example, IAA, low Pi in the soil, and *RSL4* expression are all interlinked (Figure 1).

Recently, two novel RSL4 properties were uncovered. First, RSL4 is able to self-activate, enhancing its own expression [5]. Second, the rate of RSL4 synthesis determines the final size of the root hair cell and, because it contains a D-BOX motif, its protein stability is regulated by 26S proteasome degradation [10]. Accordingly, a mutated form of RSL4 that is stable and resistant to proteolysis develops abnormally long root hairs [10]. More importantly, RSL4 controls the expression of 124 genes (84 genes determined in [9], 29 in [11], and 11 in [12]) containing a root hair-specific *cis*-element (RHE) in their regulatory regions [5]. These RSL4-activated genes function in ROS homeostasis, cell wall synthesis and remodeling, metabolism, and signaling, and represent the smallest subset of genes necessary to trigger root hair growth [5]. Together, these properties make RSL4 a key master regulator of final cell size that integrates environmental, hormonal, and developmental cues (Figure 1). Other TFs and transcriptional components not described here (e.g., LRL1-LRL3, mediator 25/PFT1, etc.) act in an RSL4-independent manner to regulate the expression of root hair genes to trigger its growth.

Concluding Remarks

In summary, recent findings highlighted here represent a significant step toward understanding RSL4-mediated regulation of cell size. Nevertheless, it remains to be determined how *RSL4* expression is controlled or balanced under conflicting growth signals or when plants are exposed to additive or synergistic cues. Thus, detailed studies of the effect of each individual factor and combined signals on *RSL4* expression are needed to unravel how the cell sizing process is fine-tuned. The identification and dissection of all components involved in this regulatory network (Figure 1) remain tasks for future research, and will require a concerted effort by the plant research community.

Author Contributions

J.M.E conceived the project and, with E.M., C.B., S.P.D.J., and S.M., wrote the article. E.M., C.B., and S.P.D.J. also provided technical assistance.

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and boron (Br), and catechol] also stimulate root hair cell growth. Since RSL4 contains a D-BOX motif in its protein sequence, it is under 26S proteasomal degradation, which regulates its lifetime. RSL4 integrates internal and external cues by triggering the expression of a core of RHE genes (~124 putative direct targets) to control root hair cell size. In addition, other TFs (e.g., LRL1-LRL3) also regulate root hair gene expression in an RSL4-independent manner. Abbreviations: Aux-RE, auxin responsive element; CYCP, P-type cyclins; D, D-BOX motif (RXXLXXXN); EXP, expansins; GTs, glycosyltransferases; LRL, *Lotus japonicus* Roothairless Like; LRX, leucine-rich extensin; NAGS, amino-acid acetyltransferase; PER, type-III peroxidases; PRP, proline-rich proteins; RBOH, respiratory burst oxidase homolog proteins; RHE, root hair-specific element; RIC, Rop-interactive Crib motif-containing protein; RLKs, receptor-like kinases; RNS, rinonuclease; TET, tetraspanin.

Spotlight

Plant Breeding Goes Microbial

Zhong Wei[1] and
Alexandre Jousset[1,2,*]

Plant breeding has traditionally improved traits encoded in the plant genome. Here we propose an alternative framework reaching novel phenotypes by modifying together genomic information and plant-associated microbiota. This concept is made possible by a novel technology that enables the transmission of endophytic microbiota to the next plant generation.

Plant Breeding for Sustainable Food Production

Feeding a growing human population without jeopardizing resources is one of the major challenges of future decades. Breeding better plants is an essential part of the solution. Plants can be bred for a range of characteristics needed for low-input, high-yield conditions. They may be selected for growth, nutritional quality, pathogen immunity, or stress tolerance. However, genetic improvement is a complicated process made even harder by the prevailing regulations. Help may come from the microscopic world. Traditionally, plants have been bred by altering their genomic information with little consideration of their interaction with surrounding organisms. Recently, a paradigm shift has taken place by considering plants as a holobiont, an ecological and evolutionary unit encompassing both the host and its associated microbiome [1]. Plants are associated with billions of bacteria and fungi that colonize inside as well as outside surfaces, such as roots, vascular vessels, and leaves [2]. Plant-associated microbiota play a fundamental role in the regulation of plant physiology and affect a range of traits involved in plant yield. Some microbial species alter the hormonal balance by producing growth hormones such as auxin or gibberellin or by reducing levels of the stress-response hormone ethylene. Other microbiota stimulate plant immunity, shifting resource allocation from growth to defense pressure [2]. Together, plant—microbe interactions can generate a range of new phenotypes without altering plant genomic information.

