectively referred to as the plant microbiota, engage in symbiotic relationships with their host ranging from parasitism to mutualism (78). For instance, members of the microbiota can provide their host with enhanced access to mineral nutrients and pathogen protection (52), representing an attractive alternative to nonrenewable inputs and interventions in agriculture. The plant microbiota is not randomly assembled from the environment; rather it is the result of a step-wise selective process: Soil microbes define a major source of inoculum for the microbiota populating the root–soil interface, whose composition and function are fine-tuned, at least in part, by the plant genome (13, 24). A prediction of these observations is that the microbiota and its functional potential could be brought to center-stage in plant breeding programs for sustainable crop production (59, 102). However, this potential has not yet been fully realized. A detailed understanding of the genetic relationships between the microbiota and their host plants is a critical step to exploiting the beneficial functions of the microbiota for sustainable crop production.

In this review, we discuss the impact of human selection on crops and their associated microbiota, with an emphasis on the microbial communities proliferating at the root–soil interface, as these communities play a pivotal role in biogeochemical cycling and sustainable agriculture (84). Our review focuses broadly on the wider crop microbiota, i.e., beyond microbes known to be symbionts, while we invite the reader interested in more established models of plant–microbe symbiosis, i.e., nitrogen-fixing rhizobia and the more generalist arbuscular mycorrhizal fungi, to read the existing literature (61, 64). We aim to provide the reader with a critical appraisal of recent manuscripts describing the plant genetic mechanisms underpinning host–microbiota interactions. We highlight the potential of genetic association studies to discover novel determinants of the plant microbiota. We further elaborate on the potential contribution to plant performance of the component of the microbiota shaped by host genetics. We conclude by commenting on strategies and challenges to exploit host genetic diversity for the manipulation of the plant microbiota.

2. HOST–MICROBIOTA INTERACTIONS WITHIN A PLANT DOMESTICATION AND DIVERSIFICATION FRAMEWORK

Plants have been engaging in relationships with soil microorganisms since land's colonization (26). Similar to other host–microbial assemblages, the relationship between plants and their microbiotas has likely been shaped by the evolutionary pressure faced by those organisms, as either independent or coevolving entities of a wider holobiome (85). Cornerstones in the evolutionary history of cultivated plants are domestication and subsequent crop diversification, man-made selection processes that progressively differentiated modern varieties from their wild relatives (72). These processes manifested through the selection of favorable traits, such as ease of cultivation and increased yield, which in turn resulted in a more uniform genetic pool of the so-called cultivated germplasm (1). This human footprint on the evolutionary history of cultivated plants appears to have also impacted plant-associated microorganisms (70). For instance, common garden experiments, in which distinct plant types are grown side-by-side in the same soil, enabled scientists to identify a genotype-dependent compositional shift in the microbiota populating the root–soil interface in wild and domesticated plants of staple crops such as barley (*Hordeum* subsp.) (3, 12), bean (*Phaseolus vulgaris* subsp.) (67), maize (*Zea mays* subsp.) (11), sugar beet (*Beta vulgaris* subsp.) (110), sunflower (*Helianthus annus*) (49), and wheat (*Triticum aestivum* subsp.) (101). Intriguingly,

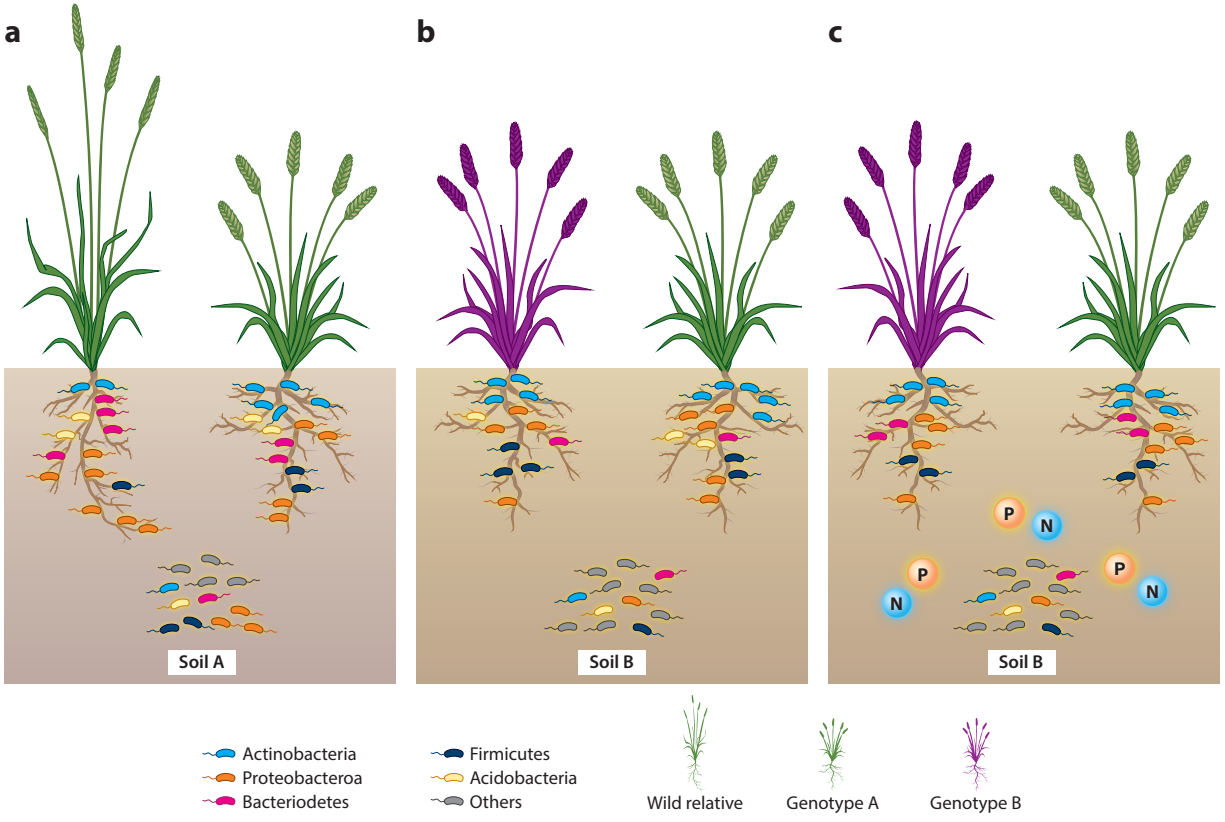

Figure 1

Crop–microbiota interactions at the crossroads of plant genetics and environmental drivers. (*a*) When grown in the same soil, wild and domesticated genotypes assemble distinct microbiotas in the rhizosphere, with a bias for members of the phyla Actinobacteria and Bacteroidetes. (*b*) The differential recruitment is modulated, at least partly, by environmental variables, with distinct soils triggering the recruitment of distinct, genotype-specific microbiotas. (*c*) Exposure to the external inputs, e.g., fertilizers, impacts the magnitude of the host genetic control of the microbiota, possibly mirroring a link between genotype-specific microbiota composition and adaptation to growth-limiting conditions.

