

# Trends in Plant Science

## Breeding towards improved ecological plant-microbiome interactions

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<b>Corresponding Author:</b>	Raffaella Balestrini Istituto per la Protezione Sostenibile delle Piante Consiglio Nazionale delle Ricerche Torino, Italy ITALY
<b>First Author:</b>	Luca Nerva
<b>Order of Authors:</b>	Luca Nerva Marco Sandrini Loredana Moffa Riccardo Velasco Raffaella Balestrini Walter Chitarra
<b>Abstract:</b>	Domestication processes, amplified by breeding programs, have allowed for the selection of more productive genotypes and more suitable crop lines capable to cope with the changing climate. Notwithstanding these advancements, the impact of plant breeding on the ecology of plant-microbiome interactions have not been adequately considered yet. This includes the possible exploitation of beneficial plant-microbe interactions to develop crop with improved performance and better adaptability to any environmental scenario. Here we will discuss the exploitation of customized synthetic microbial communities in agricultural systems, to develop more sustainable breeding strategies, based on the implementation of multiple interactions between plants and their beneficial associated microorganisms.

## **Breeding towards improved ecological plant-microbiome interactions**

Luca Nerva<sup>1,3†</sup>, Marco Sandrini<sup>1,2†</sup>, Loredana Moffa<sup>1,2†</sup>, Velasco Riccardo<sup>1</sup>, Raffaella Balestrini<sup>3†\*</sup> & Walter Chitarra<sup>1,3†</sup>

<sup>1</sup> Research Centre for Viticulture and Enology, Council for Agricultural Research and Economics (CREA-VE), Via XXVIII Aprile 26, 31015 Conegliano (TV), Italy.

<sup>2</sup> University of Udine, Department of Agricultural, Food, Environmental and Animal Sciences, Via delle Scienze 206, 33100, Udine (UD), Italy

<sup>3</sup> National Research Council of Italy - Institute for Sustainable Plant Protection (IPSP-CNR), Strada delle Cacce, 73, 10135 Torino (TO), Italy

†These authors contributed equally to this work as first authors

‡These authors contributed equally to this work as senior authors

### **\*Corresponding author details**

Raffaella Maria Balestrini – [raffaella.balestrini@ipsp.cnr.it](mailto:raffaella.balestrini@ipsp.cnr.it)

Institute for Sustainable Plant Protection (IPSP-CNR)

Strada delle Cacce, 73; 10135 Torino (TO), Italy.

**Highlights**

Domestication process and breeding programs, focused exclusively on the selection and improvement of specific plant traits, has negatively affected the recruitment and the functionality of beneficial associations in agricultural systems.

Rhizosphere and root-associated microorganisms play important roles in plant growth and resilience.

Breeding programs aiming to rationally manipulate root traits in order to recruit beneficial microorganisms or to deter pathogens need to be developed.

The development and application of synthetic communities (SynComs) represent a sustainable way to improve plant growth and resilience.

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4 Walter Chitarra<sup>1,3†</sup>

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6 (CREA-VE), Via XXVIII Aprile 26, 31015 Conegliano (TV), Italy.

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10 delle Cacce, 73, 10135 Torino (TO), Italy

11 †These authors contributed equally to this work as first authors

12 ‡These authors contributed equally to this work as senior authors

13 **\*Corresponding author details**

14 Raffaella Maria Balestrini – raffaella.balestrini@ipspp.cnr.it

15 Institute for Sustainable Plant Protection (IPSP-CNR)

16 Strada delle Cacce, 73; 10135 Torino (TO), Italy.

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1 **Abstract**

2 Domestication processes, amplified by breeding programs, have allowed for the selection of more  
3 productive genotypes and more suitable crop lines capable to cope with the changing climate.  
4 Notwithstanding these advancements, the impact of plant breeding on the ecology of plant-  
5 microbiome interactions have not been adequately considered yet. This includes the possible  
6 exploitation of beneficial plant-microbe interactions to develop crop with improved performance and  
7 better adaptability to any environmental scenario. Here we will discuss the exploitation of customized  
8 synthetic microbial communities in agricultural systems, to develop more sustainable breeding  
9 strategies, based on the implementation of multiple interactions between plants and their beneficial  
10 associated microorganisms.

11

12 **Domestication syndrome and plant-microbiomes**

13 **Plant domestication** (see **Glossary**) is the outcome of a selection process that leads to increased  
14 adaptation of plants to cultivation and utilization by humans [1]. This process is based on the  
15 implementation of breeding programs via the selection and modification of wild plant species and  
16 aims to select for useful plant traits for human requirements. Increasing crop yield has consistently  
17 been one of the most important goals during the domestication process to provide humans with a  
18 continuous and constant food supply [2]. However, plants do not have unlimited quantity of energy  
19 and the allocation of limited carbon sources is consequently influenced by a growth-defence trade-  
20 off [3]. This phenomenon is based on the concept that the limited carbon sources produced by  
21 photosynthesis are allocated toward growth or defence processes to maximize the plant's adaptation  
22 strategies and fitness costs in diverse environments [4]. If plants focus their energy mainly on  
23 growing, they automatically have less ability to deal with different kind of stresses such as pathogen  
24 infections or harsh environmental conditions. Furthermore, domesticated plants are much more  
25 nurtured than their wild parents, through fertilization, irrigation, and other protective measures, so  
26 they are characterized by less ability to interact with or adapt to the surrounding environment.

27 The ability of plants to interact with thousands of microorganisms that are surrounding and supporting  
28 them in dealing with both biotic and abiotic stresses, is one of the most important traits that should  
29 be reinforced. Several studies have already demonstrated that plant-associated microorganisms are  
30 essential to improve plants' wellness and sustainability of agricultural systems [5,6]. Modern  
31 agriculture is entering into a second green revolution and the exploitation of beneficial soil  
32 microorganisms is playing a significant role with several microorganism-based products currently  
33 coming to the market [7]. However, there is evidence that domestication processes have profoundly  
34 altered the interactions between plant hosts and associated microorganisms [8,9]. Recent studies have

1 found significant differences between the microbiome of commercial genotypes with that of their  
2 relative wild types. It has been shown that wild ancestors and primitive landraces of wheat (*Triticum*  
3 *aestivum*), breadfruit (*Artocarpus altilis*), and maize (*Zea mays*) can benefit more from mycorrhizal  
4 symbiosis [10,11] compared to selected cultivars, suggesting that the modified microbiome due to  
5 domestication is not beneficial to the plant. Studies on other plants species, including arabidopsis  
6 (*Arabidopsis thaliana*) [12], sugar beet (*Beta vulgaris*) [13], barley (*Hordeum vulgare*) [14], and  
7 lettuce (*Lactuca sativa*) [15], also suggested that human-centered breeding led to compositional  
8 changes in root-associated microbiomes. How this negative trend can be mitigated, and the plant-  
9 microbiome equilibrium restored are still open questions. Although the tight interactions of  
10 indigenous crops with the associated microbiota reinforce the ability and flexibility of crops to deal  
11 with diverse environmental stresses, a difficulty for exploiting their potential is correlated with the  
12 low ability of separating the targeted functional microbes [16]. We also still need to understand which  
13 plant traits maybe involved in the interactions with beneficial microorganisms and how we can  
14 identify them and select for them in future breeding programs (see **Outstanding questions**).

## 15 **The neglected shortcomings of traditional and new plant breeding techniques**

### 16 ***Intersection of anthropocentric breeding and plant-microbiome interactions***

17 The incessant selection of genomic plant traits, and the considerable number of inputs needed to  
18 sustain the selected genotypes, negatively influence the interactions among plants and beneficial  
19 microorganisms [17,18]. Breeding processes reduced in fact the microbial biodiversity and  
20 functioning associated to plants in agricultural systems, hampering the essential interactions that  
21 make wild species more resilient to biotic and abiotic stresses (Fig. 1) [18]. This effect is most  
22 probably due to the fact that the selected crop cultivars might have lost some of the genetic traits  
23 needed to recruit host-specific microbiota as compared to their wild relatives. It was shown that long-  
24 term nitrogen fertilization resulted in the recruitment of less and less-functional rhizobacteria in  
25 leguminous species, providing so fewer benefits to the host [19]. Similarly, Kiers et al. [20]  
26 demonstrated that older soybean cultivars had a higher ability to reach their full symbiotic potential  
27 in the presence of a rhizobia-strain cocktail, with different symbiotic effectiveness compared to newer  
28 soybean cultivars. Furthermore, Chaluvadi and Bennetzen [21] have demonstrated that there are  
29 species-specific differences in the belowground microbiome associated to wild and domesticated  
30 *Setaria*, highlighting how crop domestication plays an important role in selecting prokaryotes present  
31 in the rhizosphere. There is also evidence about the impact of plant breeding on the assembly of  
32 rhizosphere fungal communities that seem to be strongly influenced by host genotype [22]. Recent  
33 studies have shown that **root exudates** are essential for plants to assemble a functional microbiome  
34

