

# Rhizosphere plant-microbe interactions under water stress

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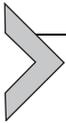
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## Abstract

Climate change, with its extreme temperature, weather and precipitation patterns, is a major global concern of dryland farmers, who currently meet the challenges of climate change agronomically and with growth of drought-tolerant crops. Plants themselves compensate for water stress by modifying aerial surfaces to control transpiration and altering root hydraulic conductance to increase water uptake. These responses are

complemented by metabolic changes involving phytohormone network-mediated activation of stress response pathways, resulting in decreased photosynthetic activity and the accumulation of metabolites to maintain osmotic and redox homeostasis. Phylogenetically diverse microbial communities sustained by plants contribute to host drought tolerance by modulating phytohormone levels in the rhizosphere and producing water-sequestering biofilms. Drylands of the Inland Pacific Northwest, USA, illustrate the interdependence of dryland crops and their associated microbiota. Indigenous *Pseudomonas* spp. selected there by long-term wheat monoculture suppress root diseases via the production of antibiotics, with soil moisture a critical determinant of the bacterial distribution, dynamics and activity. Those pseudomonads producing phenazine antibiotics on wheat had more abundant rhizosphere biofilms and provided improved tolerance to drought, suggesting a role of the antibiotic in alleviation of drought stress. The transcriptome and metabolome studies suggest the importance of wheat root exudate-derived osmoprotectants for the adaptation of these pseudomonads to the rhizosphere lifestyle and support the idea that the exchange of metabolites between plant roots and microorganisms profoundly affects and shapes the below-ground plant microbiome under water stress.



## 1. Dryland agriculture and global climate change

Climate change is among the most important global concerns faced by farmers in the 21st century. Most general circulation models forecast significant disturbances in land and ocean temperature, extreme weather events and precipitation patterns (IPCC, 2007; Stockle et al., 2010). Droughts are expected to increase in length and severity, which will adversely affect agriculture and cause significant declines in crop production on a global scale (Lesk, Rowhani, & Ramankutty, 2016). Even today the early effects of climate change on agricultural and forest lands worldwide are apparent, and these impacts will increase with time. Farmers know the weather is unpredictable, but historically, they have depended on “climate predictability” to know when it is safe to plant and harvest and the type of pests they will need to battle. With climate change, farmers are losing “climate predictability” as 100-year weather events like floods and droughts become common events, record high temperatures occur yearly, affecting crop growth, flowering, and increasing evapotranspiration demands, and pests increase in severity or spread to new areas.

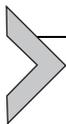
Dryland cropping systems located in semi-arid and arid parts of the world are especially vulnerable to the negative effects of changing climate conditions. Dryland systems are those where annual precipitation meets only 25–50% of potential evapotranspiration demands (Stewart & Peterson, 2015). They occupy over 40% of the land surface of the Earth and are

subdivided, based on annual precipitation, into arid (200–250 mm of annual precipitation of rain and snowfall), semi-arid (200–500 mm), and dry sub-humid (500–700 mm) regions (FAO, 2004). Drylands that occur in areas with variable rainfall and frequent droughts are especially susceptible to fluctuations in temperatures and precipitation associated with climate change. Dryland soils have a deficit of moisture, are low in soil organic matter, and are prone to erosion, fertility loss, salinization, and desertification (Reynolds et al., 2007). Despite multiple challenges, dryland areas are inhabited by over half a billion people and are an economically important source of pasture and natural vegetation for livestock, legumes, and some cereals (Rodriguez, de Voil, & Power, 2016). The continuous growth of the world population and the rise of per capita income are projected to increase food demand by over 50% by 2050 (Vos & Bellu, 2019). Dryland agriculture is essential in order to address this challenge to food security, but it will require a more holistic view of agroecosystems, more sustainable farming practices, the development of climate-resilient crops, and new technologies that incorporate better management of plant-associated microbiota including not only pathogens, but also commensal and beneficial agents that can contribute to crop health.