Given the interactive and far-reaching effects of hormonal levels on plant phenotype, microbial-induced shifts have opened a new gamut of easily reachable combinations for plant traits. In addition, some microbes provide services to the plant that can be used to replace traits lost during breeding. By making soil nitrogen, phosphorus, or iron available to the plant, root-associated microbes may and enhancing plant growth and nutrition in low input systems. By producing antibiotics, some other microbes may protect the plant from diseases against which the plant shows no or limited immunity [2].

The past years have witnessed a growing interest in considering microbiota during the breeding of new plants varieties [3,4]. For instance, selecting plants efficiently recruiting taxa suppressing pathogens [5] may alleviate the need to build disease resistance into the plant genome itself. However, the complexity of the microbiome and the still limited knowledge on the mechanisms allowing a plant to control its associated microbial communities [6,7] makes it challenging to predict microbiome transmission and function in the next generation.

We therefore propose a complementary approach: a holobiont-level breeding strategy in which microbes are one of the direct targets of the selection process that help achieve a desired plant phenotype (Figure 1).

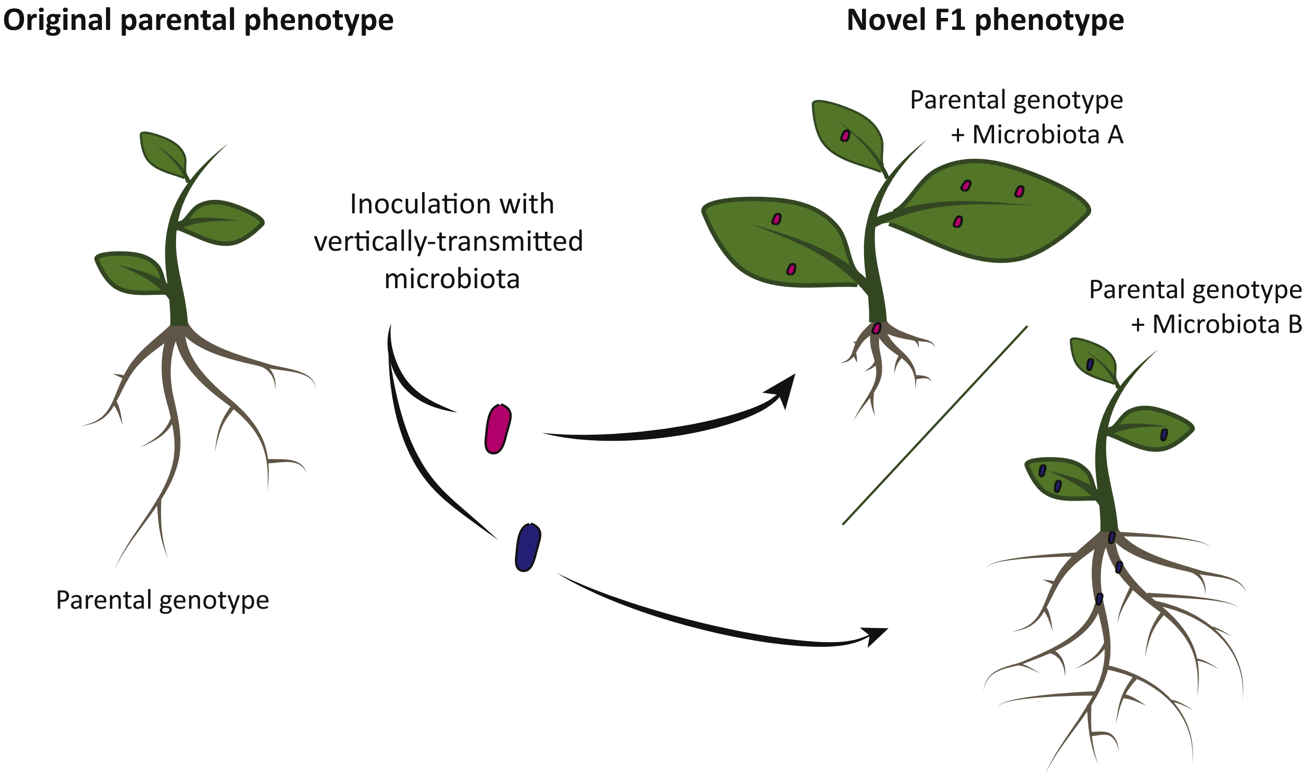

Figure 1. Conceptual Use of Vertically-transmitted Microbiota as a Basis for a Holobiont-level Breeding. The parental plant genotype can be inoculated with microbes that can modify various life history traits of the plant. Thanks to new developments enabling reliable microbial transmission to the next plant generation, the desired plant phenotype can be obtained by a combination of host- and microbial encoded traits that form together an inheritable unit.