1 and changes in plant genetics derived from breeding programs result in different root exudate  
2 composition undermining microbiome assembling and functioning [18,23], playing a role also in  
3 symbiotic relationships [24,25]. Martin-Robes stated that colonization by **arbuscular mycorrhizal**  
4 fungi is lower while infection rate by nematodes is higher in the roots of plants that grow in soils  
5 previously cultivated by domesticated plants in comparison with wild progenitors [26]. Conversely,  
6 rhizosphere microbial communities induced systemic changes in tomato root exudates, suggesting  
7 the presence of a long-distance signaling [27]. Despite the improvement in understanding root  
8 exudate composition, a few information is available so far about exudate spatial distribution and  
9 regulation in root, considering that homogenized samples are generally used [28]. Döll and colleagues  
10 dissected the root in the three fractions analyzed for tissue-specific metabolic profiles, correlating  
11 those profiles with protein abundances involved in biosynthetic pathways resolved spatially and  
12 showing that the presence of differentially abundant compounds in diverse root tissues and exudate  
13 of asparagus roots [28]. Volatile Organic Compounds (VOCs) have also a relevant role in the  
14 communications and interactions with rhizosphere-inhabiting microorganisms and they are signal  
15 molecules with a potential for application in agriculture [29]. VOCs emitted belowground can  
16 stimulate the migration of distant soil bacteria, attracting them towards the roots. Additionally,  
17 changes in the blend of root VOCs, due to a stress situation such as a fungal soilborne pathogen  
18 infection, lead to the recruitment of specific beneficial bacteria from outside the rhizosphere that  
19 might have a role to help plants to cope with the stress condition [30]. However, rhizosphere and root-  
20 associated microbiota, in addition to support plant growth, also offer a further level of genetic  
21 variability that was little considered by breeders until now [31]. Future breeding strategies should  
22 treat root features, including traits related to the microbial recruitment (Box 1), and strategies for root  
23 phenotyping should be implemented, also considering the interactions with rhizosphere microbiota.  
24 A deepen investigation of the relationships between roots and associated microbiota is particularly  
25 relevant to breeding highly efficient root systems and the consequent selection of climate-change  
26 resilient genotypes [32].

27  
28 ***Critical points of breeding based on the development and selection of the most performing plant***  
29 ***genotypes***

30 Despite breeding programs aim to produce resilient cultivars to diseases and environmental stresses,  
31 many traits are regulated by several genes (*i.e.*, polygenic resistance) and, for this reason, hardly  
32 transmissible to the progeny in a single crossing [33]. In this scenario, the monogenic resistance,  
33 exploited by conventional breeding programs, is highly effective in moving single gene traits, but it  
34 is easily suppressed by the pathogen along time [34,35]. Regarding woody plants, breeding programs

1 encounter several further limitations, such as **linkage drag**, long and laborious times needed for  
2 backcrossing and high heterozygosity degrees, hampering the development of resilient genotypes and  
3 raising the costs [36–38]. The obtained resilient/resistant genotypes are often associated to  
4 modifications that could be detrimental from the commercial point of view, such as an altered  
5 phenotype and/or biochemical profile (related to texture, taste, and/or organoleptic profiles) [39],  
6 often less accepted by consumers [40,41].

7 Application of biotechnological approaches in breeding processes has recently led to development of  
8 **New Plant Breeding Techniques** (NPBTs), able to modify specific target DNA sequences without  
9 altering other regions and, if applied with the DNA-free or marker-free approaches, without the need  
10 of long backcrossing stages [42–44]. These techniques are very promising to overcome the limits  
11 imposed by traditional breeding both in terms of time and costs. Although NPBTs-derived products  
12 are accepted in several countries, many restrictions remain, especially in Europe, [45,46] and traits  
13 related to the ability in recruiting beneficial microbiota are not so far sufficiently considered. Roots  
14 in fact share their habitat with many microorganisms, such as bacteria and fungi, that can have a  
15 positive role on plant productivity and tolerance/resilience to environmental stresses [47–52]. The  
16 exploitation of these microorganisms to improve plant traits may overcome the limits associated to  
17 both conventional breeding and NPBTs, leading to a reduced impact on the marketable characteristics  
18 of the final products, preserving the original genotype and therefore without requiring specific safety  
19 assessments on the products (*e.g.*, those for genetically modified organisms), and leading to a less  
20 expensive and time consuming application if compared to breeding programs, especially for woody  
21 plants (Fig. 2).

22  
23 A comprehensive understanding of mechanisms governing the selection of microbial community by  
24 the plant will provide useful information to improve the future agriculture. The application of novel  
25 approaches, such as i) the selection and characterization of specific microorganisms to restore growth-  
26 defence trade-off balance in commercial genotype, ii) the exploitation of complementary omics-tools  
27 and iii) the **Synthetic microbial Communities** (SynComs) application, seems a good way to reach  
28 this goal.

29  
30 ***Microbiome mediated dynamism in the allocation of plant resources***  
31 Allocation of resources to priority biological processes, restricting others, can give rise to trade-offs  
32 between different processes/responses, mainly in natural environments where plants generally cope  
33 with limiting resources [53]. Commercial genotypes are often characterized by an unbalanced  
34 allocation of energy resources, and microbiomes represent a promising tool to restore the growth-



1 defence trade-off balance in these plants, achieving an agricultural system able to survive with limited  
2 external inputs. Analyzing plant microbiomes and trying to select and identify genomes of  
3 microorganisms able to improve plant growth or resilience to several stresses could be the right way  
4 forward [53–55]. Recently, an involvement of arbuscular mycorrhizal fungi (AMF) in balancing  
5 growth-defence trade-off has been suggested in grapevine (*Vitis vinifera*), where rootstock genotypes  
6 and AMF inoculation have a relevant role in shaping the root-associated microbes and stimulating  
7 growth and defence pathways [55]. Cultivars selected for quality and/or quantity yield are usually  
8 more sensitive to biotic and abiotic stresses: using microorganisms able to place the plants in the so  
9 called “priming state” allows to restore the natural trade-off equilibrium, obtaining more resilient  
10 cultivars [55–59]. **Priming** (or acclimatization) is a complex phenomenon that consists in  
11 preconditioning the plant biotic and abiotic defence mechanisms, so that responses to stress result  
12 quicker, stronger, and more effective [60]. The simultaneous employment of biological as well as  
13 chemical agents towards further improved plant performance is gaining interest among researchers  
14 with the aim to maximize the primed status[61]. Additionally, to enabling a state of priming, several  
15 rhizosphere-associated microorganisms can perform a direct antagonism towards several pathogens  
16 acting as plant allies [62,63]. On the other hand, genotype characterized by high defense performance  
17 and low yield can be made more productive through the exploitation of plant growth promoting (PGP)  
18 microorganisms instead of modifying their own microbiome with long breeding programs.  
19 Furthermore, since most of resistant cultivars obtained by conventional breeding showed less crop  
20 quality and yield capacity, the possibility to exploit PGP microorganisms could partially restore the  
21 original features avoiding plant genetic manipulation [55,64]. ‘Resistant’ cultivars are characterized  
22 by specific resistances toward target pathogens, but they do not make provisions for the rest of biotic  
23 and abiotic stresses. An enrichment of certain taxa in microbiome composition, on the contrary,  
24 guarantee a broad-spectrum tolerance toward several pathogens contributing to plant phenotypic  
25 plasticity and adaptability to the changing environment [65,66]. It has been shown that specific  
26 microbiota members can modulate plant immunity processes through bidirectional microbiota–root–  
27 shoot mechanisms relevant for plant health [67,68]. A functional and rich microbiome can stimulate  
28 plant immunity and modulate the allocation of carbon plant resources, shifting them from growth to  
29 defence processes [69,70] (see **Outstanding questions**). Regarding grafted plants, the choice of the  
30 rootstock genotype plays an important role in shaping microbes inhabiting the rhizosphere and the  
31 allocation of carbon resources. Indeed, rootstocks differ from each other by specific growth-defence  
32 trade-off features and for the ability to recruit different microbial consortia [70]. Modulating the  
33 interactions between rootstocks and their own associated microbe, growth and defence features could

1 be managed through the bidirectional root-to-shoot mechanism, making grafted plants more suitable  
2 to sustainable practices.

3

#### 4 ***SynComs to enhance holobiont functionality***

5 Unearthing the functional relationships between plants and their microbial partners is the next step  
6 for improving plant fitness and for adopting breeding programs focused on the holobiont [71]. Natural  
7 microbial communities and the interactions between them and their host plants are known to be very  
8 complex and variable, hardly depictable and not fully understood yet. This complexity derives from  
9 the large number of microbes inhabiting the environment coupled to the often unknown functions for  
10 most of them and from the uncharacterized interactions occurring among one another [72].

11 The SynCom assembly seems to be a promising approach to exclude confounding environmental  
12 effects and to reduce the complexity of natural systems [73,74]. The establishment of a SynCom is  
13 grounded on collected knowledge of the overall composition of the root-associated microbiome  
14 needed to formulate a “**core microbiota**” [73]. Carlström et al. [75] showed how community  
15 assembly is subject to priority effects, *i.e.*, the imprint of arrival order on community structure, and,  
16 additionally, they indicated that specific strains have the greatest potential to affect community  
17 structure as keystone species. Once the keystone strain collection and the molecular identification is  
18 achieved, these microorganisms have to be tested for their antagonistic activity towards pathogens  
19 and PGP traits, and for keeping out the possibility that they can have a reciprocal inhibition effect.  
20 Microorganisms showing traits of interest can be used to establish a SynCom being tested before in  
21 controlled environments and subsequently in a natural environment [76–78]. Additionally, through  
22 the SynCom exploitation, it is possible highlighting the role of plant immune system in the  
23 assemblage of a protective microbiome [79]. Recent studies have demonstrated that plants can recruit  
24 beneficial bacteria upon pathogen infections, specifically disease resistance-inducing and growth-  
25 promoting ones [67,76].