The difficulties of farming in water-limited agroecosystems are commonly addressed through a combination of agronomic practices and the use of drought-tolerant and -resistant crops. Management practices that help conserve water and increase water use efficiency include the adoption of reduced tillage or no-till (Schillinger, Kennedy, & Young, 2007), permanent soil cover by crop residues or cover crops (Kirkegaard et al., 2014), and novel crop rotations (Whitbread, Davoren, Gupta, Llewellyn, & Roget, 2015). For example, in the low-precipitation zone of the Inland Pacific Northwest (IPNW) of the United States, crop production is limited to a two-year rotation of wheat-summer fallow, which allows water to accumulate in the soil profile during the year of fallow and is used by the crop in the following year. Conservation tillage including no-till has now been widely adopted to increase the retention of soil water and reduce soil erosion by wind and water, yet these cropping systems, which improve the environment, are heavily dependent on inorganic fertilizers and chemical pesticides, especially herbicides like glyphosate for weed control (Hansen et al., 2016). Chemical pesticides continue to face intense public criticism. Furthermore, the conversion from traditional tillage to no-till tends to initially exacerbate soilborne diseases, resulting in greater crop yield losses than occur in crops grown by using conventional management practices (Schroeder & Paulitz, 2006). A second way to improve the productivity of dryland cropping

systems involves the development of cultivars with resilience to climate stresses. In the past decade, the application of conventional breeding, field selection, quantitative trait locus (QTL) mapping, genome-wide association studies (GWAS), transcriptomics, and high-throughput phenotyping have yielded impressive advances toward understanding drought resistance mechanisms in different crops (Gupta, Rico-Medina, & Cano-Delgado, 2020). However, progress in breeding and the enhancement of crops and cultivars adapted to growing under dryland conditions remains slow. Hundreds of drought resistance genes and QTLs have been identified and mapped, but very few have been integrated into commercial cultivars by breeding programs due to the low heritability, polygenic nature, and the high level of genotype  $\times$  environment interactions of relevant traits (Hu & Xiong, 2014).

Recent studies suggest that the plant microbiome positively influences plant fitness in response to stressors associated with climate change (Coleman-Derr & Tringe, 2014). Plants foster large root-associated microbial communities that enhance the ability of plants to resist biotic and abiotic stresses by promoting nutrient uptake and plant development, suppressing pathogens, inducing resistance mechanisms, degrading soil pollutants, and modulating stress phytohormone levels (Adriaensen, Vralstad, Noben, Vangronsveld, & Colpaert, 2005; Dimkpa, Weinand, & Asch, 2009; Glick, 2012; Kuiper, Lagendijk, Bloemberg, & Lugtenberg, 2004; Lundberg et al., 2012; Marasco et al., 2012; Rolli et al., 2015; Timmusk et al., 2014; Zolla, Badri, Bakker, Manter, & Vivanco, 2013). Plant-associated soil microorganisms can also alleviate the detrimental effects of drought and heat stress (Lau & Lennon, 2012; Rodriguez et al., 2008). The exploitation of beneficial microbial communities is a promising complementary strategy that can be integrated with conventional management practices and breeding efforts to improve crop performance under a changing global climate. However, these novel strategies require a firm understanding of the physiological and molecular processes underpinning the adaptation of plants and their associated microbiomes to water stress. The topic of plant-microbial interactions under drought has been addressed in several recent reviews (De Vries, Griffiths, Knight, Nicolitch, & Williams, 2020; Naylor & Coleman-Derr, 2018; Williams & de Vries, 2020). Here, we discuss key aspects of plant and microbial responses to water stress and then focus on ecological and molecular aspects of the interaction between dryland cereals grown in semi-arid parts of the U.S. Inland Pacific Northwest and the indigenous rhizosphere *Pseudomonas* bacteria that are key members of their root microbiota.



## 2. Plant responses to water stress

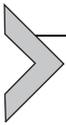
Water is critical for plant survival, and plants have evolved biochemical and physiological defense mechanisms that are activated in response to periods of drought. Depending on the species, these mechanisms may include an accelerated lifecycle before the onset of water stress (drought escape), the ability to store and maintain high water content during drought (drought avoidance), or the ability to sustain growth and cope with low tissue water content (drought tolerance) (Basu, Ramegowda, Kumar, & Pereira, 2016). On a physiological level, drought-stressed plants induce stomata closure and secrete cuticular wax, which decreases transpiration and helps retain foliar water (Moshelion, Halperin, Wallach, Oren, & Way, 2015). Another important physiological adaptation involves an increased water uptake from the soil by altering root hydraulic conductance, increasing expression of aquaporins and producing dehydrins, which are stress proteins associated with responses to drought, salinity, and development of cold hardiness and winter dormancy (Eldhuset et al., 2013; Liu, Song, Li, Yang, & Li, 2017). Plants also reduce foliar growth, thereby increasing the root/shoot ratio, and alter root system architecture in order to maximize the capture of soil water and nutrients (Dinneny, 2019).