this microbiota diversification displays a congruent pattern across plant species, manifested by the preferential association of members of the Actinobacteria phylum with modern varieties opposed to the preferential association of members of the Bacteroidetes phylum with wild relatives (19, 68) (**Figure 1*a***). Of note, evidence of plant genotype–dependent microbial recruitment has been identified within cultivated germplasm, possibly indicating a continuous selective pressure on the microbiota thriving at the root–soil interface during crop diversification. For instance, in maize, host genetic diversity in both heritage (29) and modern (66) inbred lines correlates with microbiota diversity in their rhizosphere. Likewise, plants of the two main types of cultivated rice, *japonica* and *indica* (38), host contrasting root microbiotas under field conditions (113), and wheat varieties harboring dwarfing genes, a hallmark of the Green Revolution breeding programs (8), assemble a rhizosphere microbiota significantly distinct from taller, more ancient cultivars (43). Despite the use of dedicated plant genetic material to validate the concept of host genetic control on the composition and function of rhizosphere microbes dating back to the early 1970s (58), microbiota information has rarely been used in crop selection. This would imply that microbiota

selection during domestication and crop diversification has been an unexpected consequence of selecting for macroscopic plant traits.

2.1. Edaphic Factors Modulate the Host Recruitment Cues of the Crop Microbiota

Edaphic factors are a driver of soil microbial diversity and impact on the host genetic control of the microbiota composition at the root–soil interface (13) (**Figure 1*b***). Put simply, and in analogy to many other plant traits, microbiota recruitment is influenced by an environmental component. For instance, the magnitude of host control in wild and domesticated beans depends on soil characteristics: An agricultural soil triggered a more marked plant genotype effect on rhizosphere microbial communities than a native soil (69). Likewise, environmental variables, including soil characteristics, modulated host genetic control of the maize rhizosphere microbiota in large-scale field trials (66, 95). In addition to intrinsic soil characteristics, interventions operated by farmers in their fields may impact plant–microbiota interactions (**Figure 1*c***). For example, the application of mineral nitrogen to soil, a common practice in cereal production, nearly obliterates the differential bacterial enrichment observed in the rhizosphere of wild and domesticated barley genotypes when grown in the same soil type (4). Furthermore, in maize inbred lines, the proportion of variance in the composition of the root microbiota attributable to plant genetic variation was greater under limited nitrogen compared to fertilized conditions (56).

2.2. Mechanistic Insights Into Crop–Microbiota Interactions at the Root–Soil Interface

These observations suggest that one component of host genetic control on the microbiota is hardwired into the plant genome, whereas others act as inducible traits. Mechanistically, this could be explained by the fact that edaphic factors modulate plant traits previously implicated in shaping the microbiota at the root–soil continuum, including, for example, root system architecture and rhizodeposition (see Section 3) (84). Considering that modern crops have been selected to respond to high inputs, e.g., synthetic fertilizers, rather than the establishment of beneficial plant–microbiota associations, it is legitimate to hypothesize that plant genes shaping soil microbes are a consequence of the selection for yield or other macroscopic traits (e.g., disease resistance). Mechanistically, this may have occurred as a result of the pleiotropy, linkage, or genetic drift of genes under selection for an agronomic trait, regardless of whether the microbes themselves affect or are affected by the trait. The evolution of wild relatives of crops under marginal soil conditions has provoked an interest in their microbiota as a potential untapped resource for sustainable crop production (73). The alternative scenario is that, albeit unintentionally, early farmers and plant breeders may have already selected the optimal plant-microbiota combination for a given species. For example, the responsiveness of maize to mineral fertilizers, which are an element of modern maize breeding, appears to have encouraged modern lines to recruit a richer pool of microbes involved in nitrogen biogeochemical cycling when compared to wild teosinte lines (*Z. mays* subsp. *mexicana* and *Z. mays* subsp. *parviglumis*) (28). Intriguingly, these scenarios are not mutually exclusive: Their occurrence could be dictated by specific interactions between soil characteristics and plant genotypes. The key to validating these scenarios is the identification and characterization of plant genes shaping host-microbiota interactions at the root–soil interface (26).

3. LINKING ROOT TRAITS TO MICROBIOTA RECRUITMENT

Plant genes that influence root traits are primary candidates for shaping the microbiota at the plant–soil continuum. For instance, the root system architecture, i.e., the genetically encoded

Pleiotropy: the phenomenon in which a single gene is responsible for several distinct and unrelated phenotypic effects

Linkage: association in inheritance of two or more nonallelic genes that is greater than the association expected from independent assortment

Genetic drift: a change in the allele frequency in a population due to random chance

three-dimensional way roots explore the soil profile, may expose plants to distinct microbial communities. For instance, rhizosphere communities examined along the longitudinal root axis revealed a zone-specific, compositional pattern defined by the succession of the meristematic, elongation, and lateral root formation zones (108). In barley, the *rhl1*.a and *rhp1*.b mutants are impaired in the development of root hairs and foster less complex communities than their near-isogenic parental lines (74). Likewise, specific root length (SRL), i.e., the ratio between the root length and root dry weight, emerged as a driver of microbiota composition in bean: ancestral genotypes, characterized by a high SRL, host a higher proportion of Bacteroidetes in their rhizosphere compared with modern genotypes, characterized by a lower SRL and a higher proportion of Actinobacteria (67). Consistently, mutations affecting either root hairs or lateral root formation in maize triggered differential bacterial recruitment in the rhizosphere (108). Closer inspection of these data revealed that *lrt1*, a mutant impaired in lateral root formation, preferentially enriched the rhizosphere with members of the Oxalobacteraceae family in a flavone-dependent manner. Taken together, these observations suggest that individual root traits are sufficient to modulate the rhizosphere microbiota.