26 Although the effectiveness of the SynCom application in agriculture has been proven to be often  
27 inconsistent due to low efficiency in establishment and survival of the selected taxa [80–83], the  
28 SynCom approach seems to be pivotal for capitalizing associated microorganisms, increasing the  
29 agroecosystem resilience, and finally driving new breeding programs. The main reasons of failures  
30 are related to the plant-associated microbes ability to exert their beneficial effects. These can be  
31 reduced for several reasons that are closely linked to the host plant genotype, the microbial species  
32 compatibility with the growth environment, the spatial competition with other soil microorganisms  
33 and the persistence in soil. The ecological interactions occurring with the natural occurring microbial  
34 population is one of the most important aspects that must be considered when applying SynComs in

1 real environments. The importance of ecologically based community assembly rules has been  
2 demonstrated by the survival of SynComs developed using *Pseudomonas* spp.: survival rate was  
3 directly and positively correlate to the diversity of the developed consortia [84]. Additionally, the  
4 community growth and development are affected by several factors including growth substrates and  
5 presence of other chemical compounds [85]. The still limited knowledge on the mechanisms  
6 underlying plant ability to control its associated microbial communities and how members of  
7 microbial consortia interact with one another also strongly limit their exploitation in agriculture [86–  
8 88]. Interestingly, AM responsiveness seems to be subjected to ‘genotype x environment (G x E)  
9 interaction’ that is referred to the phenomenon for which diverse genotypes respond to different  
10 environments in different manners [89].

11 Regarding the impact of human-focused breeding on microbiome assembly and functionality,  
12 additional insights into microbiomes of wild plants and native habitats could contribute to reinstate  
13 or enrich current genotypes of microorganisms with beneficial effects on plant growth, development,  
14 and health [82,90]. Thanks to a combined approach (*e.g.*, high-throughput plant phenotyping,  
15 identification of the core microbiota strictly linked with a specific genotype, inoculation of  
16 personalized SynCom and analysis of plant responses at molecular level), it is workable finding some  
17 microorganisms, strictly linked with a wild genotype, that can be used to restore traits lost during  
18 breeding programs.

19

#### 20 **Concluding remarks**

21 Domestication, focused exclusively on the selection and improvement of specific plant traits, is by  
22 its very nature an anthropocentric breeding program and has negatively affected the recruitment  
23 and/or the functionality of beneficial holobiont-associated microbes in agricultural systems. Plant-  
24 associated microorganisms are known to improve plant growth and wellness and thus the plant’s  
25 ability to interact and cooperate with microorganisms should be considered as a fundamental trait in  
26 modern breeding programs. Applying a holistic vision of plant breeding and including the  
27 exploitation of “holo-omics” techniques (Box 2), will lead to a deeper understanding of the hidden  
28 world of plant-microbiome interactions. This will open a path to try and manage these interactions as  
29 a sustainable tool to restore plant resilience against stressful factors.

1 **Box 1 – The importance of root traits**

2 Efficient root systems are essential to enhance crop productivity, indeed studies on plant genotypes  
3 better adapted to stresses are now focusing on root traits [91,92]. The use of sophisticated systems  
4 and sensors enabled researchers to follow the development of the root system and to evaluate the  
5 uptake of water and nutrients at the root level. Plants can adapt root apparatus to optimize the  
6 availability in water and nutrients, thus having an impact in plant resilience and productivity [93].  
7 However, they do not live alone in the soil and root-associated microorganisms are known to play a  
8 fundamental role in plant adaptation to adverse environmental conditions.

9 One of the main questions regarding plant-microbe interactions concerns the identification of  
10 functional mechanisms that plants exploit to shape their microbiome. It is worth noting that, if from  
11 one side root traits are able to influence the composition of the root-associated microbes, on the other  
12 hand microbes are able to interact with the plant modifying root traits [94]. Among root features,  
13 architectural traits (root depth/angle, length, density) and morphological traits (root hairs, root  
14 diameter, aerenchyma/cell size, root cap properties) are the physical properties playing important role  
15 in the two-way interaction by specifically altering the plant-microbe interaction interface [90,95,96].  
16 In parallel, the unique biochemical fingerprint profile of a plant deeply contributes to the recruitment,  
17 colonization ability and function outline of specific microbiomes [97]. The root exudates, and the cell  
18 wall composition, vary considerably according to the age, developmental stage and species also  
19 between different genotypes and varieties of the same species and constitute a primary feed source  
20 for the rhizosphere-associated microorganisms, making the soil surrounding roots more suitable for  
21 bacteria and fungi proliferation [98]. The influence of root architecture and exudates on rhizosphere  
22 and root environments, and consequently on the microbial recruitment, nowadays represent a *hotspot*  
23 in the researches aimed to study root interactions and is attracting always more attention [99–101].

24 Agricultural sustainability can be potentially boosted by implementing breeding program able to  
25 consider the above-mentioned root properties, enhancing the possibility to recruit microbiomes with  
26 beneficial traits. Linking root phenome to specific microbiomes (or related functional traits) may  
27 enhance our understanding of the interactions occurring in the rhizosphere and the role that these  
28 interactions play in generating climate-resilient agroecosystems [102]. Important aftermaths of such  
29 approaches will impact on significant soil and root-related functional aspects such as N fixation and  
30 cycling, P dynamics, C dynamics and plant water availability. Recently, Brisson et al. suggested that  
31 root exudation in response to phosphate stress was conserved during the maize domestication  
32 (teosinte vs maize), shifting the exudation levels of specific metabolites and the microbial  
33 communities, although selective recruitment of phosphate solubilizers in response to phosphate  
34 availability has not been observed[101].

1 **Box 2 - Holo-omics studies: detailed information on host-microbiota interactions**

2 To develop protocols for a new kind of breeding linked to the holobiont concept, a more detailed  
3 knowledge of the mechanisms governing plant-microbiome interactions is needed [103]. Holo-omics,  
4 *i.e.*, the incorporation of data across multiple omic levels from both host and microbiota domains,  
5 may have the potentiality to resolve the functionality of a plant microbiome ecosystem by generating  
6 an image of what is being expressed, translated, and produced during plant microbiome interactions  
7 [103,104]. The combination between host and microbial datasets provide in fact a powerful approach  
8 for the development of hypotheses and advancement in the topic of plant interactions [103]. Pairing  
9 host-centered omics-tools, such as transcriptomics, epigenomics and proteomics, in combination with  
10 the more commonly used microbial-focused techniques such as amplicon sequencing, shotgun  
11 metagenomic, metatranscriptomics, and exometabolomics, seems to be a very promising approach to  
12 achieve a more integrated knowledge on plant microbiome functions [103–105]. A great number of  
13 works grounded on this current approach are nowadays present in literature [106,107]. Recently,  
14 Castrillo et al. [108] have explored the relationship between Phosphate Starvation Response (PSR)  
15 and microbiome composition and functionality in *Arabidopsis* [108]. Through a holo-omics design  
16 (16S profiling and RNA-seq), they have demonstrated that the plant root microbiome directly  
17 connects phosphate stress response and plant immune system, and that, gene controlling PSR  
18 contributes to the root microbiome assembly. Microbial communities of PSR mutant plants were  
19 distinct from those of wild type, and inoculation of a specific SynCom enhanced the activity of a  
20 master regulator of PSR (PHR1) under limited phosphate conditions, confirming that PHR1 directly  
21 regulates a functionally relevant set of plant-microbe recognition genes. Moreover, Stringlis et al.  
22 [109] demonstrated, using metagenome and metabolome approaches, that beneficial rhizobacteria  
23 induced excretion of the metabolite scopoletin that stimulates iron uptake and suppresses soil-borne  
24 pathogens in *Arabidopsis*.

25 A combined use of omics approaches led to obtain information on the so-called “soil memory”.  
26 Particularly, Li et al. [110] applied metagenomics to characterize the peanut (*Arachis hypogaea*)  
27 rhizosphere microbiome and root metatranscriptomics on peanut plants grown in a soil with different  
28 management histories, such as monocropping and crop rotation. These authors found that the past  
29 planting record had a significant impact on the assembly of the microbial community in the peanut  
30 rhizosphere, indicating a soil memory effect affecting crop rhizosphere microbiomes and plant  
31 physiology. Thanks to multi-omics and bioinformatics technologies it is now possible to identify core  
32 interactions between plants and native microbiomes pointing out useful traits for breeding purposes.

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5 **Declaration of interests**

6 The authors have no interests to declare.

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1 **Glossary**

2 **Arbuscular mycorrhizal symbiosis:** Beneficial associations between the roots of most terrestrial  
3 plants, including several relevant crops, and the arbuscular mycorrhizal fungi, which are obligate  
4 symbiotic fungi belonging to the Glomeromycotina sub-phylum.

5 **Core microbiota/microbiome:** The set of microorganisms/microbial genes that is systematically  
6 associated with a given host plant and forms webs of interactions that can be exploited to optimize  
7 microbial functions at individual plant and ecosystem levels.