Plant responses to abiotic stresses are governed by networks of signaling pathways that involve abscisic acid (ABA), jasmonic acid (JA), ethylene (ET), and salicylic acid (SA). Among these phytohormones, ABA functions as a growth inhibitor and key regulator in the adaptation to environmental stress. It is strongly induced under drought and excessive salinity and helps plants tolerate water deficit by inducing the closure of stomata and slowing water loss via transpiration (Vishwakarma et al., 2017). JA and its derivatives regulate multiple stress response and developmental processes. These compounds accumulate in plant tissues during drought and, like ABA, help conserve water by controlling the function of stomata (Hossain et al., 2011; Wang, Song, Gong, Xu, & Li, 2020). Ethylene is also produced in response to multiple abiotic stresses, including drought, salt, and flooding (Kazan, 2015). This phytohormone regulates plant organ development and, depending on the concentration and type of stressor, influences shoot and root growth and the formation of aerenchyma (Dubois, Van den Broeck, & Inze, 2018; Ni et al., 2018). ET acts as yet another regulator of stomatal function, although its effect is modulated by ABA (Tanaka et al., 2005). Although salicylic acid is known for its role in the induction

of systemic acquired resistance to pathogens, this phytohormone also mediates plant response to drought, salinity, high temperature, and heavy metals (Hasanuzzaman et al., 2017). It has been suggested that the protective effect of SA may involve the modulation of redox homeostasis and accumulation of osmolytes in stressed plants (Herrera-Vasquez, Salinas, & Holuigue, 2015; Jagendorf & Takabe, 2001).

Drought, high salinity, and other abiotic stresses cause alterations in plant carbon metabolism, the extent of which depends on the rate of the stress, plant species, and tissue type (Cui et al., 2019). These changes are partially caused by the decrease in photosynthesis due to inhibited biochemical and photochemical processes (deactivation of RuBisCO, slow regeneration of RuBP) and closed stomata that limit CO<sub>2</sub> diffusion into the leaf mesophyll (Bota, Medrano, & Flexas, 2004; Chaves, Flexas, & Pinheiro, 2009; Flexas & Medrano, 2002). Other changes involve an osmotic adjustment that enables plants to maintain cell turgor critical for growth and survival under water stress (Turner, 2018). Osmoprotectants, or osmolytes, including soluble sugars like trehalose and charged metabolites like glycine-betaine and proline accumulate throughout plant tissues (Bohnert, Nelson, & Jensen, 1995). They act as compatible solutes that lower the cell water potential and promote water retention without interfering with normal metabolism. Under severe or persistent osmotic stress, these metabolites stabilize cellular components and help maintain redox balance by scavenging free radicals (Couee, Sulmon, Gouesbet, & El Amrani, 2005; Miller, Suzuki, Ciftci-Yilmaz, & Mittler, 2010). Drought enhances production of reactive oxygen species (ROS) that interfere with cellular metabolism by damaging lipids, proteins and other macromolecules (Helena & Carvalho, 2008). The activation of enzymatic antioxidants helps to prevent acute cellular damage and maintain redox homeostasis and membrane integrity (Pardo-Hernandez, Lopez-Delacalle, & Rivero, 2020; Verma, Srivastava, Tiwari, & Chakrabarty, 2019). This represents a crucial part of the plant defense response to abiotic stress, and there is a correlation between the induction of antioxidant pathways and peroxidases and the degree of drought tolerance (You & Chan, 2015). Water-stressed plants also produce and accumulate specialized free radical scavengers, such as flavonols and anthocyanins, that mitigate oxidative stress in plants exposed to drought (Nakabayashi, Mori, & Saito, 2014; Nakabayashi, Yonekura-Sakakibara, et al., 2014). Finally, the exposure of different plant species to drought and other abiotic stresses results in a rapid accumulation of the non-proteinogenic amino acid  $\gamma$ -aminobutyric acid (GABA) (Shelp et al., 2012). Recent studies suggest

that GABA is not just a metabolite but rather a signaling compound involved in the maintenance of carbon and nitrogen metabolism, regulation of TCA cycle, scavenging of ROS, and osmoregulation (Seifikalhor, Aliniaefard, Hassani, Niknam, & Lastochkina, 2019; Yong et al., 2017). Interestingly, plant osmolytes, phytohormones and their precursors are actively metabolized by soil bacteria, which suggests an avenue for stressed plants to select their rhizosphere microbial communities. Conversely, plant-associated microorganisms may alleviate detrimental effects of drought stress by modulating the levels of defense metabolites and phytohormones in the plant rhizosphere (Kudoyarova et al., 2019).