The contribution of individual root traits may act at a nexus of other recruitment cues, e.g., uptake of mineral nutrients, for the plant microbiota. Strikingly, both plant–soil feedback and synthetic community (SynCom) experiments mimicking the flavone-dependent Oxalobacteraceae enrichment revealed a role for the interaction between this bacterial family and the root system for plant adaptation to limited nitrogen conditions (108). Interestingly, a nitrogen impact on host genetics that shape the microbiota composition has been observed in multiple crops. For instance, allelic differences at the gene coding for the NRT1.1B nitrate transporter in the rice *indica* and *japonica* cultivar types are associated with distinct structural and functional root microbiotas (113). Remarkably, SynCom-based reconstruction of the *indica* microbiota, but not the *japonica* microbiota, promoted rice growth when supplied with organic nitrogen, implying that only the *indica*-derived SynCom could metabolize organic nitrogen into bioavailable nitrate and ammonium. Likewise, overexpression in apple (*Malus domestica*) of the nitrate transporter MdNRT2, which catalyzes a rate-limiting step for nitrate uptake under low nitrogen conditions (112), increased nitrogen uptake in the presence of bacteria of the family Rhizobiaceae compared to sterile conditions (17). This apparent integration between adaptation to growth-limiting mineral supplies and microbiota recruitment does not appear confined to the so-called macronutrients. For example, grasses secrete phytosiderophores as chelating agents to sequester the vital micronutrient iron via root transporters (60). In maize, *tom1* mutants, which are deficient in phytosiderophore secretion and reduced in iron uptake, are enriched for members of the phyla Actinobacteria in the rhizosphere compared to cognate wild-type rhizospheres (104). This actinobacterial enrichment is also a hallmark of the microbiota of sorghum exposed to drought conditions (105). Interestingly, recolonization experiments performed using *Streptomyces coelicolor Sc1*, a member of the phylum Actinobacteria that was isolated from sorghum roots, revealed that increased iron availability for plant uptake reduced the plant growth promotion potential of *Streptomyces* under drought (104). Additional experiments further supported an interdependency between phylum-specific enrichment and a plant's adaptation to growth-limiting conditions in sorghum, as demonstrated by controlled experiments with two Actinobacterial strains administered to plants maintained in sterilized substrates promoting larger root biomass in drought-stressed plants (105). Collectively, these studies suggest the existence of a genetic network linking microbiota recruitment with plant adaptation to the environment. Given the wide range of external stimuli faced by plants, what other genetic determinants may be part of this network?

Rhizodeposits, including primary and secondary plant metabolites released at the root–soil interface, have historically been implicated as major determinants of the rhizosphere microbiota

Synthetic community (SynCom): a combination of microorganisms mimicking the taxonomic composition and/or functional potential of a target microbiota

Rhizodeposits: all material lost from plant roots, including water-soluble exudates, secretions of insoluble materials, lysates, dead fine roots, and gases

Biological nitrification inhibition: capacity of certain plant species to release organic compounds that suppress the activity of nitrifier microorganisms, thus reducing soil nitrification

(15, 39). Results gathered from independent labs working with maize brought benzoxazinoids (BXs), which are indole-derived secondary metabolites with defensive properties found in grasses (103), to center-stage in plant–microbiota interactions (14, 20, 37, 46). Almost invariably, members of the phylum Proteobacteria emerged as the most responsive phylum to perturbations in BXs, as revealed by the comparison between wild-type and transposon insertion lines at individual genes in the BX biosynthetic pathway. Closer inspection of these data suggests that this effect is fine-tuned by soil characteristics (14), root type (20), developmental stage (46), and microhabitats within the host (37, 46). These investigations also revealed that soil conditioned with BXs, i.e., soils in which inbred lines producing these compounds have been grown, are associated with increased expression of plant defensive responses and herbivore growth suppression as well as decreased primary metabolite accumulation and plant growth in the next plant generation (37).

The species *Hordeum vulgare*, encompassing cultivated barley, produces the indol-alkaloid compound gramine instead of BXs (31). Gramine is a defensive and allelopathic secondary metabolite preferentially accumulated by wild barley genotypes (*H. vulgare* subsp. *spontaneum*) compared to domesticated ones (*H. vulgare* subsp. *vulgare*) (55). Interestingly, the exogenous application of gramine to the rhizosphere of modern, elite varieties is sufficient to trigger a differential enrichment of individual members of the endogenous barley microbiota (54). Likewise, cultivated sorghum (*Sorghum bicolor*) secretes the species-specific secondary metabolite sorgoleone exclusively by root hairs (21), and this metabolite was previously implicated in biological nitrification inhibition (82). Investigations conducted with sorghum RNA-interference lines of the *ARS1* and *ARS2* genes, which secreted significantly reduced amounts of sorgoleone compared to wild-type plants, revealed that sorgoleone impacts the abundance and growth of bacterial taxa beyond simply nitrifying bacteria (96). Interestingly, and in analogy with BXs, the control exerted by sorgoleone on the sorghum microbiota displayed a clear host microhabitat effect (i.e., endosphere versus rhizosphere): The taxonomic composition of the microbial communities residing outside the root corpus emerged as significantly affected compared with the endophytic ones (96). Another example of a clade-specific secondary metabolite capable of shaping microbiota composition is represented by avenacins, antimicrobial saponins from a cultivated type of oat (*Avena strigosa*). Avenacin decreases eukaryotic diversity (i.e., Amoebozoa and Alveolata) in the rhizosphere as revealed by a metatranscriptomic comparison of wild-type plants and the avenacin-deficient mutant *sad1* (87).

Additional examples of how the secretion of secondary metabolites may modulate microbiota composition at the root–soil interface come from cucurbits. The triterpenoid cucurbitacin, which is excreted into the rhizosphere, triggers the differential enrichment of bacteria protecting against the fungal pathogen *Fusarium oxysporum* (114).

Linking specific plant characteristics, such as secondary metabolites, to microbiota recruitment requires testing predefined genes and their coded traits for their capacity to modulate microbes at the root–soil interface. However informative, this approach prevents the opportunity to unveil other or additional classes of genes putatively shaping the crop microbiota. In the next section, we discuss strategies and recent results from studies that avoid the limitations intrinsic to predicting roles for specific host genes and their encoded traits.