8 **Holobiont:** Assemblage of a host and the many other species living in or around it, which together  
9 form a discrete ecological unit.

10 **Hologenome:** The union of all the genes in an holobiont.

11 **Linkage drag:** It refers to the usually undesirable effect of moving unwanted genes in the progeny  
12 that are linked to the gene(s) or QTL of interest.

13 **New Plant Breeding Techniques:** Methods allowing the development of new plant varieties with  
14 desired traits by modifying specific DNA sequences.

15 **Plant domestication:** Co-evolutionary process in which human select wild plants based on their  
16 adaptation to cultivation and human-derived purposes.

17 **Priming:** The state that can be reached following treatments with various inducers such as chemical  
18 compounds or beneficial microbes. The primed-plants are able to display faster and stronger defense  
19 responses.

20 **Root exudates:** Range of compounds released by the roots into the surrounding soil with key role in  
21 supporting plant development and interactions with rhizosphere microbes, facilitating root  
22 associations with beneficial microbes and suppressing pathogens.

23 **Synthetic microbial communities (SynComs):** Comprehensible system of reduced complexity.  
24 Wild microbial communities are composed of mixed microbes with several unknown function,  
25 SynComs allow the generation of defined system with known taxonomic as well as functional  
26 profiles.

27 **Traditional Breeding:** Genetic enhancement of adaptation traits, disease and pest resistance, abiotic  
28 stress tolerance, nutritional and water use, to improve crop yield and product quality.

29

1 **Figure legends**

2 **Figure 1. Comparison between wild and domesticated plant species.** On the left, wild relative  
3 displays a better adaptation to environmental stresses (both biotic and abiotic), a balanced growth-  
4 defence trade-off, a rich associated-microbiome, but a low yield. On the right side, domesticated plant  
5 shows an improved productivity, but a reduction of both stress resilience and ability to recruit plant-  
6 associated microbes. To cope with the increased susceptibility, human practices such as irrigation,  
7 fertilization and pesticide application are needed.

8 **Figure 2. Overview of the classical breeding, new plant breeding techniques and the microbial**  
9 **breeding approaches for woody plants.** On the left, classical breeding relies on the possibility to  
10 transfer traits (*e.g.*, related to biotic or abiotic stress resilience) from wild sexually compatible species  
11 to domesticated species of high economically importance through crossing. The main limitation in  
12 such approach, especially for woody species, is the presence of juvenile stages, which increase the  
13 time for back-crossing, and the transmission of undesired traits (linkage drag). In the middle, new  
14 plant breeding techniques allow to reduce the time needed by conventional breeding, but they still  
15 present limitations such as the need of specific tissues and/or cellular types (*e.g.*, embryogenic calli)  
16 and several limitations related to GMO regulations. On the right, microbial breeding approach that  
17 can overcome the limitation of the classical breeding techniques, preserving the original genotype  
18 and reducing development times and costs of a synthetic community (SynCom).

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1 **Breeding towards improved ecological plant-microbiome interactions**

2  
3 ~~Nerva~~-Luca ~~Nerva~~<sup>1,3†</sup>, ~~Sandrini~~-Marco ~~Sandrini~~<sup>1,2‡</sup>, ~~Moffa~~-Loredana ~~Moffa~~<sup>1,2‡</sup>, Riccardo Velasco<sup>1</sup>,  
4 ~~Balestrini~~-Raffaella ~~Balestrini~~<sup>3†\*</sup> & ~~Walter~~ Chitarra-~~Walter~~<sup>1,3†</sup>

5 <sup>1</sup>Research Centre for Viticulture and Enology, Council for Agricultural Research and Economics  
6 (CREA-VE), Via XXVIII Aprile 26, 31015 Conegliano (TV), Italy.

7 <sup>2</sup>University of Udine, Department of Agricultural, Food, Environmental and Animal Sciences, Via  
8 delle Scienze 206, 33100, Udine (UD), Italy

9 <sup>3</sup>National Research Council of Italy - Institute for Sustainable Plant Protection (IPSP-CNR), Strada  
10 delle Cacce, 73, 10135 Torino (TO), Italy

11 †These authors contributed equally to this work as first authors

12 ‡These authors contributed equally to this work as senior authors

13 **\*Corresponding author details**

14 Raffaella Maria Balestrini – raffaella.balestrini@ipspp.cnr.it

15 Institute for Sustainable Plant Protection (IPSP-CNR)

16 Strada delle Cacce, 73; 10135 Torino (TO), Italy.

17  
18 **Keywords**

19 Domestication Syndrome, Holo-omics Approaches, Holistic Breeding, Mycorrhizal Symbiosis, Plant  
20 Microbiome



## 1 **Abstract**

2 ~~Over the years,~~ Domestication processes, amplified by breeding programs, have allowed for the  
3 selection of more productive genotypes and ~~of~~ more suitable crop lines capable to ~~contrast~~ cope with  
4 the changing climate ~~change~~. Notwithstanding these advancements, the impact of plant breeding on  
5 the ecology of plant-microbiome interactions ~~have not been adequately considered yet, as well as~~ This  
6 includes the possible ~~the~~ exploitation of beneficial plant-microbe interactions to develop crop with  
7 improved performance and ~~more-better~~ adaptability ~~ed~~ to any environmental scenario, ~~have not~~  
8 ~~adequately considered yet.~~ Here w ~~here~~ We will ~~here~~ discuss the exploitation of customized synthetic  
9 microbial communities in agricultural systems, to develop more sustainable breeding strategies, based  
10 on the implementation of multiple interactions among-between plants and their beneficial associated  
11 microorganisms.

12

## 13 **Domestication syndrome and plant-microbiomes**

14 **Plant domestication** (see **Glossary**) is the outcome of a selection process that leads to increased  
15 adaptation of plants to cultivation and utilization by humans ~~being~~ [1]. This process is ~~grounded~~ based  
16 on the implementation of breeding programs ~~finalized to~~ via the selection and modification of wild  
17 plant species and aimed to ~~achieve~~ select for useful ~~characteristics~~ plant traits for human  
18 requirements. ~~The~~ increasing crop yield has consistently been one of the most important goals  
19 ~~reached by humans~~ during the domestication process ~~and it has allowed~~ to provide humans with a  
20 continuous and constant food supply [2]. However, plants do not have unlimited quantity of energy  
21 and the allocation of limited carbon sources is consequently influenced by a growth-defence trade-  
22 off [3]. This phenomenon is based on the concept that the limited carbon sources produced by  
23 photosynthesis are allocated toward growth or defence processes to maximize the plant's adaptation  
24 strategies and fitness costs in diverse environments [4]. If plants focus their energy mainly on  
25 growing, they automatically have less ability to deal with different kind of stresses such as pathogen  
26 infections or harsh environmental conditions. Furthermore, domesticated plants are much more  
27 ~~nurtured than their wild parents, through kept in safe in a cotton wool, constantly~~ fertilizationed,  
28 irrigationed, and other protective measures ~~ed by anthropic inputs~~, so they are characterized by less  
29 ability to interact with or adapt to the surrounding environment ~~compared to wild parents~~.

30 The ability of plants to interact with thousands of microorganisms that are surrounding and supporting  
31 them in dealing with both biotic and abiotic stresses, is one of the most important traits that should  
32 ~~be shelved~~ reinforced. Several ~~works~~ studies have already demonstrated that plant-associated  
33 microorganisms are essential to improve plants' wellness and sustainability of agricultural systems  
34 [5,6]. ~~The~~ modern agriculture is entering into a second green revolution and the exploitation of

1 beneficial soil microorganisms is ~~playing a significantly increasing role~~ with several microorganism-  
2 based products ~~currently~~ coming to ~~the~~ market [7]. However, there is ~~more~~ evidence that  
3 domestication processes have profoundly altered the interactions ~~among-between~~ plant hosts and  
4 associated microorganisms [8,9]. Recent studies have ~~compared~~ ~~found significant differences~~  
5 ~~between~~ the microbiome of commercial genotypes ~~and-with that of~~ their relative wild types,  
6 ~~emphasizing the significant differences among them~~. It has been shown that wild ancestors and  
7 primitive landraces of wheat (*Triticum aestivum*), breadfruit (*Artocarpus altilis*), and maize (*Zea*  
8 *mays*) can benefit more from mycorrhizal symbiosis [10,11] ~~with respect-compared~~ to selected  
9 cultivars, ~~suggesting that the modified microbiome due to domestication is not beneficial to the plant~~.  
10 - Studies on other plants species, including *arabidopsis* (*Arabidopsis thaliana*) [12], sugar beet (*Beta*  
11 *vulgaris*) [13], barley (*Hordeum vulgare*) [14], and lettuce (*Lactuca sativa*) [15], ~~also~~ suggested that  
12 human-centered breeding led ~~to~~ compositional changes in root-associated microbiomes. ~~However,~~  
13 ~~how~~ this negative trend ~~could-can~~ be mitigated, and the plant-microbiome equilibrium restored ~~are~~  
14 ~~still open questions~~. ~~Although the tight interactions of indigenous crops with the associated~~  
15 ~~microbiota reinforce the ability and flexibility of crops to deal with diverse environmental stresses, a~~  
16 ~~difficulty for exploiting their potential is correlated with the low ability of separating the targeted~~  
17 ~~functional microbes~~ [16] ~~[Chen et al.]~~. ~~We also still need to understand, and how human can address~~  
18 ~~breeding-to-shape~~ which plant traits ~~maybe~~ involved in the interactions with beneficial  
19 microorganisms ~~and how we can identify them and select for them in future breeding programs are~~  
20 ~~points that have to be still investigated~~ (see **Outstanding questions**).