### 3. Rhizosphere microbiome alleviates drought stress in plants

Two lines of research provide experimental evidence that plant-associated rhizobacteria have a key role in alleviating drought stress in plants. The first group of studies tested the ability of individual strains to promote plant growth under conditions of water deficit. A non-exhaustive list of non-symbiotic species with documented ability to confer plant tolerance to water stress includes numerous species of *Pseudomonas* and *Bacillus* (reviewed by Ngumbi and Kloepper (2016)), *Paenibacillus* (Timmusk et al., 2014), *Variovorax* (Garcia Teijeiro, Belimov, & Dodd, 2020), *Phyllobacterium* (Bresson, Varoquax, Bontpart, Touraine, & Vile, 2013), *Achromobacter* (Castillo, Escalante, Gallardo, Alemanno, & Abdala, 2013; Mayak, Tirosch, & Glick, 2004), *Burkholderia* (Fan et al., 2015), *Enterobacter* (Naveed, Mitter, Reichenauer, Wieczorek, & Sessitsch, 2014), and *Alcaligenes* (Naseem & Bano, 2014b). More recently, a meta-analysis of 52 published papers evaluated the extent to which rhizobacteria influence root and shoot mass and seed yield in plants under optimal as well as water-stressed conditions (Rubin, van Groenigen, & Hungate, 2017). Most studies included in that analysis focused on interactions between maize, wheat, sunflower, and lettuce with *Pseudomonas*, *Azospirillum*, *Azotobacter*, and *Bacillus*. These studies were selected such that drought was imposed through irrigation deficit rather than by manipulating salinity or including polyethylene glycol to impose matric stress, as these techniques often led to confounding errors. Results of the meta-analysis revealed that beneficial rhizobacteria consistently promoted plant growth by increasing root mass, shoot mass and seed yield (35%, 28%, and 19%, respectively) under well-watered conditions. Under water stress, the effect was even more evident, with

the percentage increase in root mass, shoot mass and reproductive yield being 43%, 45%, and 40%, respectively (Rubin et al., 2017). These findings agree with the results of an earlier meta-analysis that examined the effect of microbial symbionts such as arbuscular mycorrhizal fungi on the growth and yield of plants exposed to water stress (Jayne & Quigley, 2014). Biomass measurements revealed that plants colonized by mycorrhizae grew significantly better and had a greater reproductive response compared to the non-mycorrhizal control plants. The magnitude of the mycorrhizal effect varied among annual and perennial plants, herbaceous and woody plants, and depended on water stress levels. The results also indicated that C3 plants responded less strongly than C4 plants. Similar beneficial effects were observed in response to colonization of drought stressed plants with symbiotic rhizobia (Goicoechea, Antolín, & Sanchez-Díaz, 1997), certain strains of the ascomycete *Trichoderma* (Estevez-Geffriaud, Vicente, Vergara-Díaz, Narvaez Reinaldo, & Trillas, 2020) and endophytes from the order *Sebaciales*, such as *Piriformospora indica* (Tsai et al., 2020).

Another clear line of evidence for the ability of rhizobacteria to influence plant growth under water-limited conditions is provided by attempts to engineer the rhizosphere microbiome for drought relief. In contrast to using individual strains, these studies use an accelerated selection process to promote the assembly of microbial communities capable of enhanced plant growth promotion under water stress. For example, Lau and Lennon (2012) cultivated multiple generations of *Brassica rapa* in drought-stressed soil mesocosms. Soil microbial communities that evolved under continuous water stress differed significantly from those maintained under well-watered conditions. Plants grown in association with such a drought-adapted microbiome maintained greater fitness under water stress, which was manifested as accelerated flowering and smaller drop in fruit production relative to controls. A similar cycling approach was recently used by Jochum, McWilliams, Pierson, and Jo (2019) to evolve a drought-adapted rhizosphere microbial community in wheat. That host-mediated microbiome engineering (HMME) experiment involved growing six continuous rounds of wheat seedlings in the same drought-stressed soil. Soil communities recovered at the end of the sixth HMME cycle significantly improved water stress tolerance, which was evident from the increased biomass, root length, and leaf surface area. The beneficial effect could be transferred by adding 1–0.1 part of the drought adapted soil to a regular soil and was destroyed by autoclaving. A 16S rRNA-based analysis of the drought-adapted

