


Bacterial gas-lighting of lateral root formation

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The root system is a major determinant of plant performance. It forms an expansive three-dimensional structure that forages the soil for water and nutrients. The combination of root growth and the formation of branches, called lateral roots, shapes the root system architecture. Although these processes are regulated via well-defined genetic programs, both are responsive to external factors, such as humidity, nutrient availability, and soil compaction (1, 2). This encoded plasticity allows plants to adapt to local soil conditions. Besides the abiotic factors, roots are also continuously exposed to different types of opportunistic and commensal soil-dwelling microorganisms that can be either neutral, detrimental, or beneficial for the plant, dependent on the particular condition. Thus, roots need to constantly process a plethora of signals, not only to discern friend from foe but also to respond correctly to a given fluctuation in its environment. One clear example where selectivity is extremely important, is the symbiosis between highly specific rhizobacteria or fungi that results in the formation of new plant structures to host the symbiont, i.e., root nodules or mycorrhizal arbuscles respectively (3, 4). However, among "the dark matter" of microorganisms in the soil, many have been reported to induce changes in root system architecture (5, 6). Despite this, most studies have been descriptive, and understanding of the molecular mechanism(s) exploited by the microorganisms to evoke changes in root system architecture is still lacking.

In this issue of PNAS, Gonin et al. (7) address this question by means of a large collection of bacteria that recapitulate the phylogenetic diversity present in the root environment of healthy, *Arabidopsis* plants. They confirm that lateral root

modification is a common feature among bacteria, as most of their bacterial strains tested altered the root system architecture. Although changes in lateral root density are often correlated with changes in primary root growth, the detailed analysis performed by Gonin et al. (7) reveals that variations in root length does not fully explain the observed changes in lateral root density. This is remarkable as it suggests that some bacteria can manipulate lateral root formation independent of primary root development. Thus, this process is likely to be an important bacterial feature, which has so far been overlooked.

In *Arabidopsis*, lateral roots are formed from so-called founder cells, which are a set of periodically patterned, specialized cells in the pericycle adjacent to the xylem (8). The developmental process from founder cell to emerged lateral root is controlled by the phytohormone auxin (9). Since some bacterial genomes contain auxin biosynthetic or auxin degrading operons, Gonin et al. tested whether the effect on lateral root density was correlated with the presence of such

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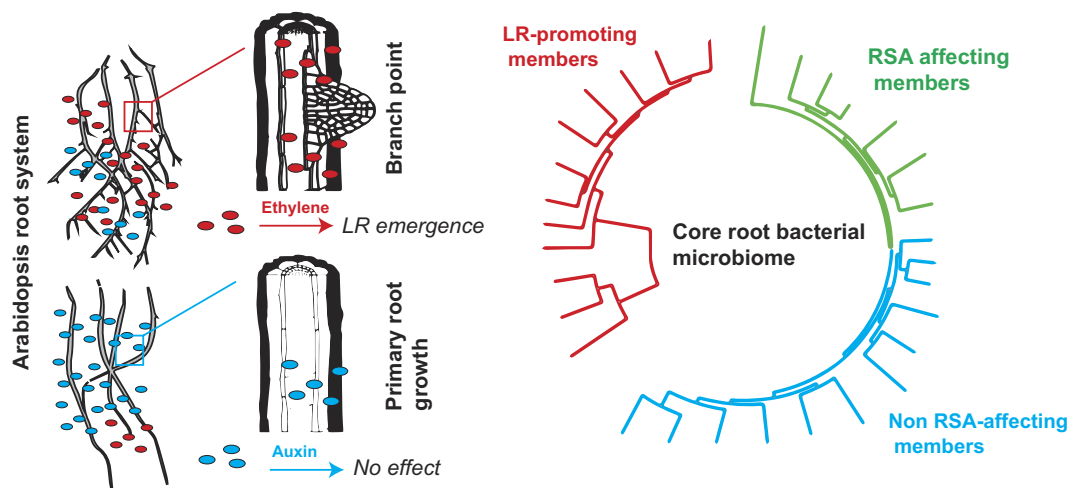


Fig. 1. Defining a subset of the core *Arabidopsis* bacterial microbiome that affects LR emergence. Root systems consists of branch points (lateral roots, LR), which increases the coverage of the system and provide an overall architecture (Root system architecture, RSA). The work by Gonin et al. provides novel insights into how bacterial members of the core root microbiome can affect this. Their work reveal that certain members of the bacterial community can, via the phytohormone ethylene, promote branching independently of primary root growth—a feature that, from a plant developmental point of view, is dependent on another hormone, namely auxin. This finding is very exciting and will lead to new agriculturally relevant models of how to optimize root systems under diverse stress conditions.

operons. Interestingly, this was not the case, and it led the authors to look for other aspects of root development besides auxin homeostasis to affect root system architecture. To get further insights into this Gonin et al. (7) cleverly tested whether bacteria that affected lateral roots in *Arabidopsis* were able to induce root branching in the basal land plant *Selaginella moellendorffii* (*Selaginella*), in which lateral roots (LR) formation is unresponsive to auxin (10). A significant number of these either induced or repressed branching in *Selaginella* roots, thereby providing strong evidence that an auxin-independent regulatory mechanism indeed controls bacteria-induced root branching. Subsequently, Gonin et al. (7) tested whether the selected bacteria also could affect lateral root density in a set of *Arabidopsis* auxin signaling mutants that have clear lateral root phenotypes. Although there was some variability in their capacity, this analysis clearly supported their hypothesis as some strains enhanced lateral root development even in mutants with extreme root branching phenotypes such as *arf7arf19* and *gnom*¹⁸⁴ mutants (11, 12).