## 23 **The neglected shortcomings of traditional and new plant breeding techniques**

### 24 ***Intersection of anthropocentric breeding and plant-microbiome interactions***

25 The incessant selection of genomic plant traits, and the considerable number of inputs needed to  
26 sustain the selected genotypes, negatively influence the interactions among plants and beneficial  
27 microorganisms [17,18]. Breeding processes reduced in fact the microbial biodiversity and  
28 functioning associated to plants in agricultural systems, hampering the essential interactions that  
29 make wild species more resilient to biotic and abiotic stresses (Fig. 1) [18]. This effect is most  
30 probably due to the fact that the selected crop cultivars might have lost some of the genetic traits  
31 needed to recruit host-specific microbiota as compared to their wild relatives. It was shown that long-  
32 term nitrogen fertilization resulted in the recruitment of less and less-functional rhizobacteria in  
33 leguminous species, providing so fewer benefits to the host [19]. Similarly, Kiers et al. [20]  
34 demonstrated that older soybean cultivars had a higher ability to reach their full symbiotic potential

1 in the presence of a rhizobia-strain cocktail, with different symbiotic effectiveness compared to newer  
2 soybean cultivars. Furthermore, Chaluvadi and Bennetzen [21] have demonstrated that there are  
3 species-specific differences in the belowground microbiome associated to wild and domesticated  
4 *Setaria*, highlighting how crop domestication plays an important role in selecting prokaryotes present  
5 in the rhizosphere. There is also evidence about the impact of plant breeding on the assembly of  
6 rhizosphere fungal communities that seem to be strongly influenced by host genotype [22]. Recent  
7 studies have shown that **root exudates** are essential for plants to assemble a functional microbiome  
8 and changes in plant genetics derived from breeding programs result in different root exudate  
9 composition undermining microbiome assembling and functioning [18,23], playing a role also in  
10 symbiotic relationships [24,25]. Martin-Robes stated that colonization by **arbuscular mycorrhizal**  
11 fungi is lower while infection rate by nematodes is higher in the roots of plants that grow in soils  
12 previously cultivated by domesticated plants ~~in~~ in comparison with wild progenitors [26]. Conversely,  
13 rhizosphere microbial communities induced systemic changes in tomato root exudates, suggesting  
14 the presence of a long-distance signaling [27]. Despite the improvement in understanding root  
15 exudate composition, a few information is available so far about exudate spatial distribution and  
16 regulation in root, considering that homogenized samples are generally used [28]-. Döll and  
17 colleagues dissected the root in the three fractions analyzed for tissue-specific metabolic profiles,  
18 correlating those profiles with protein abundances involved in biosynthetic pathways resolved  
19 spatially and showing that the presence of differentially abundant compounds in diverse root tissues  
20 and exudate of asparagus roots [28]. Volatile Organic Compounds (VOCs) have also a relevant role  
21 in the communications and interactions with rhizosphere-inhabiting microorganisms and they are  
22 signal molecules with a potential for application in agriculture [29]. VOCs emitted belowground can  
23 stimulate the migration of distant soil bacteria, attracting them towards the roots. Additionally,  
24 changes in the blend of- root VOCs, due to a stress situation such as a fungal soilborne pathogen  
25 infection, lead to the recruitment of specific beneficial bacteria from outside the rhizosphere that  
26 might have a role to help plants to cope with the stress condition [30]-. However, rhizosphere and  
27 root-associated microbiota, in addition to support plant growth, also offer a further level of genetic  
28 variability that was little considered by breeders until now [31]. Future breeding strategies should  
29 treat root features, including traits related to the microbial recruitment (Box 1), and strategies for root  
30 phenotyping should be implemented, also considering the interactions with rhizosphere microbiota.  
31 A deepen investigation of the relationships between roots and associated microbiota is particularly  
32 relevant to breeding highly efficient root systems and the consequent selection of climate-change  
33 resilient genotypes [32].

34

1 ***Critical points of breeding based on the development and selection of the most performing plant***  
2 ***genotypes***

3 Despite breeding programs aim to produce resilient cultivars to diseases and environmental stresses,  
4 many traits are regulated by several genes (*i.e.*, polygenic resistance) and, for this reason, hardly  
5 transmissible to the progeny in a single crossing [33]. In this scenario, the monogenic resistance,  
6 exploited by conventional breeding programs, is highly effective in moving single gene traits, but it  
7 is easily suppressed by the pathogen along time [34,35]. Regarding woody plants, breeding programs  
8 encounter several further limitations, such as **linkage drag**, long and laborious times needed for  
9 backcrossing and high heterozygosity degrees, hampering the development of resilient genotypes and  
10 raising the costs [36–38]. The obtained resilient/resistant genotypes are often associated to  
11 modifications that could be detrimental from the commercial point of view, such as an altered  
12 phenotype and/or biochemical profile (related to texture, taste, and/or organoleptic profiles) [39],  
13 often less accepted by consumers [40,41].

14 Application of biotechnological approaches in breeding processes has recently led to development of  
15 **New Plant Breeding Techniques** (NPBTs), able to modify specific target DNA sequences without  
16 altering other regions and, if applied with the DNA-free or marker-free approaches, without the need  
17 of long backcrossing stages [42–44]. These techniques ~~promise are very promising~~ to overcome the  
18 limits imposed by traditional breeding both in terms of time and costs. Although NPBTs-derived  
19 products are accepted in several countries, many restrictions remain, especially in Europe, [45,46]  
20 and ~~despite their interesting features~~, traits related to the ability in recruiting beneficial microbiota  
21 are not so far sufficiently considered. Roots in fact share their habitat with many microorganisms,  
22 such as bacteria and fungi, that can have a positive role on plant productivity and tolerance/resilience  
23 to environmental stresses [47–52]. The exploitation of these microorganisms to improve plant traits  
24 may overcome the limits associated to both conventional breeding and NPBTs, leading to a reduced  
25 impact on the marketable characteristics of the final products, preserving the original genotype and  
26 therefore without requiring specific safety assessments on the products (*e.g.*, those for genetically  
27 modified organisms), and leading to a less expensive and time consuming application if compared to  
28 breeding programs, especially for woody plants (Fig. 2).

29

30 ***Holistic vision of plant breeding***

31 ~~Plants cannot be considered as standalone entities and a more holistic vision is needed. Plants and all~~  
32 ~~associated microorganisms are now considered as a unique organism called “holobiont” [5], to which~~  
33 ~~the “hologenome” (*i.e.*, the entire set of genomes within the holobiont) is associated [49]. It thus~~  
34 ~~becomes necessary including microbiota, particularly soil microorganisms, as a target in plant~~

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~~breeding approaches to limit the loss of microbial genetic diversity, inherent in current agricultural systems [50]. Research is now focusing on the interactions among plants and their microbiomes as alternative or in combination to the selection of specific traits such as high yield or genetic resistances to pathogens [6,51–53].~~

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A comprehensive understanding of mechanisms governing the selection of microbial community by the plant will provide useful information to improve the future agriculture. The application of novel approaches, such as i) the selection and characterization of specific microorganisms to restore growth-defence trade-off balance in commercial genotype, ii) the exploitation of complementary omics-tools and iii) the **Synthetic microbial Communities** (SynComs) application, seems a good way to reach this goal.

#### *Microbiome mediated dynamism in the allocation of plant resources*

~~Allocation of resources to priority biological processes, restricting others, can give rise to trade-offs between different processes/responses, mainly in natural environments where plants generally cope with limiting resources [53].~~ Commercial genotypes are often characterized by an unbalanced allocation of energy resources, and microbiomes represent a promising tool to restore the growth-defence trade-off balance in these plants, achieving an agricultural system able to survive with limited external inputs. Analyzing plant microbiomes and trying to select and identify genomes of microorganisms able to improve plant growth or resilience to several stresses could be the right way forward [53–55]. ~~Recently, an involvement of arbuscular mycorrhizal fungi (AMF) in balancing growth-defence trade-off has been suggested in grapevine (*Vitis vinifera*), where rootstock genotypes and AMF inoculation have a relevant role in shaping the root-associated microbes and stimulating growth and defence pathways [55].~~ Cultivars selected for quality and/or quantity yield are usually more sensitive to biotic and abiotic stresses: using microorganisms able to place the plants in the so called “priming state” allows to restore the natural trade-off equilibrium, obtaining more resilient cultivars [55–59]. **Priming** (or acclimatization) is a complex phenomenon that consists in preconditioning the plant biotic and abiotic defence mechanisms, so that responses to stress result quicker, stronger, and more effective [60]. ~~The simultaneous employment of biological as well as chemical agents towards further improved plant performance is gaining interest among researchers with the aim to maximize the primed status[61].~~ Additionally, to enabling a state of priming, ~~many of several~~ rhizosphere-associated microorganisms can perform a direct antagonism towards several pathogens acting as plant allies [62,63]. On the other hand, genotype characterized by high defense performance and low yield can be made more productive through the exploitation of plant growth promoting (PGP) microorganisms instead of modifying their own microbiome with long breeding