4. MAPPING THE GENETIC BASIS OF PLANT–MICROBIOTA INTERACTIONS

The implementation of genetic mapping experiments for plant microbiota determinants offers an unbiased approach to discovering novel regions of plant genomes putatively shaping microbiota recruitment. The foundation of this experimental approach has two grounds. First, although

HIDDEN GEMS

The seed microbiota refers to seed-inhabiting microbes that can be either transferred vertically from mother plants or acquired horizontally from various environments (81). The heritability of these microbes is associated, at least partially, with the evolutionary pressure for functions related to seed dormancy, germination, and seedling establishment exerted during plant domestication and crop diversification (41). Seed microbes feature adaptations to survive in the seed environment, such as tolerance to desiccation (44) and amylase activity to resume activity upon germination (25). Yet in some instances, the maternal-to-offspring pathway may be hijacked by microbial pathogens to ensure their persistence in the next plant generation (10). In turn, the wider seed microbiota can host microbes capable of protecting plants from pathogens, as exemplified by recent investigations conducted with rice (53) and maize (65). Intriguingly, this microbial reservoir mirrors the taxonomic composition of the microbiota associated with other plant organs (86), suggesting that the seed microbiota represents a starter inoculum from which at least some community members are assembled in successive generations, although this appears to be modulated in an environment- and species-specific fashion (32, 40).

microbes can be vertically transmitted by offspring plants (see the sidebar titled Hidden Gems), microbial acquisition from the environment, in particular from the soil, represents a pivotal component of microbiota diversification (13). Although the so-called genotype effect explains a relatively minor proportion of the microbiota variation, usually not exceeding 10% (33), it retains a critical role in regulating functions provided by microbes to their host. The deterministic component of microbial community assembly that is ultimately caused by host genetics is also referred to as microbiota heritability (91). Second, ecological indices and multivariate statistical analyses computed on microbial counts, the most common descriptors of sequencing surveys of the microbiota, have a numerical nature that can be treated as a quantitative phenotype in genetic investigations.

A pioneering work predating the widespread use of next-generation sequencing in the field used maize recombinant inbred lines and an ecological index as a quantitative phenotype in a mapping experiment that led to the identification of six chromosomal regions significantly associated with microbiota diversity in the phyllosphere (5). Interestingly, some of these regions demonstrated an environmental component, i.e., their effect was only observed when plants were exposed to UV-B radiation, and comap with quantitative trait loci (QTLs) for resistance against Southern leaf blight, which is caused by the fungus *Bipolaris maydis* (5). Despite the relatively large mapping intervals, by mining sequencing resources available at that time, the authors identified a gene coding for a glutamate decarboxylase as a priority candidate for one of the identified QTLs, although a validation of this finding was not performed (5).

The advent of high-throughput sequencing approaches provided scientists with a wealth of microbiota information. Perhaps not surprisingly, given the complexity of plant microbiota interactions, the relatively simple genetic model *Arabidopsis thaliana* rapidly gained center-stage in this type of investigation. In a seminal work for the field, scientists used the abundances of individual bacteria and fungi identified in the phyllosphere to perform a genome-wide association study (GWAS) in a panel of diverse worldwide *Arabidopsis* genotypes (36). In this study, the phenotype for mapping consisted of the main community-level traits (i.e., composition and species richness), abundance, and presence/absence of the most dominant taxa. Heritability was identified after removing rare taxa, suggesting that the host control might be exerted primarily over the more abundant members of the phyllosphere microbiota. Gene-ontology enrichment on the identified plant genetic loci associated with these communities pointed at the immune system as a driver of microbiota quantitative variation with terms such as defense response, kinase activity, and cell

Heritability: the proportion of variability that can be attributed to inherited genetic factors as opposed to that caused by the environment

Genome-wide association study (GWAS): statistical approach to identify chromosomal regions that significantly affect the variation in quantitative traits in a large pool of unrelated genotypes

wall modifications for bacteria. More recently, another GWAS was implemented for a worldwide panel of *Arabidopsis* using richness and principal coordinates data as quantitative phenotypes for the root-associated microbiota. This study investigated both the bacterial and the fungal components; heritability was higher in the latter (6). Consistent with the previous investigation, genes underpinning the observed heritability were putatively involved in processes related to immunity, cell wall integrity, and root-hair development. Interestingly, genes encompassing these categories were different for the bacterial versus fungal communities (6).

Among crop plants, maize has been the subject of distinct association mapping studies for the microbiota using next-generation sequencing data. In the first example, a GWAS was conducted for genes regulating the phyllosphere communities (94). Despite having tested hundreds of microbial traits, only a relatively small number of associations with the maize genome were identified, suggesting that microbial traits may have low power for mapping so that each association explained a low variance proportion (94). More recently, an investigation targeting belowground communities revealed hundreds of significant associations in the maize genome with the abundance of 150 selected diagnostic microbial markers (56). The heritability of those traits was higher than the one obtained for phyllosphere microbes and was influenced by an environmental component (i.e., nitrogen availability), reinforcing the notion that both microhabitat and environment modulate the host genetic control of the microbiota (56).

By deploying an ingenious strategy, scientists capitalized on what could have been considered microbial contamination of whole-genome sequence data of the 3000 Rice Genomes Project to infer abundances of the associated phyllosphere microbes and conduct a GWAS experiment. Interestingly, this approach revealed genes related to stress responses, carbon metabolism, and regulation of gene expression among the primary candidates shaping the rice phyllosphere microbiota (75). An additional GWAS in the root and rhizosphere of the perennial crop tea (*Camellia sinensis*) revealed compartment-specific genetic determinants of the associated microbes by using microbial abundances and compositions as phenotypes (83). Although the metabolism of metal ions and organic compounds gene categories were overrepresented in the rhizosphere, cell wall metabolism and carbon catabolism were more prominent terms in the endosphere.

Other examples of association mapping studies targeting microbiota recruitment include investigations of diversity panels in sorghum (23), foxtail millet (*Setaria italica*) (97), and switchgrass (*Panicum virgatum*) (89). In the first of these studies, the characterization of 200 diverse genotypes of field-grown sorghum led to the identification of a major peak on chromosome 4 controlling the abundances of specific rhizosphere bacteria by a GWAS. Exploring publicly available RNA-seq data sets, the authors could select candidate genes with a root-specific expression on this major peak, including a gamma carbonic anhydrase-like 2, a putative beta-1,4 endoxylanase, and the nucleotide-binding leucine-rich repeat (NLR) gene *RGA2* (23). In the second study, the investigation of 827 foxtail millet cultivars identified hundreds of genetic associations between SNPs in the foxtail millet genome and bacterial members of the rhizoplane microbiota. Interestingly, a gene-ontology enrichment analysis revealed a functional specialization of host genetic determinants for members of given bacterial orders, with a bias for plant genes related to metabolites, hormone signaling, nutrient uptake, and immunity, including the ortholog of the archetypal plant immune receptor *Fls2*, as candidates for microbiota diversification (97). The last of these investigations focused on the fungal component of the microbiota populating the phyllosphere of switchgrass across seasons and different growing sites. Despite the impact of environmental variables on phyllosphere microbes, scientists were able to map a major host genetic determinant of microbiota composition on chromosome 2. RNA-seq experiments and the microbiota characterization of a set of switchgrass genotypes harboring contrasting alleles at the locus of interest pointed at genes coding for receptor-like kinases (RLKs) as primary candidates for this trait (89).