Breeding the Holobiont: Challenges and Opportunities

Plant-associated microbiota offer a range of properties enabling them to alter plant traits to obtain a phenotype similar to the one resulting from classical plant breeding. The idea to use microbes for optimizing plant phenotype is not new. To date, however, plant enhancement by microbes has been constrained by the lack of an integrative conceptual framework and by technological limitations.

From a conceptual perspective, desired impacts of microbiota on plants have long been lumped into an appealing but relatively undefined concept of ‘plant growth promotion’. Plant-associated microbiota can however influence multiple plant regulatory cascades which will together define plant phenotype. Moreover, the effect of the altered phenotype on plant growth will be context-dependent as a function of abiotic and biotic environmental parameters.

A further complication emerges from the complexity of microbial communities associated with plants. Microbe-microbe interactions have a strong influence on plant—microbe interactions [7], complicating their predictability. Finally, the effect of externally applied microbes is often unreliable due to the low survival of the introduced microbiota and is not inherited by the next generation [4,8], hampering their use as part of a breeding strategy. Yet recent conceptual and technological enhancements have made the development of a holobiont breeding closer to reality.

First, the past few years have witnessed a better integration of plant physiology, regulatory cascades, and host-microbe interactions [1,3,9]. Modern plant—microbes interaction studies will typically evaluate the effects of microbes on specific regulatory cascades [10] while at the same time remaining aware of tradeoffs between plant traits [11]. This novel approach to ‘beneficial’ microbes enables the selective use of microbes to manipulate plant characteristics and thereby achieve what a breeding program ought to do.

In order to breed the plant holobiont efficiently, we need to be able to generate an inheritable assemblage of plant and microbiota. Thus far, the large-scale application of microbiota has been a limiting factor. This constraint may be solved by a recent technological breakthrough. By inoculating flowers with specific microbiota, a team of scientists centered around Birgit Mitter was able to transfer them reliably into seed material, passing them to the next plant generation [10]. The microbes could survive both as endophytes within the plants and from there grow in the rhizosphere. Mitter and colleagues demonstrate the usefulness of this approach by transmitting vertically beneficial microbes that speed up flowering. The timing of flowering is a major component of yield and a typical target for plant breeding [11]. By introducing specific endophytic microbes through the flowers, the timing of flowering in wheat could be optimized without manipulating the plant genotype. The effect of microbes on plant phenotype can thus be seen—at least for the F1 generation—as a holobiont-level breeding altering the host traits by the inclusion of specific microbiota. This breakthrough may open completely new venues for plant breeding. Instead of only modifying the plant genome, future plant enhancement strategies could rely on a joint modification of the plant genome in combination with microbes that will further modify plant phenotype (Figure 1). Together, the genotype x microbiota integrative approach may offer a flexible and powerful tool to create custom plants rapidly that suit the needs of individual farmers. For instance, the same reference plant genotype could be combined with different microbiota to generate easily customized phenotypes. As an example, microbes enhancing nutrient acquisition may facilitate growth in poor soils, while those that accelerate flowering [10] may help utilize the short vegetation period in cold areas.

Perspectives

Despite all the recent improvements, further studies are still needed to implement holobiont-level breeding fully. A first limitation stems from the limited vertical transmission of the microbes. Even when bacteria could be reliably transmitted to the F1, they disappeared in further plant generations [10]. However, this is only a marginal disadvantage when seeds are produced by dedicated companies. Plants grown for seed production could be inoculated on large scale. With a F1 generation being grown for human or animal consumption, the lack of microbial transmission to its seeds would not be relevant.

On a conceptual level, we call for a more integrative view on the effects on plant traits by the microbiota introduced. Plant response is very variable and depends on both the plant genotype and the environmental conditions [6]. Applications of beneficial microbiota thus remain a tedious process of trial and error. Adopting a trait-based approach matching alterations of plant regulatory cascades to specific microbial genes would provide a more nuanced framework that may serve to better predict the effects of microbiota on plant phenotype, ultimately unlocking the ability of plants to prosper under the desired conditions. Finally, to ensure a stable plant transfection and a controlled expression of the desired microbial traits, we need to gain more insight on the ecology and evolution of endophytic microbes, including the mechanisms determining the survival within the plant tissues and the transmission to the next generation.

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