Wired to the roots

Impact of root-beneficial microbe interactions on aboveground plant physiology and protection

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Keywords: rhizosphere, root-shoot signalling, roots, stomata, rhizobacteria

Often, plant-pathogenic microbe interactions are discussed in a host-microbe two-component system, however very little is known about how the diversity of rhizospheric microbes that associate with plants affect host performance against pathogens. There are various studies, which specially direct the importance of induced systemic defense (ISR) response in plants interacting with beneficial rhizobacteria, yet we don't know how rhizobacterial associations modulate plant physiology. In here, we highlight the many dimensions within which plant roots associate with beneficial microbes by regulating aboveground physiology. We review approaches to study the causes and consequences of plant root association with beneficial microbes on aboveground plant-pathogen interactions. The review provides the foundations for future investigations into the impact of the root beneficial microbial associations on plant performance and innate defense responses.

Beneficial association of the microorganisms with the plants can protect plants from the vagaries of phyto-infestation without being in close approximation of the pathogens.^{3,4} The association of the beneficial plant growth promoting rhizobacteria (PGPR) leads to resistance in the leaves and also protection against the pathogens through induced systemic defense (ISR) responses.⁵ Local variations in the below ground microorganisms have been shown to have a significant impact on the plant performance. However, no signals of co-evolution were observed in the below-ground interactions between the plant species and nematodes or soil microbial communities.⁶ Whether these organisms or their metabolic exudates have profound impacts on the aboveground plant protection still remains to be answered. The present review is an attempt to bring to focus on the effect of beneficial root associated microorganisms on above-ground protection in plants. This review provides a snapshot of the existing literature pertaining to the importance of root association of beneficial microbes in the aboveground physiological changes in plants.

Introduction

Plants, despite being sessile, have developed copious ways to overcome the barriers to their survival. A wealth of information supports that a plant performs several decisive operations to sustain life. With a huge foliar leaf surface when compared with roots, stress signals are largely perceived by the aerial plant parts. However, the concept that roots too can serve an important role in plant protection was best understood when in tobacco plants aboveground nicotine production increased post root insect infestation thereby increasing the resistance to above ground herbivores.¹ Rhizosphere has long been the main source of nutrients for the surviving microorganisms in the soil. Being a rich source of nutrients in the form of “rhizodeposits,” they are the driving forces of the microbial diversity and their activities. Roots are in continuous and constant association with microbiota below-ground, wherein they encounter both beneficial and pathogenic microorganisms. Plant growth promoting rhizobacteria (PGPR) are beneficial microorganisms that dwell in the rhizospheric socket of plants driving the growth of plants and protecting plants from pathogens i.e., biocontrol and soil remediation.²

Microbial Inhabitation in the Phyllosphere

Epiphytic inhabitation of microbes occurs due to the direct and inevitable exposure of plants to the atmosphere. This colonization is considered to be more complex than it was thought, for it was observed that the phyllosphere is colonized by a group of microbes that could not be detected by the traditional culture based methods.⁷ Bacteria on the leaf surfaces are localized at the base of the trichomes, stomata and along the leaf veins. Phyllosphere microbes are exposed to a variety of changes in the atmospheric conditions and they survive a wholesome hostile environment with rapid fluctuations in the environment.^{8,9} A majority of these microbes survive at high numbers without causing any diseases whereas a few enter the plant body to cause diseases. Phyllobacteria employ a range of egression and ingression strategies to gain entry into the leaf.⁷ Phytopathogenic bacteria on the compatible hosts achieve high internal titers as compared with the non-pathogenic bacteria. Using culture-independent studies, it is now possible to characterize several hundreds of bacterial taxa and communities dwelling on the leaf surface, and surprisingly, these communities are shown to be varied across plant species.^{8,10,11} This diversity in the microbial populations has been more severe among the plants belonging to the same

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Submitted: 09/11/12; Accepted: 09/24/12
<http://dx.doi.org/10.4161/psb.22356>

Table 1. Shoot-to-root and root-to-shoot signaling and the physiological effects elicited by beneficial rhizobacteria