Examples of mapping experiments for microbiota determinants have recently been performed within a domestication framework. For instance, we recently used a segregating population between an elite variety (*H. vulgare* subsp. *vulgare*) and a wild barley accession (*H. vulgare* subsp. *spontaneum*) to scan the barley genome for significant associations with an external quantitative phenotype defined as the abundances of rhizosphere bacteria differentially enriched between the parental lines (27). This approach, conceptually different than a GWAS as it focuses on a biparental population, allowed us to identify several regions of the barley genome significantly associated with the bacterial composition in the rhizosphere. One of these regions, located on chromosome 3H and designated *QRMC-3HS*, emerged as a major determinant of the barley microbiota due to its association with a group of taxonomically diverse bacteria. Rhizosphere microbiota sequence and root RNA-seq data of sibling lines, derived from the original mapping population and harboring contrasting alleles at *QRMC-3HS*, allowed us to show that this locus impacts the bacterial, but not the fungal, component of the microbiota. Furthermore, we identified three candidate genes, encoding an unknown product, an NLR, and a xyloglucan endotransglucosylase/hydrolase enzyme, that will be prioritized in further investigations (27). In a similar approach, scientists used recombinant inbred lines of crosses between a wild tomato (*Solanum pimpinellifolium*) relative and a modern tomato cultivar (*Solanum lycopersicum* var. Moneymaker) to resolve the genetic basis of microbiota recruitment in this horticultural crop (62). A distinctive feature of this investigation was combining amplicon sequencing data with metagenome-assembled genomes (MAGs), enabling the identification of regions of the tomato genome significantly associated with both taxonomic composition and functional potential of the rhizosphere communities. This approach revealed several associations with both amplicon data and a *Streptomyces* sp. MAG (62); these included a ~6-Mbp region on chromosome 6 harboring 84 root-expressed genes, including those coding for the iron regulator FIT and the water channel aquaporins.

Taken together, these studies, summarized in **Table 1**, revealed three patterns. First, the existence of a relationship between regions of the crop genomes significantly associated with microbial traits and regions underlining other plant traits subjected to human selection. For example, the GWASs conducted in both the maize rhizosphere and foxtail millet rhizoplane revealed that microbiota traits were significantly correlated with agronomic traits and measures of plant performance (56, 97). Although correlations do not necessarily imply causal relationships, during crop selection the microbiota could have been unintentionally altered, either because they affect yield directly or because the selected plant traits have side effects on the microbiome. Second, in a conceptual analogy of what has been extensively documented for the model plant *Arabidopsis* (30, 34), receptors and other components of the plant immune system gained momentum as candidates for influencing the assembly of the crop microbiota for both aboveground and belowground communities. Finally, footprints of human selection on the genetic characteristics of a plant's determinants of microbiota composition could be identified in maize, where the genomic regions associated with bacteria are under purifying selection (56), in barley where the *NLR* candidate gene maps in an area of structural variation of the genome (27), and in tomato where microbiota QTLs encompassed so-called domestication sweeps (62). In the next section, we discuss strategies to expedite the translation of these discoveries into applications for crop development.

Metagenome-assembled genomes (MAGs): single-taxon assemblies reconstructed using metagenomic sequences that have been asserted to be a close representation of an actual individual genome

Purifying selection: a form of selection purging deleterious mutations that are produced in each generation

Structural variation: a large-scale genomic difference, e.g., chromosomal deletion, duplication, insertion, or inversion, that is inherited and polymorphic in a species

Domestication sweeps: the result of a selection leading to a reduction or elimination of genetic variation near the genes underpinning domestication traits

5. ESTABLISHING CAUSAL RELATIONSHIPS BETWEEN HOST GENETIC CONTROL OF THE MICROBIOTA AND PLANT TRAITS

The interdependency between plant macroscopic traits and microbiota composition implies that focusing on the genetic variation existing for the former may facilitate the characterization of host genetic components controlling the latter (91). Yet this approach alone may fail to elucidate

Table 1 Genetic approaches and microbial phenotypes used as external quantitative traits in association studies conducted with crops

Plant/crop	Environment	Population	Microbial sampling	Phenotype	Mapping	Validation	Reference
Arabidopsis	Autoclaved soil in glasshouse for 19 days and transferred to the field	196 worldwide accessions	Metabarcoding Phyllosphere DNA	Microbial abundances, P/A, PCA, CCA, species richness	mGWAS	NA	36
Arabidopsis	Autoclaved soil in glasshouse for 19 days and transferred to the field	196 worldwide accessions	Metabarcoding Root endosphere and rhizoplane DNA	Species richness and PCA	mGWAS	NA	6
Barley	Glasshouse, agricultural soil	Nested association mapping population HEB-25, 54 lines	Metabarcoding Rhizosphere DNA	Microbial abundances	QTL	Lines with contrasting (wild/elite) alleles at locus *QRMC-3HS*, RNA-seq, variant calling	27
Foxtail millet	Field, one location	827 cultivars	Metabarcoding Rhizoplane DNA	Microbes with significant correlation with growth/yield traits	GWAS MWAS on growth and yield traits and mGWAS	Marker strains impact on growth traits and plant RNA-seq responses	97
Maize	Field, one location	IBM advanced RILs	TRFLP Phyllosphere DNA	Simpson index	QTL	NA	5
Maize	Field, one location	Goodman Maize Association Panel, 300 accessions	Metabarcoding Phyllosphere RNA	Microbial abundances, alpha-diversity, PCoA	mGWAS	NA	94
Maize	Field, one location, low and high nitrogen fertilizer	Maize diversity panel, 230 accessions	Metabarcoding Root endosphere and rhizosphere DNA	150 microbial traits	mGWAS	NA	56

(*Continued*)

Table 1 (*Continued*)