The paper by Gonin et al., provides novel mechanistic insights into how root-associated bacteria affect plant development in terms of root system architecture.

The process of *Arabidopsis* lateral root development, from initiation to emergence can be divided in eight distinct stages. By quantifying these stages after inoculation with different bacterial strains, Gonin et al. (7) additionally revealed that certain bacteria can promote the development of already-formed lateral root primordia. However, and quite interestingly, they also found that there was no difference in the periodic formation of pre-branch sites, a process that regulates the spatiotemporal distribution of lateral roots along the primary root (8). Moreover, the authors were able to demonstrate that auxin biosynthesis was not targeted by the bacteria to stimulate lateral root formation—an assumption that could be made based on putative downstream responses. Thus, the authors were able to raise the question of bacteria might stimulate the transition from a quiescent to an active primordium that can produce auxin?

Next, Gonin et al. (7) performed a transcriptional analysis to ask which genetic program is targeted by the lateral root formation-promoting bacteria? for this, they compared changes in gene expression evoked by inoculation with the different bacterial strains. This also included several of the auxin signaling mutants used earlier in which inoculation with the bacterial strains could promote lateral root formation. Interestingly, this analysis revealed several ethylene-responsive genes that showed a remarkably strong differential regulation, including several transcription factors related to ethylene signaling. This was observed in wild-type plants as well as in auxin-related mutants and was independent of the presence of ethylene biosynthetic operons in the genomes of the used bacterial strains. Comparing the effect of inoculation with lateral root formation-promoting bacteria on wild-type plants and different ethylene signaling mutants revealed that their effect of bacterial inoculation was strongly repressed in the tested ethylene mutants, without clear differences in the

bacterial colonization of the roots. Since changes in ethylene signaling can alter auxin homeostasis through inhibition of auxin biosynthesis, Gonin et al. (7) went one step further and used auxin biosynthesis inhibitors, precursors, and inhibitors of ethylene signaling in the different ethylene signaling mutants to provide convincing evidence that the bacteria target ethylene signaling to stimulate lateral root formation. Since ethylene signaling also recently was shown to be involved in the regulation of primary root growth dependent on the soil compaction (2), intuitively, compacted soil would have less diffusion of ethylene and hence bacteria-mediated stimulation of lateral root formation would be repressed, allowing the plant to invest resources in the primary root growth to escape the compacted soil. It will be interesting to see whether these two types of responses are linked.

Intriguingly, Gonin et al. (7) noticed a negative correlation between the induction of defense genes and lateral root formation in their transcriptome analysis of bacterial inoculated roots. Indeed, when comparing the responsiveness of a set of known defense marker genes, they did not find any significant differences in the expression of these genes or a correlation between expression levels and the observed lateral root density. Thus, the capacity to stimulate lateral root formation is not per se dependent on changes in the activation of the plant immune system.

Finally, the authors created synthetic communities from the individual strains that could stimulate lateral root formation, which could recapitulate the effects on lateral root formation. Also in this case, the effect was gone when ethylene signaling mutants were exposed to these two types of bacterial communities. They then used this as a tool to investigate effects on LR formation under abiotic stress conditions. Intriguingly, even when plants were grown under stress conditions that normally do not favor lateral root formation such as high salt and low iron, in both cases the inoculated plants formed more lateral roots compared to non-inoculated plants. In case of the high salt, the observed effects evoked by the two types of bacterial communities were much lower compared to when plants were grown under nutrient-sufficient conditions. Thus, the lateral root developmental program is capable to both integrate the microbial effect as well as environmental fluctuations, thereby increasing root plasticity. This not only provides a good starting point to better understand how plants can adapt to a changing environment under more complex conditions such as natural soils, but also creates an agriculturally relevant toolbox for answering questions related to the role of biotic factors under abiotic stress conditions. Since previously the same group had shown that suberin deposition is related to root-microbiota interactions (13), they also tested mutants that were affected in their root suberin load. This revealed that the presence or absence of suberin did not affect the capacity of the bacteria to induce lateral root formation. In parallel, it was excluded that the bacterial effects on lateral roots were caused by nutrient deficiencies originating from plant-bacterium competition.

Overall, the paper by Gonin et al. provides novel mechanistic insights into how root-associated bacteria affect plant development in terms of root system architecture. This will without doubt be an important piece of work that will shape

this direction in the future. One immediate question to ask is why would bacteria bother to do this? What do they gain? By enhancing the emergence of lateral roots, the bacteria might have more access to sugars that are transported by the phloem or have more potential entry points. Moreover, the site of lateral root emergence also provides an opportunistic

access point to the root vasculature that is normally shielded from entry of microorganisms by barriers (14). Moreover, intermicrobial communication may also play a role, it would be interesting to study if combinations of bacteria and fungi cooperate at sites where the bacteria provide access for the fungi by the promotion of lateral root emergence (Fig. 1).

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