community revealed a significant decrease in alpha diversity accompanied by shifts in the abundance of *Betaproteobacteria*, *Acidobacteria*, and *Gemmatimonadetes* (Jochum, McWilliams, Pierson, & Jo, 2019). Although carried out under controlled conditions, both studies cited above demonstrate how changes in the rhizosphere microbiome can accelerate plant responses and adaptation to new abiotic stressors. These studies also open a potential avenue for the development of microbial inoculants that will be effective at improving plant tolerance to water deficit.



#### 4. How rhizobacteria protect plants from drought stress

The molecular mechanisms by which rhizosphere microorganisms protect plants from water stress are multifaceted and include the modulation of plant hormone levels and the synthesis of osmolytes, antioxidants, and humectants. Despite their hypothesized involvement, most of these microbial traits are shared by multiple unrelated taxa and are nonspecific, especially the ability of microorganisms to control pathogens and stimulate plant growth and nutrient acquisition (Finkel, Castrillo, Herrera Paredes, Salas Gonzalez, & Dangl, 2017). Most claims about the contribution of certain microbial mechanisms to the alleviation of water stress come from correlational studies carried under controlled conditions. There is limited evidence that plant inoculation with beneficial microorganisms selected in the lab provides advantages to drought-stressed plants in the field. The ability of rhizobacteria to improve the nutritional status of plants has been covered by several thorough reviews and will not be discussed here. Instead, we will briefly address the ability of microorganisms to impart drought tolerance by modulating phytohormones and producing osmoprotectants and exopolysaccharides.

As we have outlined above, abscisic acid (ABA) plays a key role in the ability of plants to mount a defense response to water stress. Although the production of ABA by bacteria has been described (Cohen, Bottini, & Piccoli, 2008; Shahzad et al., 2017), this trait appears to be rare and, in contrast to plants and fungi, the genetic basis for the synthesis of ABA in bacteria remains unknown. Bacterial catabolism of ABA (Hasegawa, Poling, Mayer, & Bennett, 1984) is equally poorly characterized, but both the breakdown and synthesis of this phytohormone have been attributed to the ability of some rhizosphere microorganisms to alleviate drought stress in plants (Belimov et al., 2014; Cohen et al., 2015; Cohen, Travaglia,

Bottini, & Piccoli, 2009). Other studies have reported that the application of beneficial rhizobacteria coincides with changes in the levels of endogenous ABA in drought-stressed plants (Salomon et al., 2014; Zhang, Xie, et al., 2008), although it is difficult to determine whether the effects were direct or mediated by changes in other signaling networks.

Ethylene (ET) regulates multiple different aspects of plant growth and development, and its levels are induced by wounding, pathogen infection, flooding, extreme temperature, and drought (Kazan, 2015; Morgan & Drew, 1997). ET mediates plant adaptation to stress by interfering with auxin response factors, constraining growth and development, and expediting senescence. The ability to catabolize the immediate precursor of ET, 1-aminocyclopropane-1-carboxylate (ACC), is considered the key mechanism by which rhizosphere bacteria exert beneficial effects on plants affected by drought and other abiotic stresses (Glick, 2014). Specifically, microorganisms secreting the enzyme 1-aminocyclopropane-1-carboxylate deaminase convert ACC to ammonia and  $\alpha$ -ketobutyrate, thus lowering ethylene levels and promoting growth in stressed plants (Glick et al., 2007). The beneficial effect of ACC-deaminase producers on drought-stressed plants has been documented in multiple crops treated by such diverse rhizobacteria as *Bacillus* (Lim & Kim, 2013), *Variovorax* (Dodd, Jiang, Teijeiro, Belimov, & Hartung, 2009), *Burkholderia* (Naveed et al., 2014), *Achromobacter* (Mayak et al., 2004), and different species of *Pseudomonas* (Carlson et al., 2020; Cheng, Park, & Glick, 2007; Niu, Song, Xiao, & Ge, 2018; Zahir, Ghani, Naveed, Nadeem, & Asghar, 2009). This list is by no means exhaustive because studies of this type are numerous and ACC-deaminase is highly prevalent among divergent *Proteobacteria*, *Firmicutes*, and *Actinobacteria* (Nascimento, Rossi, Soares, McConkey, & Glick, 2014).