Plant/crop	Environment	Population	Microbial sampling	Phenotype	Mapping	Validation	Reference
Rice	Field, two locations	International Rice Gene Bank Collection and China National Crop Gene Bank, 3,000 accessions	Metagenomics Phyllosphere DNA	Microbial abundances (Braken) on hub taxa	mGWAS	NA	75
Sorghum	Homogenized field soil mix in a growth chamber and field transplantation, one location	US sorghum association panel, 200 accessions	Metabarcoding Root endosphere and rhizosphere DNA	Microbial abundances	mGWAS	Rhizosphere bulk segregant analysis, publicly available RNA-seq	23
Switchgrass	Field, four locations	Diversity panel	Metabarcoding Phyllosphere DNA	NMDS	mGWAS	Lines with contrasting alleles at locus Chr02N_57831909, RNA-seq	89
Tea	Field, one location	100 cultivars	Metabarcoding Phyllosphere, endosphere, and rhizosphere DNA	Microbial abundances and PCoA	mGWAS	NA	83
Tomato	Glasshouse, agricultural soil	F8 RIL population derived from cv. Moneymaker and CGN14498, 100 lines	Metabarcoding Metagenomics Rhizosphere DNA	Microbial abundances, Shannon index, PCoA, SNV allele read counts	QTL	Bulk segregant analysis	62

Abbreviations: Braken, Bayesian re-estimation of abundance after classification with kraken; CCA, canonical correspondence analysis; GWAS, genome-wide association study; IBM, B73 × Mo17 maize inbred lines; mGWAS, microbe genome-wide association study; MWAS, microbe-wide association study; NA, not available; NMDS, nonmetric multidimensional scaling; P/A, presence/absence; PCA, principal component analysis; PCoA, principal coordinate analysis; QTL, quantitative trait locus; RIL, recombinant inbred line; SNV, single nucleotide variant; TRFLP, terminal restriction fragment-length polymorphism.

microbial contributions to plant traits, this latter contribution representing the host–microbiota component of a given plant trait's heritability. To this end, approaches uncoupling individual components of the partnership, i.e., the plant, the microbiota, and the environment, and recoupling them in defined combinations place scientists in the position to infer causal relationships between structural and functional microbial configurations and given plant phenotypes. Reductionistic approaches capitalizing on indexed, genome-annotated collections of microbes isolated from plant organs, often assembled into SynComs, combined with the inoculation of axenically grown plants have become a powerful tool to test Koch's postulates for the plant microbiota (90).

Hub microbes: microbes that are disproportionally important in shaping microbial communities

Heterosis: the phenomenon by which the progeny of diverse genotypes or crosses, called hybrids, exhibit greater biomass, speed of development, and fertility than both parents

Works conducted in *Arabidopsis* paved the way for the use of this approach in characterizing the host genetic basis of plant–microbiota interactions. In a seminal work for the research field, Bodenhausen and coworkers used a 7-strain SynCom, representative of the main bacterial phyla of the endogenous *Arabidopsis* microbiota, to systematically assess the contribution of 55 mutations at multiple plant traits to microbial assembly in the phyllosphere (7). This approach allowed the authors to identify cell surface components as determinants of the phyllosphere microbiota and demonstrate that mutations affecting cuticle formation were associated with a two-pronged perturbation of leaf communities manifested by an increase in bacterial abundance and enrichment of *Variovorax* sp. compared to wild-type plants. In another elegant investigation, scientists used sulfatase activity, i.e., the microbial-mediated mineralization of organic sulfur, from soils previously used for *Arabidopsis* cultivation as a quantitative phenotype in a GWAS. This led to the identification of *CYP71A27*, encoding a novel component of the camalexin synthesis pathway, as a primary candidate gene for plant–microbiota interactions in soil (45). Strikingly, loss-of-function *cyp71a27* mutants, when grown under axenic conditions, failed to benefit from the growth-promotion potential conferred by the beneficial bacterial strains *Pseudomonas* sp. CH267, *Pseudomonas simiae* WCS417r, and *Paraburkholderia phytofirmans PsJN* to wild-type plants. Conversely, the mutants were more susceptible to the growth inhibition caused by the root pathogen *Burkholderia glumae* PG1, providing a functional significance for *CYP71A27* in discriminating between microbial friends and foes at the root–soil interface (45). More recently, Brachi and coworkers (9) conducted a GWAS in *Arabidopsis* plants grown in different environments and showed that genetic signatures of the host's impact on the microbiota were conserved across these environments. They also showed that the host genetics correlated with ecologically relevant hub microbes, i.e., microbes identified as interacting with a high number of other microbes in network analyses. As hub microbes have the potential to control the proliferation of other members of the community (2), a host genetic control exerted on hub microbes would represent an efficient way for plants to modulate the entire community and/or its effect on plants. Consistently, an exemplar of one of the hub microbes identified in the aforementioned Brachi et al. (9) study, a *Brevundimonas* sp. isolated from *Arabidopsis* leaves, provided growth benefits to plants when inoculated under axenic conditions.

Examples of similar investigations are now becoming available for crop plants. For example, in foxtail millet, isolates representative of bacteria whose abundances in a sequencing survey were either positively or negatively correlated with growth traits were used in recolonization experiments with axenically grown plants. Regardless of the substrate used for plant growth, i.e., either sterile plates or sterilized soil, scientists identified a consistent output, whereby the inoculation of individual isolates previously positively correlated with growth traits promoted plant performance, whereas negatively correlated ones failed to do so (97). In maize, a seven-strain SynCom and axenically grown plants have been used to investigate the impact of the microbiota on heterosis, a plant breeding target in several crop species in which progeny plants called hybrids exhibit superior traits to their parents. Interestingly, this approach revealed an unexpected outcome: When SynCom-inoculated and mock-inoculated plants were compared, the hybrid outperformed its parental lines due to SynCom-mediated growth suppression of the parental lines

rather than enhanced growth of the hybrid. These results were the first to suggest a microbial role in heterosis. Strikingly, scientists were able to replicate this differential phenotype when plants were grown in the presence of more complex microbial communities, i.e., a soil slurry, derived from farm soil, and in two distinct field sites, when compared to sterilized controls (93). Despite the fact that contrasting fields had contrasting effects on the impact of soil sterilization on heterosis, these results unequivocally point to a microbial contribution to this plant trait.