Beneficial Microbes/ metabolites	Plant	Extracellular Metabolites	Effects on plants	Intra plant signaling	References
<i>B. subtilis</i>	<i>A. thaliana</i>	unknown	Pathogen defense	Shoot-root	25
<i>B. subtilis</i>	<i>A. thaliana</i>	unknown	decreased stomatal conductance, pathogen defense	Shoot-root, root-shoot	26
	<i>Phaseolus vulgaris</i>	Homoserine lactone	increased stomatal conductance, transpiration rate	Root-shoot	30
<i>Bradyrhizobium japonicum</i> WB74, CB756	<i>Glycine max</i> , <i>Vigna subterranea</i> , <i>Phaseolus vulgaris</i> , <i>Eleusine cora- cana</i> , <i>Sorghum bicolor</i> , <i>Zea mays</i>	lumichrome	increased or decreased stomatal conductance	Root-shoot	31
<i>B. subtilis</i>	<i>Lactuca sativa</i>	zeatin riboside	shoot and root growth	Root-shoot	33
<i>Bacillus</i> sps <i>Pseudomonas</i> , <i>Rhizobium</i> sps.		unknown	herbivory protection	Shoot-root, Root-shoot	24

biogeographical area due to changes in the leaf characteristics.⁹ In spite of the knowledge that various biogeographical conditions support different bacteria in the phyllosphere, the understanding as to how these microbes affect overall physiological events in plants is still at its infancy.

Importance of Stomata for Pathogen Entry

Stomata are the natural pores existing on the abaxial leaf surface of plants facilitating gaseous exchange. These pores, besides helping in carbon fixation, have been reported to be the port of entry for benign microbes and pathogens.^{12–17} Though the stomata have been one among the several entry points for microbial invasion, the underlying fact that they involve complex interactions in facilitating microbial entry into the plant body has been evident from the works of Melotto et al. (2006).¹⁸ Using isolated epidermal peels from the leaves of *Arabidopsis thaliana*, the authors demonstrate the behavior of the stomatal guard cells in response to the pathogenic bacteria *Pseudomonas syringae* pv *tomato* (hereafter *PstDC3000*). Stomatal closure in the presence of bacteria is caused by microbe-associated molecular patterns (MAMPs) regulated by the defense hormone salicylic acid (SA). In the presence of the pathogen, stomata closed within 1h of exposure to the pathogen as a result of the innate immune response. However, the pathogen *PstDC3000* reopened the closed stomata using the virulence factor coronatine (COR) to gain entry into *A. thaliana* and cause pathogenesis at later time points. Besides COR, stomata of the *Arabidopsis* plants, have been shown to perceive multiple MAMPs including flagellin (flg22), lipopolysaccharide (LPS) and the elongation factor (EF-TU).^{19–21} Among these, cognate pathogen recognition receptors (PRRs) have been identified for a few MAMPs. An important PRR is the FLS2 receptor, whose presence in the stomata is required for flagellin perception.^{18–20} The importance of this receptor has been shown by developing *fls2* mutants where stomatal closure was unaffected in the presence of the pathogen *PstDC3000*. A striking feature of the study is that though the stomata closed due to innate immune response toward *PstDC3000* and the human pathogen *Escherichia coli* O157:H7, *PstDC3000* alone was capable of

overcoming the stomatal defense, to gain entry. Having known that stomata play an important role in protecting the plants from invading pathogens, it is worth investigating whether the below-ground microbiota and their association have an effect on the above ground protection of the plant from pathogens or how the plant root selectively recruits microorganisms for sustaining plant protection?

Influence of Belowground Microbiota on Above Ground Protection and Survival

Plant root associated rhizobacteria have been enlisted as plant growth promoting microorganisms which live in the nutrient rich rhizosphere of the plants. They are frequently referred to as bio-fertilizers, rhizo-remediators, phyto-stimulators and stress controllers.² The addition of rhizobacteria and their effect on the induction of the induced systemic resistance (ISR) response in the treated plants have been well documented.²² Primarily, the belowground biota and its interactions are capable of stimulating systemic defense signals from the root to the above ground parts. These signals could either be air-borne or vascular.²³ There is a substantial amount of literature that focus on the importance of belowground associations on plant-insect interactions.²⁴ However, the concept of belowground microbial associations directly impacting plant physiological events to augment pathogen infection is still not rigorously tested. A practical insight into the root associated microbes and their exudates on aerial plant protection and survival has been shown in the studies of Rudrappa et al. (2008).²⁵ It was demonstrated that the aerial infection of the foliar pathogen *PstDC3000* led to the recruitment of the rhizobacteria *Bacillus subtilis* FB17 in *A. thaliana* roots due to increase in the L-malic acid secretion (Table 1).²⁵ The biofilm formation by the beneficial rhizobacteria was shown in a mono-axenic system, and, in the presence of the aerial foliar pathogen, the biofilm formation increased thereby making the plant more tolerant to infection by the pathogen. The studies offer a valuable addition to the literature already known regarding the beneficial effects of PGPRs on plant protection. However, the study raised several questions: Does the beneficial association