1 programs. Furthermore, since most of resistant cultivars obtained by conventional breeding showed  
2 less crop quality and yield capacity, the possibility to exploit PGP microorganisms could partially  
3 restore the original features avoiding plant genetic manipulation [55,64]. ‘Resistant’ cultivars are  
4 characterized by specific resistances toward target pathogens, but they do not make provisions for the  
5 rest of biotic and abiotic stresses. ~~An enrichment of certain taxa in microbiome composition~~  
6 ~~microbiome composition~~, on the contrary, guarantee a broad-spectrum tolerance toward several  
7 pathogens contributing to plant phenotypic plasticity and adaptability to the changing environment  
8 [65,66]. It has been shown that specific microbiota members can modulate plant immunity processes  
9 through bidirectional microbiota–root–shoot mechanisms relevant for plant health [67,68]. A  
10 functional and rich microbiome can stimulate plant immunity and modulate the allocation of carbon  
11 plant resources, shifting them from growth to defence processes [69,70] (see **Outstanding**  
12 **questions**). Regarding grafted plants, the choice of the rootstock genotype plays an important role in  
13 shaping microbes inhabiting the rhizosphere and the allocation of carbon resources. Indeed,  
14 rootstocks differ from each other by specific growth-defence trade-off features and for the ability to  
15 recruit different microbial consortia [70]. Modulating ~~thus~~ the interactions between rootstocks and  
16 their own associated microbe, growth and defence features could be managed through the  
17 bidirectional root-to-shoot mechanism, making grafted plants more suitable to sustainable practices.

### 19 *SynComs to enhance holobiont functionality*

20 Unearthing the functional relationships between plants and their microbial partners is the next step  
21 for improving plant fitness and for adopting breeding programs focused on the holobiont [71]. Natural  
22 microbial communities and the interactions between them and their host plants are known to be very  
23 complex and variable, hardly depictable and not ~~well-fully~~ understood yet. This complexity derives  
24 from the large number of microbes inhabiting the environment coupled to the often unknown  
25 functions for most of them and from the uncharacterized interactions occurring among one another  
26 [72].

27 The SynCom assembly seems to be a promising approach to exclude confounding environmental  
28 effects and to reduce the complexity of natural systems [73,74]. The establishment of a SynCom is  
29 grounded on collected knowledge of the overall composition of the root-associated microbiome  
30 needed to formulate a “**core microbiota**” [73]. Carlström et al. [75] showed how community  
31 assembly is subject to priority effects, *i.e.*, the imprint of arrival order on community structure, and,  
32 additionally, they indicated that specific strains have the greatest potential to affect community  
33 structure as keystone species. Once the keystone strain collection and the molecular identification is  
34 achieved, these microorganisms ~~need-have~~ to be tested for their antagonistic activity towards ~~different~~

1 pathogens ~~and, for their~~ PGP traits, and for keeping out the possibility that they can have a reciprocal  
2 inhibition effect. Microorganisms showing traits of interest can be used to establish a SynCom being  
3 tested before in controlled environments and subsequently in a natural environment [76–78].  
4 Additionally, through the SynCom exploitation, it is possible highlighting the role of plant immune  
5 system in the assemblage of a protective microbiome [79]. Recent studies have demonstrated that  
6 plants can recruit beneficial bacteria upon pathogen infections, specifically disease resistance-  
7 inducing and growth-promoting ones [67,76].

8 Although the effectiveness of the SynCom application in agriculture has been proven to be often  
9 inconsistent due to low efficiency in establishment and survival of the selected taxa [80–83], the  
10 SynCom approach seems to be pivotal for capitalizing associated microorganisms, increasing the  
11 agroecosystem resilience, and finally driving new breeding programs. The main reasons of failures  
12 are related to the plant-associated microbes ability to exert their beneficial effects. These can be  
13 reduced for several reasons that are closely linked to the host plant genotype ~~and the growth~~  
14 environment, the microbial species compatibility with the growth environment, the spatial  
15 competition with other soil microorganisms and the persistence in soil. The ecological interactions  
16 occurring with the natural occurring microbial population is one of the most important aspects that  
17 must be considered when applying SynComs in real environments. The importance of ecologically  
18 based community assembly rules has been demonstrated by the survival of SynComs developed using  
19 Pseudomonas spp.: survival rate was directly and positively correlate to the diversity of the developed  
20 consortia [84]. Additionally, the community growth and development are affected by several factors  
21 including growth substrates and presence of other chemical compounds [85]. The still limited  
22 knowledge on the mechanisms underlying plant ability to control its associated microbial  
23 communities and how members of microbial consortia interact with one another also strongly limit  
24 their exploitation in agriculture [86–88]. Interestingly, AM responsiveness seems to be subjected to  
25 'genotype x environment (G x E) interaction' that is referred to the phenomenon for which diverse  
26 genotypes respond to different environments in different manners [89].

27 Regarding the impact of human-focused breeding on microbiome assembly and functionality,  
28 additional insights into microbiomes of wild plants and native habitats could contribute to reinstate  
29 or enrich current genotypes ~~for~~ of microorganisms with beneficial effects on plant growth,  
30 development, and health [82,90]. Thanks to a combined approach (*e.g.*, high-throughput plant  
31 phenotyping, identification of the core microbiota strictly linked with a specific genotype, inoculation  
32 of personalized SynCom and analysis of plant responses at molecular level), it is workable finding  
33 some microorganisms, strictly linked with a wild genotype, that can be used to ~~replae~~ restore traits  
34 lost during breeding programs.

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## Concluding remarks

Domestication, ~~process~~ focused exclusively on the selection and improvement of specific plant traits,  
is by its very nature a, ~~amplified by~~ anthropocentric breeding programs ~~and focused exclusively on~~  
~~the selection and improvement of specific plant traits,~~ has negatively affected the recruitment and/or  
the functionality of beneficial holobiont-associated microbes in agricultural systems. Plant-associated  
microorganisms are known to improve plant growth and wellness and ~~thus~~ the plant's ability to  
interact and cooperate with ~~them~~ microorganisms should be considered as a fundamental trait in  
modern breeding programs. ~~Starting~~ Applying from a holistic vision of plant breeding and ~~through~~  
including the exploitation of “holo-omics” techniques (Box 2), ~~it is possible to~~ will lead to a deeper ~~ly~~  
understanding of the hidden world of plant-microbiome interactions. This will open a path to ~~and~~ try  
~~and to~~ manage it as these interactions as a sustainable ~~weapon~~ tool to restore plant resilience against  
stressful factors.



## 1 **Box 1 – The importance of root traits**

2 Efficient root systems are essential to enhance crop productivity, indeed studies on plant genotypes  
3 better adapted to stresses are now focusing on root traits [91,92]. The use of sophisticated systems  
4 and sensors enabled researchers to follow the development of the root system and to evaluate the  
5 uptake of water and nutrients at the root level. Plants can adapt root apparatus to optimize the  
6 availability in water and nutrients, thus having an impact in plant resilience and productivity [93].  
7 However, they do not live alone in the soil- and root-associated microorganisms are known to play a  
8 fundamental role in plant adaptation to adverse environmental conditions.

9 One of the main questions regarding plant-microbe interactions concerns the identification of  
10 functional mechanisms that plants exploit to shape their microbiome. It is worth noting that, if from  
11 one side root traits are able to influence the composition of the root-associated microbes, on the other  
12 hand microbes are able to interact with the plant modifying root traits [94]. Among root features,  
13 architectural traits (root depth/angle, length, density) and morphological traits (root hairs, root  
14 diameter, aerenchyma/cell size, root cap properties) are the physical properties playing important role  
15 in the two-way interaction by specifically altering the plant-microbe interaction interface [90,95,96].  
16 In parallel, the unique biochemical fingerprint profile of a plant deeply contributes to the recruitment,  
17 colonization ability and function outline of specific microbiomes [97]. The root exudates, and the cell  
18 wall composition, vary considerably according to the age, developmental stage and species ~~(but~~ also  
19 between different genotypes and varieties of the same species) and constitute a primary feed source  
20 for the rhizosphere-associated microorganisms, making the soil surrounding roots more suitable for  
21 bacteria and fungi proliferation [98]. The influence of root architecture and exudates on rhizosphere  
22 and root environments, and consequently on the microbial recruitment, nowadays represent a hotspot  
23 in the researches aimed to study root interactions and is attracting always more attention [99–101].  
24 ~~There is a significant potential to boost a~~ Agricultural sustainability can be potentially boosted by  
25 implementing breeding program able to consider the above-mentioned root properties, enhancing the  
26 possibility to recruit microbiomes with beneficial traits. ~~In addition,~~ Linking root phenome to  
27 specific microbiomes (or related functional traits) may enhance our understanding of the interactions  
28 occurring in the rhizosphere and the role that these interactions play in generating climate-resilient  
29 agroecosystems [102]. Important aftermaths of such approaches will impact on significant soil and  
30 root-related functional aspects such as N fixation and cycling, P dynamics, C dynamics and plant  
31 water availability. Recently, Brisson et al. ~~+~~ suggested that root exudation in response to phosphate  
32 stress was conserved during the maize domestication (teosinte vs maize), shifting the exudation levels  
33 of specific metabolites and the microbial communities, although selective recruitment of phosphate  
34 solubilizers in response to phosphate availability has not been observed[101].