Although this review focuses on the host genetics side of plant–microbiota interactions, it is worth highlighting the potential of metagenomic sequencing in elucidating causal relationships between the host genetic control of the microbiota and plant traits. For instance, the use of MAGs as a phenotype in the tomato mapping experiments allowed scientists to identify bacterial genes involved in the metabolism of plant polysaccharides, iron, sulfur, trehalose, and vitamins among the ones significantly associated with plant QTLs (62). This, in turn, may inform the selection of plant candidate genes for further characterization. Likewise, the in silico reconstruction of individual microbial genomes may expedite the isolation of microbial hubs that could be tested for their association with specific plant traits/genes, as efficiently demonstrated for the identification of microbes underpinning disease suppression (16, 47).

6. REWIRING HOST–MICROBIOTA INTERACTIONS FOR SUSTAINABLE CROP PRODUCTION

Intuitively, once plant genes shaping microbes are known and the resulting impacts of those microbes on plant performance have been established, translation of this knowledge requires generating plants that harbor these genes (or their contrasting alleles). Yet this conceptually simple step may prove to be challenging to translational applications for agriculture. For instance, scientists have been trying to engineer cereal crops to form nodules like legumes do for several years (18). Although the molecular pathway is well understood in both plants and bacteria, this approach has not yet succeeded (63). These observations prompt a legitimate question: Despite our increased understanding of the genetics of plant–microbiota interactions, how realistic is the possibility of engineering plants to recruit a superior microbiota than that molded by thousands of years of domestication and breeding selection? We argue that a positive answer to this question may be based, at least in part, on two observations. First, unlike nodulating rhizobia, the majority of the microbiota thriving at the root–soil interface does not require specialized plant organs to thrive; therefore, this may facilitate the manipulation of community composition via host genetics. Second, microbiota hubs could be the primary target of the manipulation, which, in turn, can then be extended to the rest of the community. The latter is akin to a counter-selection for pathogenic microbes, against which individual plant genes have proved to be effective.

An attractive avenue where these concepts may have a tangible impact is the improvement of currently input-demanding elite varieties for low-input agriculture. Given the influence played by the environment in shaping host–microbiota interactions, a promising approach to develop microbiota-ready varieties could be represented by the so-called focused identification of germplasm strategy (**Figure 2*a***), which is based on matching accessions to environments where a target trait, in this case microbiota recruitment, may confer an adaptive advantage (48). Wild relatives and locally adapted varieties, and the putative benefits conferred by their associated microbiotas (73), may represent an ideal target for this strategy. Operationally, this approach requires access to ecologically referenced accessions as well as information linking microbiota recruitment to eco-geographic adaptation, as exemplified for barley (3, 76, 79). Once relevant genes for microbiota recruitment are identified and validated, hybridization and selection, as well as gene editing, can be then used to mobilize them into a desired genetic background. This

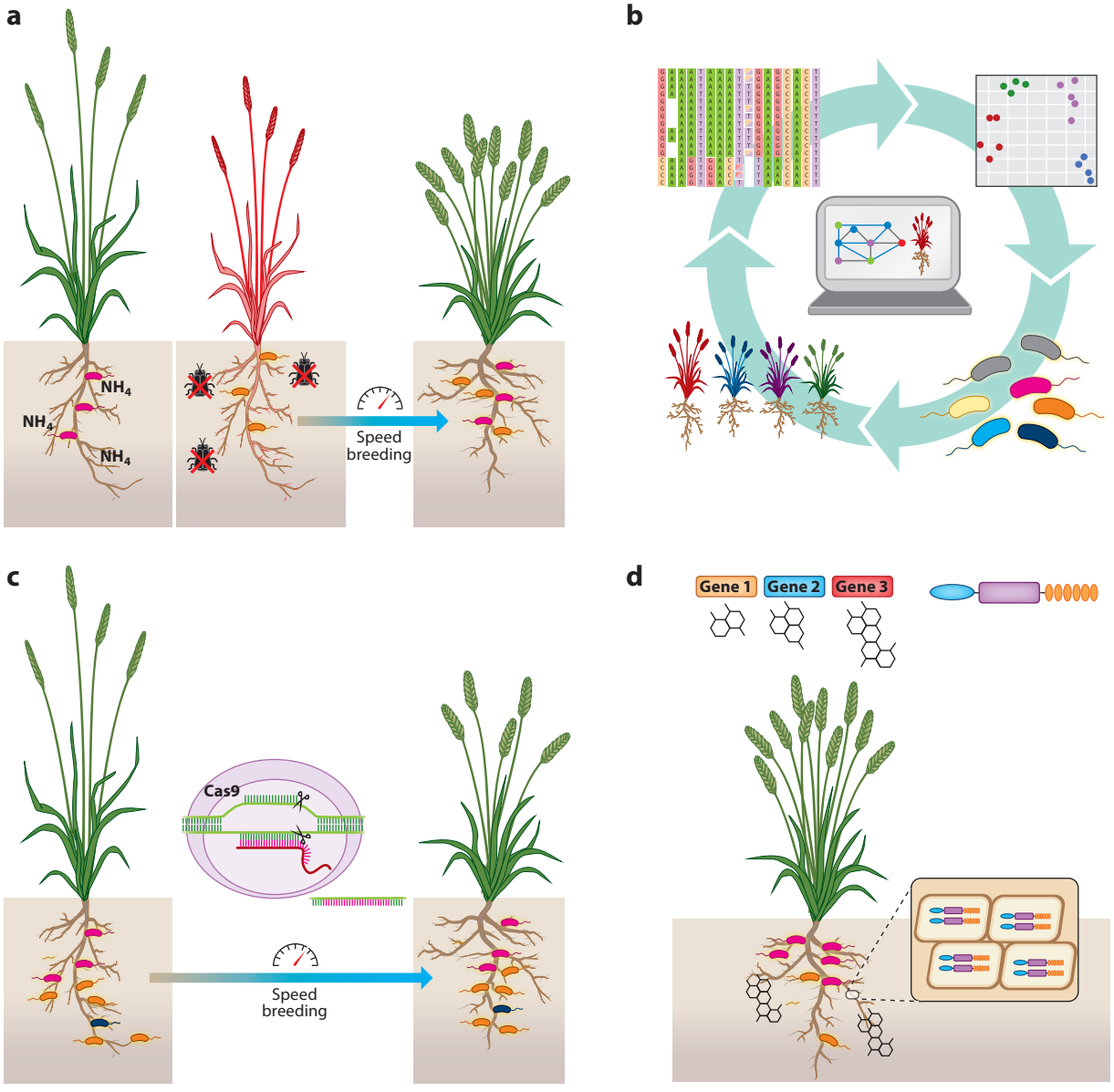

Figure 2

Strategies to rewire the host–microbiota interactions in crops. (*a*) The characterization of environmentally adapted genotypes may lead to the identification of major plant genetic determinants of the microbiota to be mobilized into elite material using, for example, speed breeding. (*b*) To facilitate the mobilization of more complex host–microbiota polygenic inheritance, genomic selection can be deployed to predict the outcome of novel genetic material (e.g., crosses) on microbial assemblages integrating plant's genotypic and phenotypic data with microbiota information, including community composition and individual microbial genomes. (*c*) De novo domestication can be used to confer key domestication traits to wild relatives already adapted to given microbiotas and soil conditions. (*d*) In a targeted approach, candidate genes (e.g., biosynthetic gene clusters producing secondary metabolites) or immune receptors (e.g., nucleotide-binding leucine-rich repeats) can be integrated into given plant genotypes to modulate microbiota composition.