Figure 1. Root inoculation with *Bacillus subtilis* FB17 causes stomatal closure in *A. thaliana* Col-0 plants as determined using Cryo-Scanning Electron Microscopy (Cryo-SEM) (A) Stomata in leaves of Col-0 plants. (B) Stomata in Col-0 plants root inoculated with FB17 (bar = 10 μ m). (C) Stomata of Col-0 plants leaf inoculated with FB17 (~ 0.1 OD₆₀₀) (D) Stomata in leaves after co-inoculation with *PstDC3000* (~ 0.1 OD₆₀₀) in leaves and FB17 in roots. The micrographs are selected as representative images from $n = 100$ stomata.

have a direct or indirect effect on pathogen multiplication? How does aerial infection alter the *in planta* physiological effects? Since the pathogen *PstDC3000* uses stomata as one of the main entry points to cause disease in *A. thaliana*, do root associated microbes alter stomatal properties?

Recently it was demonstrated that the addition of the rhizobacteria, *B. subtilis* FB17 to the roots of the *A. thaliana* plants restricted the entry of the foliar pathogen *PstDC3000* through the stomata (See also Figure 1).²⁶ The root-inoculation apparently triggered an *in planta* signal that resulted in the closure of the guard cells, which was more pronounced in the presence of the foliar pathogen *PstDC3000*. Both abscisic acid (ABA) and SA were shown to be involved in the early closure of the stomata thus minimizing the pathogen entry points on the leaf surface.

Prior root inoculation with FB17, resulted in the decrease of stomatal pore sizes and reduced the *in planta* *PstDC3000* titers.²⁶ The study also explains how the addition of the MAMPs such as the LPS and flagellin to the plant roots alter stomatal aperture sizes, thereby protecting the plants from *PstDC3000* entry. The rhizobacteria not only confers protection toward the live pathogen but elicits similar symptoms of stomatal closure post leaf exposure to the phytotoxin, coronatine. The influence on the stomatal closure was observed as a general phenomenon with the entire *Bacillus* species tested, which indicates that members of this genus are capable of modulating the stomatal phenotype. In addition, the rhizobacteria does not alter stomatal aperture sizes when added to the leaves (Fig. 1). The study per se portrays the involvement of the primary signaling components, mainly

SA and ABA, during the beneficial interaction of *B. subtilis* FB17 with the plant root and its effect on the stomatal behavior. However, in the absence of an active competitor during root colonization, the extensive growth of the beneficial rhizobacteria on the roots is quite uncommon. Under natural selection conditions, the reminiscence of the colonization scenario mentioned above is unlikely to happen. In those growth conditions, how does a plant make a judicious recruitment of a beneficial? or Does the selective soil microbiota associate with the plant at the time of distress and in turn protects the plant? The latter part of the above argument is partially supported by the findings of Herman et al. (2007).²⁷ Under field conditions when the plant is exposed to a pathogen, the plant would be equipped to mount a defense response.^{27,28} This was proved in tomato plants, where, after an initial treatment with ASM (Acibenzolar-S-methyl); a SA functional analog, there was a significant increase in the defense gene expression when compared with untreated plants. However, when the same plant was treated again with ASM, there defense gene expression was even higher. Nevertheless, the findings reinstate that plants are indeed “protected to an extent” under natural field conditions. As described by Sampath kumar et al. (2012)²⁶ either the microbe or the MAMP such as LPS could cause stomatal closure which indicates that the plant roots are exposed to both beneficial and pathogenic microbes which are capable of “priming” the plants for protection. But the priming responses might raise the question of cost effectiveness for the plant. Whether there is above ground herbivory or pathogen attack, carbon allocation to belowground plant parts increase.^{25,29} But how these associations fall in place at the time of stress is complicated and needs empirical evidence. The switch between defense and benefit is crucial as it concerns plants’ survival.