1 **Box 2 - Holo-omics studies: detailed information on host-microbiota interactions**

2 To develop protocols for a new kind of breeding linked to the holobiont concept, a more detailed  
3 knowledge of the mechanisms governing plant-microbiome interactions is needed [103]. Holo-omics,  
4 i.e., the incorporation of data across multiple omic levels from both host and microbiota domains,  
5 ~~studies~~ may have the potentiality to resolve the functionality of a plant microbiome ecosystem by  
6 generating an image of what is being expressed, translated, and produced during plant microbiome  
7 interactions [103,104]. The combination between host and microbial datasets provide in fact a  
8 powerful approach for the development of hypotheses and advancement in the topic of plant  
9 interactions [103]. Pairing host-centered omics-tools, such as transcriptomics, epigenomics and  
10 proteomics, in combination with the more commonly used microbial-focused techniques such as  
11 amplicon sequencing, shotgun metagenomic, metatranscriptomics, and exometabolomics, seems to  
12 be a very promising approach to achieve a more integrated knowledge on plant microbiome functions  
13 [103–105]. ~~Regarding this holistic approach, Nyholm et al. [93] coined the term “holo omics” to~~  
14 ~~describe experiments that integrate data across multiple omics levels from both host and microbiota~~  
15 ~~domains.~~ A great number of works grounded on this current approach are nowadays present in  
16 literature [106,107]. Recently, Castrillo et al. [108] have explored the relationship between Phosphate  
17 Starvation Response (PSR) and microbiome composition and functionality in *Arabidopsis* [108].  
18 Through a holo-omics design (16S profiling and RNA-seq), they have demonstrated that the plant  
19 root microbiome directly connects phosphate stress response and plant immune system, and that, gene  
20 controlling PSR contributes to the root microbiome assembly. Microbial communities of PSR mutant  
21 plants were distinct from those of wild type, and inoculation of a specific SynCom enhanced the  
22 activity of a master regulator of PSR (PHR1) under limited phosphate conditions, confirming that  
23 PHR1 directly regulates a functionally relevant set of plant-microbe recognition genes. Moreover,  
24 ~~using metagenome and metabolome approaches~~ Stringlis et al. [109] demonstrated, using  
25 metagenome and metabolome approaches, that beneficial rhizobacteria induced excretion of the  
26 metabolite scopoletin that stimulates iron uptake and suppresses soil-borne pathogens in  
27 a *Arabidopsis*.

28 A combined use of omics approaches led to obtain information on the so-called “soil memory”.  
29 Particularly, Li et al. [110] ~~[103]~~ applied metagenomics to characterize the peanut (*Arachis hypogaea*)  
30 rhizosphere microbiome and root metatranscriptomics ~~of the roots of on~~ peanut plants grown in a soil  
31 with different management histories, such as monocropping and crop rotation. ~~They~~ These authors  
32 found that the past planting record had a significant impact on the assembly of the microbial  
33 community in the peanut rhizosphere, indicating a soil memory effect affecting crop rhizosphere  
34 microbiomes and plant physiology. Thanks to multi-omics and bioinformatics technologies it is now

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- 1 possible to identify core interactions between plants and native microbiomes pointing out useful traits
- 2 for breeding purposes.

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5 **Declaration of interests**

6 The authors have no interests to declare.

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1 **Glossary**

2 **Arbuscular mycorrhizal symbiosis:** Beneficial associations between the roots of most terrestrial  
3 plants, including several relevant crops, and the arbuscular mycorrhizal fungi, which are obligate  
4 symbiotic fungi belonging to the Glomeromycotina sub-phylum.

5 **Core microbiota/microbiome:** The set of microorganisms/microbial genes that is systematically  
6 associated with a given host plant and forms webs of interactions that can be exploited to optimize  
7 microbial functions at individual plant and ecosystem levels.

8 **Holobiont:** Assemblage of a host and the many other species living in or around it, which together  
9 form a discrete ecological unit.

10 **Hologenome:** The union of all the genes in an holobiont.

11 **Linkage drag:** It refers to the usually undesirable effect of moving unwanted genes in the progeny  
12 that are linked to the gene(s) or QTL of interest.

13 **New Plant Breeding Techniques:** Methods allowing the development of new plant varieties with  
14 desired traits by modifying specific DNA sequences.

15 **Plant domestication:** Co-evolutionary process in which human select wild plants based on their  
16 adaptation to cultivation and human-derived purposes.

17 **Priming:** The state that can be reached following treatments with various inducers such as chemical  
18 compounds or beneficial microbes. The primed-plants are able to display faster and stronger defense  
19 responses.

20 **Root exudates:** Range of compounds released by the roots into the surrounding soil with key role in  
21 supporting plant development and interactions with rhizosphere microbes, facilitating root  
22 associations with beneficial microbes and suppressing pathogens.

23 **Synthetic microbial communities (SynComs):** Comprehensible system of reduced complexity.  
24 Wild microbial communities are composed of mixed microbes with several unknown function,  
25 SynComs allow the generation of defined system with known taxonomic as well as functional  
26 profiles.

27 **Traditional Breeding:** Genetic enhancement of adaptation traits, disease and pest resistance, abiotic  
28 stress tolerance, nutritional and water use, to improve crop yield and product quality.

29

1 **Figure legends**

2 **Figure 1. Comparison between wild and domesticated plant species.** On the left, wild relative  
3 displays a better adaptation to environmental stresses (both biotic and abiotic), a balanced growth-  
4 defence trade-off, a rich associated-microbiome, but a low yield. On the right side, domesticated plant  
5 shows an improved productivity, but a reduction of both stress resilience and ability to recruit plant-  
6 associated microbes. To cope with the increased susceptibility, human practices such as irrigation,  
7 fertilization and pesticide application are needed.

8 **Figure 2. Overview of the classical breeding, new plant breeding techniques and the microbial**  
9 **breeding approaches [for woody plants](#).** On the left, classical breeding relies on the possibility to  
10 transfer traits (*e.g.*, related to biotic or abiotic stress resilience) from wild sexually compatible species  
11 to domesticated species of high economically importance through crossing. The main limitation in  
12 such approach, especially for woody species, is the presence of juvenile stages, which increase the  
13 time for back-crossing, and the transmission of undesired traits (linkage drag). In the middle, new  
14 plant breeding techniques allow to reduce the time needed by conventional breeding, but they still  
15 present limitations such as the need of specific tissues and/or cellular types (*e.g.*, embryogenic calli)  
16 and several limitations related to GMO regulations. On the right, microbial breeding approach [that](#)  
17 can overcome the limitation of ~~both—the previously techniques~~[classical breeding techniques](#),  
18 preserving the original genotype and reducing development times and costs of a synthetic community  
19 (SynCom).

## **Breeding towards improved ecological plant-microbiome interactions**

Luca Nerva<sup>1,3†</sup>, Marco Sandrini<sup>1,2†</sup>, Loredana Moffa<sup>1,2†</sup>, Velasco Riccardo<sup>1</sup>, Raffaella Balestrini<sup>3†\*</sup> & Walter Chitarra<sup>1,3†</sup>

<sup>1</sup> Research Centre for Viticulture and Enology, Council for Agricultural Research and Economics (CREA-VE), Via XXVIII Aprile 26, 31015 Conegliano (TV), Italy.

<sup>2</sup> University of Udine, Department of Agricultural, Food, Environmental and Animal Sciences, Via delle Scienze 206, 33100, Udine (UD), Italy

<sup>3</sup> National Research Council of Italy - Institute for Sustainable Plant Protection (IPSP-CNR), Strada delle Cacce, 73, 10135 Torino (TO), Italy

†These authors contributed equally to this work as first authors

‡These authors contributed equally to this work as senior authors

### **\*Corresponding author details**

Raffaella Maria Balestrini – [raffaella.balestrini@ipsp.cnr.it](mailto:raffaella.balestrini@ipsp.cnr.it)

Institute for Sustainable Plant Protection (IPSP-CNR)

Strada delle Cacce, 73; 10135 Torino (TO), Italy.

### **Outstanding questions**

How does the loss of plant traits during domestication negatively influence the ability of current genotype to dealing with the surrounding environment and soil beneficial microorganisms?

What is the impact of diverse environmental conditions, including the climate changes, on the outcomes of the interactions between host plant and rhizosphere microbiota?

How does root microbial communities influence root exudation, root architecture and root morphology, impacting the belowground interactions?

How do diverse plant genotypes recruit a diverse microbial community?

How do mutualistic microorganisms modify the root traits (physical and chemical properties) to promote and maintain symbiosis?

### Editorial comments

When revising the manuscript

1. Please clarify/consider the points highlighted by the referees and detail your response/the changes you introduced in a letter accompanying your revision.

A: We have introduced all the requested changes and we have prepared a detailed rebuttal letter.

2. From an editorial perspective, I thought that your article is overall very well written and I very much enjoyed reading it. Therefore, I have nothing to add to the reviewer comments at this point, but please note that your manuscript will be copy-edited after acceptance.

A: Thank you for your positive comments!