approach may be further expedited by applying speed breeding, which significantly reduces plant generation time (98). Central to the success of this approach is the identification of a limited number of major determinants of the microbiota and the likelihood of recombination occurring between those loci and the ones associated with unwanted traits. This latter aspect is pivotal when no other approaches (e.g., genetic transformation) can be applied.

Conversely, when the number of loci putatively involved in microbiota recruitment exceeds a few units, as in several of the examples outlined in the above section, this introgression approach appears less practical because of the overwhelming number of combinations to be selected. In plant genetics, genomic selection, whereby phenotypes are inferred from whole genotypic information, has been proposed to tap into complex genetic diversity (109). For host genetic control of the microbiota, this could be implemented by identifying the minimum number of plant genotypes required to recapitulate microbial diversity and function of a given genetic pool, capitalizing also on deep-learning techniques that dissect microbiota data (35) and using them as founding members of breeding families to be characterized in multilocational trials (**Figure 2*b***), as recently illustrated for widening the genetic pool of yellow rust resistance in wheat (80).

Another intriguing approach is represented by de novo domestication, a paradigm shift for plant breeding in which a limited number of genes underpinning the so-called domestication syndrome traits are mobilized from elite material into wild plants to capitalize on the broad genetic diversity of the latter (107). As several domestication traits are coded by loss-of-function alleles of genes existing in the wild germplasm, this approach can be rapidly deployed using CRISPR-Cas9 mutagenesis when protocols for genetic transformation are available, as elegantly demonstrated through the de novo domestication of the wild tomato *S. pimpinellifolium* into plants with quality and yield characteristics comparable to cultivated tomatoes (115). This approach appears particularly suited to exploit microbial associations underpinning complex plant traits that are not currently in the cultivated germplasm (**Figure 2*c***), such as the mucilage-secreting, aerial roots of maize landraces conducive to the activity of nitrogen-fixing bacteria (88).

An alternative strategy may be represented by a priori systematic manipulation of candidate genes underpinning plant–microbiota interactions leading to novel, isogenic genotypes to be tested for their capacity to shape the microbiota and confer adaptation to given environments. In light of recent discoveries, genes encoding plant immune receptors, e.g., *NLRs* and *RLKs*, are particularly attractive targets for this approach thanks to the established technique, e.g., resistance gene enrichment sequencing, designated RenSeq, expediting their identification (42, 51) and mobilization (106) among plant genomes (**Figure 2*d***). Yet it is important to mention that the strength of the relationship between resistance genes and microbiota recruitment is modulated at species and microhabitat levels (92) and likely requires a case-by-case validation.

Likewise, leveraging transcriptomic resources may lead to the identification of biosynthetic gene clusters (BGCs) putatively involved in the production of secondary metabolites shaping the microbiota composition (**Figure 2*d***). These BGCs can then be tested using genetic techniques (e.g., exploiting natural variation for given crops, CRISPR-Cas9 mutagenesis) in an approach conceptually similar to an experiment recently described for the discovery of defense-related molecules in wheat (71).

Speed breeding: an approach to crop improvement that uses increased light duration and intensity to accelerate plant development

Genomic selection: a phenotypic prediction approach in which individuals are selected based on their genotype represented by genome-wide markers

Domestication syndrome traits: phenotypic changes associated with adaptation to cultivation under domestication and often shared across a broad array of crops (e.g., loss of seed dispersal, larger fruits, changes in photoperiod sensitivity)

Biosynthetic gene clusters (BGCs): physically clustered groups of two or more genes that together encode a biosynthetic pathway to produce a specialized metabolite

7. CONCLUSION AND FUTURE DIRECTIONS

Recent advances in experimental and computational approaches have given scientists the opportunity to gain unprecedented insights into the heritability of host–microbiota interactions in crops. Similar to the heritability of agronomic traits, the host genetic determinants of the microbiota have been shaped by plant domestication and crop diversification. Rather than being

a mere consequence of a conscious selection for plant microscopic traits though, the heritable component of microbiota recruitment represents, at least partially, a selection for microbial functions underpinning the growth, development, and health of their hosts. Soil and other environmental variables drive the magnitude of the heritability of microbiota recruitment, which is functionally akin to an external quantitative trait and is fine-tuned at a species and microhabitat level.

Further experimentation is required to gain a precise understanding of when and how microbiota heritability can be deployed for breeding purposes. Examples of missing heritability in macroscopic traits, e.g., the genotype × environment component of flowering time in *Arabidopsis* (77), or complex traits for which the contribution of genotype × microbiota interactions has recently been determined, e.g., the establishment of the root rot complex in legumes (99, 100), represent promising targets for said experiments.

In addition, a greater understanding of microbiota heritability will likely impact the application of microbial inoculants to crop production. For instance, it has been extensively demonstrated that exogenous application of individual or consortia of plant- and soil-derived microorganisms has the potential to promote plant performance, although such inoculants often yield inconsistent results under field conditions (50). Host genetics may be a factor contributing to this variable performance, particularly in the face of a lack of breeding to select for a positive response to such inoculants. Although strategies for the integration of microbial inoculants into the seed industry have been established (57), dedicated programs involving the identification and characterization of crop genetic determinants underpinning responsiveness to inoculants are required to fully exploit the potential of agricultural biologicals for sustainable crop production.

Taken together, the examples discussed in this review illustrate how the crop microbiota can be integrated, as an external quantitative trait, in the characterization and selection of plant genetic resources. Considering the fast-paced development of crop (pan-)genomics (22, 111), the way is paved for the successful implementation of breeding for the microbiota in the years to come.

DISCLOSURE STATEMENT

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Errata

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