Soil Microbiota, Metabolites and Aboveground Physiological Responses

Very few reports explain the importance of the root associated microbes and their exuded products on plant stomata. Joseph and Phillips, (2003),³⁰ have reported on the effect of the homoserine lactones (HSLs) on the stomatal responses in the plants. HSLs were reported to modulate marked increase in the stomatal conductance and leaf transpiration rate in common bean seedlings (Table 1). Whereas, addition of 5nM of lumichrome: a rhizosphere signal molecule resulted in the decrease of stomatal conductance and the transpiration rate.³¹ The authors also conducted parallel experiments wherein the surface sterilized seeds were soaked in the signal molecule lumichrome (5 nM), ABA (10 nM) or the commercial inoculants *Bradyrhizobium japonicum* strain WB74 and *Bradyrhizobium* strain CB756. By including a series of legumes and also monocots in their study: soybean, cowpea, barley, pea, lupin, maize, sorghum and bambara groundnut, a species dependent variation in the responses toward the addition of the signal molecules and the commercial bio-inoculants was observed. Except for pea and sorghum, notable decrease in the stomatal conductance and the leaf transpiration rate was observed in the species of soybean and the bambara groundnut ($p \leq 0.05$) with both the rhizobial treatments. This is perhaps the

only report that has substantiated the findings, of the effect of the addition of the beneficials to the roots, and their impact on the leaf stomatal conductance and the transpiration rate by the live infective rhizobial cells. Since the outcome of their research using the purified compound was of the same magnitude as compared with live cell effect, the authors concluded that the lumichrome released by the live cells in the rhizosphere was responsible for the observed physiological variations in the stomata. However, these studies did not enumerate the differences in the stomatal phenotype. Indeed, the study calls for an investigation into the effect of the addition of rhizobia and its effect on the stomatal phenotype. Genetic lesions for some of the signal molecules will help us to narrow down other signaling components altering stomatal phenotype.

Microbially-Derived Exo-Metabolites and Abiotic Stress

Drought has been shown to alter cytokinin levels in the plants and this factor has been accounted for as an important adaptation for water limitation and survival of the plants under stressful environmental conditions.³² *Bacillus* (strain IB-22) produces zeatin riboside as a complex in the bacterial culture media³³ (Table 1). Cytokinins promote the opening of the stomata³⁴ and account for other biometric responses such as increase in shoot growth and decrease in the root growth.³³ Inoculation of cytokinin producing *Bacillus* to the roots of the lettuce plants was shown to increase the levels of the shoot cytokinin and ABA levels. However, the stomatal conductance did not change in the inoculated plants, as the shoots became less sensitive to the increase in the ABA concentrations. In contrast to the above observation, Sampath kumar et al. (2012)²⁶ have shown that the addition of the *B. subtilis* FB17 to the roots of the *A. thaliana* plants resulted in a decrease in the stomatal apertures due to increasing titers of ABA *in planta* as a flux in phytohormone ABA plays a pivotal role in altering the physiology of the stomatal guard cells.³⁵ Whether the rhizobacteria FB17 produces the cytokinins such as zeatin riboside or other cytokinins remains unknown. But the capacity of the root inoculated bacteria and its ability to influence the stomatal aperture through ABA is quite clear. The studies also raise a question whether the addition of the rhizobacteria and its influence on the stomatal behavior and physiology could be manipulated to the plant’s advantage.

The studies by Sampath kumar et al. (2012),²⁶ have not only shown that the stomata respond to biotic stimuli but provide an alternative method for studying stomatal behavior as a whole plant response vs. the use of the isolated epidermal peels for analyzing stomatal responses. However, it is important to mention that the stomatal aperture studies conducted by Sampath kumar et al. (2012)²⁶ did not account for the stomatal behavior as an effect of the bacterial metabolites exuded by the rhizobacteria FB17. Further experimentation using root exudates after aerial *Pst*DC3000 infection will certainly explain the physiological changes occurring within the bacterium for the release of the metabolites which alter stomatal phenotype. An interesting scenario would be when the observed stomatal closure due to aerial

Figure 2. Schematic showing two-way signaling in plants under tritrophic interactions. Plants under rhizobacterial treatment (*B. subtilis* FB17) inflict a root-to-shoot signaling involving interplay in between SA and ABA leading to stomatal closure and disease evasion. In contrast, plants facing aerial pathogens (*P. syringae* PstDC3000) induce a shoot-to-root signaling leading to rhizobacterial binding and induced systemic defense response (ISR). Dashed lines indicate unknown mechanisms.

pathogen attack and belowground recruitment of FB17 could be reverted back. This could be performed by using a split root system wherein one part of the root colonized by the beneficial rhizobacteria can be severed off to observe an effect for the reverted phenotype i.e., opening of the stomata.