3. Please note that it is your responsibility to obtain permission to reproduce copyrighted material (i.e. figures, tables or excerpts that have been published online or in print) from the publishers of the original material. This is also relevant to figures that have been altered in any way (however, if additional information is added to figures it is sufficient to cite the original material in the figure legend). Note that while permission is usually always given, almost all publishers still require a formal request be made to reproduce material from their publications. Depending on the publisher, permission can be obtained in various ways as explained in our author guidelines. You should retain the completed form on its return from the copyright holder. Please note that it is courteous to inform the author of the original material of your intent to use their published work.

A: No permissions are needed.

4. The Publisher now requires authors to declare any conflicts of interest that relate to papers accepted for publication in this Journal. However, there is no need to add any statement, if you have no conflict of interest.

A: No conflict of interest.

### Referee Comments

Reviewer #1: The concepts of the plant holobiome and synthetic communities to investigate plant microbiome and to support plant production are established, and several reviews on these subjects have been recently published. Same applies on the idea of plant breeding towards sustainability by using plant microbiome, which is the topic of this manuscript. Luca et al. cover several themes in their review, and many sections are of general knowledge from other reviews, not focused on science reports but merely list ideas from reviews or opinion papers. Perhaps one means of restructuring the paper would be to focus on the breeding process with the microbiome, how would it take place? And relating it to the use of SynComs. In this form the two topics are separated. The language needs also strong editing of a native speaker. In the following I have tried to give some comments how to improve the manuscript.

A: Thank you for the comments and suggestions. In the revised version we have tried to improve the manuscript following them as well as the comments by the other reviewers.

P1. rewrite lines 23-25

A: Done



P2 lines 5-7: There are some ideas already in the literature. The authors find some from this paper: Nature Food. 2021 Apr;2(4):233-40.

A: Thank you we have added the reference.

P3 Add plant secondary metabolites in the tissues, use Frontiers in Plant Science, 12, 300. Add root volatiles. During biotic stress plants can promote the acquisition of certain beneficial bacteria to their rhizosphere. Active emission of volatile organic compounds (VOCs) is an identified mechanism utilized by plants for this process.

A: Thank you for the suggestion. About the comment on secondary metabolites in the tissues we have added a new citation in the revised version. We have also added a part on root VOCs.

P5 5-12 remove this general section.

A: Done

P5 21-25 rewrite and elaborate by using the cited references.

A: We have slightly changed this part also adding a new reference. Since this is an opinion article we have not space to add details on all the several treated points, but we tried to encounter the reviewer's comments and suggestions.

P5 26-28 as well as P6 1-3: use original contributions that show this.

P5 5-7 The references show that an enrichment of certain taxa induce the phenotype, not a rich microbiome.

A: Thank you for the comments. We have changed accordingly.

P6 9-11 rewrite. The references do not show what was stated. The rest of the paragraph is speculative, as no functional importance is given for the different microbiomes of the rootstocks in the cited paper.

A: We have revised this part adding a sentence on the major outputs.

P7 6-10. the basic information of SynComs has been reviewed well lately. Problems in field scale should be elaborated. The use of SynComs for agricultural production is limited mostly due to problems in their resistance to field conditions. the authors should add the problems in carrier material selection, biofertilizer formulation, promise of selecting thermo-tolerant/drought-tolerant/moisture-tolerant and rhizosphere compatible/ plant colonising members of the SynCom, and methods of application (seeds, application on seedlings, systemically invading strains on leaves, adjusting fertilization to support the function of the SynCom etc.

A: Thank you for your comments and precious suggestions. We have revised the manuscript adding some sentences and novel references related to these aspects, but we have not added specific examples in the text due to the fact that opinion papers have a limited space. However, specific references have been added and they could be useful for the reader.

Commented [RB1]: Lascerei a voi sono pagina 6 e 7 nel PDF

Conclusions are too general. They should realistically state what has to be worked on more and what problems should be solved to obtain SynCom based products..

A: Conclusions have been revised.

BOX1 22-24 cite papers where this has been proven, not reviews.

A: We have added some more recent citations.

BOX2 introduce the term holo-omics in more detail and refer rather to the more general treatise Microbiome 9, 69 (2021)

A: DONE, the suggested reference has been added.

Reviewer #2: This is a timely and very important opinion paper on a topic of broad interest within plant biology, plant breeding and microbiology. In general, the manuscript is well written and very well organized. The authors nicely discuss the short comes of traditional and new plant breeding techniques and the potential of synthetic microbial communities to develop sustainable breeding strategies. The manuscript is worthwhile for publication in Trends in Plant Science.

A: Thank you for the positive comments.

I have just a few minor comments.

1. The authors should include in the Glossary the words "priming" and "core microbiota"

A: Done

2. Page 3, line 33: Please delete in

A: DONE

3. Page 6, line 30: It is unclear to me what the authors mean with "community assembly is subject to priority effects". Please explain this idea briefly.

A: DONE

4. Page 7, lines 6-7: It is stated that the effectiveness of the application of SynComs in agriculture has been shown to be inconsistent. Please provide a few examples and critically discuss them.

**Commented [RB2]:** Io ho aggiunto qualcosa, ma forse voi avete la bibliografia più recente alla mano

5. Page 7, line 30: "menage" should read as "manage".

**Commented [RB3]:** Non sono riuscita a trovarlo

Reviewer #3: The opinion article by Luca and colleagues provides a nice discussion on the timely topic of novel breeding approaches towards improved plant-microbiome interactions. Such an improvement would be greatly beneficial in order to achieve important traits such as abiotic and biotic stress tolerance/resistance. The piece is written nicely and is easy to follow, while keeping up with up-to-date relevant literature. It will also appeal to a large audience. I am generally highly receptive of this article being published in TIPS, provided that some (relatively minor) points are addressed (appearing in random order):

A: Thank you for the positive comments.

- I am not entirely sure what the basis is for focusing on 'ecological' interactions, as the breeding approaches and use of syncomms etc is targeted for improvement of traits important to agricultural production. Would the authors care to comment on why such a focus is placed in the title?

- The manuscript, although well-written in general, does require careful language editing, as several grammar and syntax errors can be found throughout (e.g. see second highlight, p. 2 l. 6 ('have not been adequately...'), p. 4, l. 11 (despite breeding programs aiming to...) etc.

A: Thank you. Done.

- The reference cited in support of the concept of priming (p. 5, l. 30) does not seem to be the most appropriate one, considering that it is relevant to such a major concept. Perhaps other, groundbreaking reports in the field would be more appropriate (such as Conrath's and Mauch-Mani's works).

A: Thank you, done. We have added a reference by Martinez-Medina et al. Trends in Plant Science.

- Authors discuss the use of microorganisms towards improved growth under diverse conditions, also under the (biological) priming concept. A new direction of research has been the simultaneous employment of biological as well as chemical agents, with the latter constituting a well-established approach, towards further improved plant performance. Perhaps the authors could very briefly discuss/introduce this direction as well.

A: thank you for the suggestion. We have added a sentence on this.

- As a last note, which might sound slightly unorthodox, I noticed that the two models/figures presented depict grapevines, which is quite interesting. I am not suggesting that grapevines are not important crops (far from it), but considering that the current opinion article discusses new plant breeding techniques, wouldn't it be more appropriate if the authors had used other, more widely used crops in such approaches such as tomato or even cereals? Just a thought.

A: Done

Reviewer #4: The article by Nerva and colleagues presents an opinion on a research topic of great interest to the plant science community - the reciprocal interactions between plants and their beneficial associated microorganisms - pointing out critical steps to be undertaken for the successful exploitation of such knowledge in breeding strategies to increase plant resilience to stresses as well as agricultural sustainability.

Overall, I find that the paper is accurately written and accessible to a wide readership. A glossary box and (two) figures are included to explain the most difficult terms and concepts, respectively, for facilitating the readers.

I agree with the hints herein provided by authors on the necessity to both consider plants and all associated microorganisms as a unique "holobiont" and to amplify our understanding on the mechanisms governing plant-microbiome interactions.

I'd like to provide hereafter some minor comments:

- in the second highlight "plays" should be replaced with "play";
- pag. 3 - line 33: "in in" should be replaced with "in";
- pag. 7 - line 19: I'd use the term "restore" instead of "replace";
- pag. 7 - line 30: I'd use the term "tool" instead of "weapon";
- pag. 9 - line 31: "point out" should be replaced with "pointing out";
- The last sentence in the figure 2 legend should be rephrased to facilitate the comprehension.

**Commented [RB4]:** Cambiare la figura

A: Thank you for the positive comments. We have revised the manuscript accordingly the reviewers' suggestions.

Dear Editor,

please consider the revision of the attached manuscript PLANTS-D-22-00057, " Breeding towards improved ecological plant-microbiome interactions " by Nerva et al. We have carefully revised the manuscript and we have addressed all the comments and suggestions raised by the Referees. All the changes made are highlighted in the text (we have prepared and uploaded a file with the clean version followed by the version with tack-changes) and detailed in the point-by-point reply included in the rebuttal letter. We hope that our manuscript can now be fully considered for publication in Trends in Plant Science.

Grateful for your kind attention and for the time to handle this manuscript

Best wishes,

Raffaella Balestrini

*Corresponding author details*

Raffaella Maria Balestrini

[raffaella.balestrini@ipsp.cnr.it](mailto:raffaella.balestrini@ipsp.cnr.it)

Institute for Sustainable Plant Protection (IPSP-CNR)

Strada delle Cacce, 73; 10135 Torino (TO), Italy.

Telephone 0039-347-7515291