Root to Shoot Intraplant Signal and Aboveground Responses

Plants have been proposed as mediators during multitrophic interactions linking the attackers and beneficials (Fig. 2). Microbial association with plant roots activates defense genes both within the root and the shoot (Table 1). This favors transport of several signals to above ground parts of the plants evoking a response which may be beneficial or harmful. Due to the beneficial action of the root associated microbes, the aerial plant parts receive a positive effect wherein they become more palatable for the insects and herbivores. Besides eliciting these responses, the beneficial microbes also trigger ISR. Apart from PGPRs, other soil organisms such as the herbivorous insects³⁶ and arbuscular mycorrhizal fungi (AMF)³⁷ also associate with plant roots. Intraplant communication in plants, especially after root inoculation or infestation has been more actively studied in plant root herbivory studies. During root herbivory, factors such as plant age, microbial species

specificity, abiotic stresses and genotype all affect communication within a plant.^{36,38} Recently Erb et al. (2011)³⁹ have shown that root herbivory by *Spodoptera littoralis* results in a marked increase in the maize leaf resistance mechanisms. The authors concluded that, instead of a classical JA-dependent response in the leaves, there occurs an increase in the ABA content due to the water loss caused by root herbivory. Their studies are a staunch support for the rapid root-to-shoot communication in plants. The references cited above are clear indicators of the fact that the roots are actively involved in plant protection, and that they are also capable of modulating the shoot defense responses against the pathogens. Similarly in the case of PGPRs, the findings of Rudrappa et al. (2008)²⁵ clearly illustrate that there exists a communication within the plants after root inoculation of *B. subtilis* FB17. The study highlights the active communication between the root-to-shoot and shoot-to-root, by showing increased colonization of the rhizobacteria by way of CFUs post foliar infection with the hemi-biotrophic pathogen *PstDC3000*. Following which Sampath kumar et al. (2012)²⁶ also describe how the addition of rhizobacteria FB17 result in intra-plant root-to-shoot signaling, which helps in the closure of the stomata due to the binding of the rhizobacteria and elevation of shoot ABA concentrations. The observations in the stomata were found to be as a result of an active signal communication within a 3 h time period

that overlaps with the active ingress mechanism of the foliar pathogen *Pst*DC3000. The studies further our insight regarding a possible mechanism by which rhizobacteria might regulate the stomatal apertures through intraplant messengers mainly SA and ABA as both these defense hormones were necessary for stomatal closure. Further understanding into the actual involvement of ABA in regulating the guard cell closure and opening due to root derived signals can be obtained by generating grafts using the ABA deficient root-stocks with wild type scions or generating double knock outs deficient in both ABA and SA. The study would gain importance when the observations are replicated in commercial crop plants such as Tomato. The response of plants in relation to the natural pathogens would open possibilities for understanding how plants have evolved to protect against pathogen or microbial invasion.

Future Insights

Microbial association with plant roots and the successive signaling within the plants are all dynamic events that are in continuum under natural environmental conditions. Under a

gnotobiotic condition, the microbial colonization increases and reaches a rather highly stressful biotic condition which may result in the activation of defense signaling components. From the agriculture stand point, the beneficials and their necessity for plant protection is undeniable. But would it be possible to tailor a plant's need for a beneficial during distress? Do a species specific pathogen and a plant beneficial relationship occur? The recent report on the specificity of *Arabidopsis* microbiome^{40,41} may help guide the future research to understand beneficial microbe association in plants at the molecular and ecological level. It would be interesting to know the candidate microbe(s) recruited by the plant in a mixture of naturally occurring soil borne organisms and it would be worthwhile to investigate the magnitude of protection.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

H.P.B. acknowledges the support from University of Delaware Research Foundation (UDRF) and NSF Award IOS-0814477.

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