Auxins such as indole-3-acetic acid (IAA) regulate plant growth and development by influencing the elongation of shoots and roots, differentiation of vascular tissues, the formation of lateral and adventitious roots, and root gravitropism (Aloni, Aloni, Langhans, & Ullrich, 2006). It has been suggested that over 80% of culturable rhizobacteria carry pathways for the synthesis of IAA and have the capacity to produce auxins (Duca, Lorv, Patten, Rose, & Glick, 2014; Patten & Glick, 1996; Spaepen & Vanderleyden, 2011). The production of IAA by microorganisms plays an important role in plant-microbe signaling and symbiotic relationships of bacteria with plants (Spaepen, Vanderleyden, & Remans, 2007). Several studies also suggest that IAA-producing rhizobacteria can alleviate drought stress in plants. For example, treatment of wheat and maize with IAA-producing strains of

*Bacillus* and *Enterobacter* increased the drought tolerance in both plant species (Jochum et al., 2019). In both cases, the application of bacteria significantly increased root length, surface area and the number of root tips. Some beneficial rhizobacteria seem to manipulate plant auxin levels indirectly. For example, the treatment of *Arabidopsis* with *Phylobacterium brassicacearum* modified root architecture and increased the length of lateral roots, which coincided with improved drought tolerance (Bresson et al., 2013). Subsequent work revealed that the effect was mediated not by the release of microbial IAA, but rather by changes in auxin distribution and homeostasis in the colonized plant. Interestingly, the effect of auxins on plant roots is concentration-dependent in that low levels of endogenous IAA stimulate the elongation of the primary root, while high levels inhibit this growth and induce the formation of lateral roots and root hairs (Patten & Glick, 2002). Inoculation of plants with IAA-producing bacteria seems to mirror this trend, and species with moderate levels of auxin have a beneficial effect, while overproducers inhibit root growth and elongation (Xie, Pasternak, & Glick, 1996).

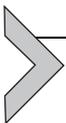
Other suggested mechanisms for the alleviation of drought stress involve the ability of microorganisms to balance osmotic pressure across cellular membranes under hypertonic conditions by impacting plant levels of compatible solutes (De Vries et al., 2020). In addition, the application of beneficial rhizobacteria to water-stressed plants often coincides with the induction of enzymatic antioxidants such as glutathione reductase, catalase (CAT), superoxide dismutase (SOD), peroxidase, and ascorbate peroxidase (APX). Both phenomena have been demonstrated in maize, tomato, rice, beans and *Arabidopsis* exposed to different individual strains or simple consortia of plant growth-promoting rhizobacteria (Vurukonda, Vardharajula, Shrivastava, & Sk, 2016). For example, treatment of plants by different *Pseudomonas* or *Bacillus* strains correlated with the accumulation of proline (Ansary et al., 2012; Armada, Roldan, & Azcon, 2014) or choline and glycine betaine (Zhang et al., 2010). Similar effects were observed in drought-stressed maize and beans treated, respectively, with trehalose-overproducing strains of *A. brasilense* (Rodriguez, Suarez, Caballero, & Itturiaga, 2009) and *Rhizobium etli* (Suarez et al., 2008). Interestingly, the macroarray profiling of root nodules inoculated with trehalose-overproducing *R. etli* revealed an upregulation of multiple genes involved in nodulation, stress tolerance, carbon and nitrogen metabolism, suggesting that trehalose acts as a signaling compound (Suarez et al., 2008). Similarly, changes in levels of plant antioxidant enzymes have been recorded in multiple studies, suggesting a link

between the presence of rhizobacteria and activation of ROS scavenging systems during water stress (Vurukonda et al., 2016). For example, the treatment of plants with strains of *Pseudomonas*, *Bacillus*, *Bradyrhizobium*, or *Enterobacter* coincided with a significant increase in the activity of APX, SOD, CAT, and alleviation of water stress (Gururani et al., 2013; Tiwari, Lata, Chauhan, & Nautiyal, 2016; Vardharajula, Zulfikar Ali, Grover, Reddy, & Bandi, 2011; Wang et al., 2012). While the correlation between the presence of rhizobacteria and changes in the levels of plant osmolytes and antioxidant enzymes is well documented, the explicit mechanism behind this phenomenon remains elusive. Relevant studies often use strains secreting ACC-deaminase and/or auxins, and it is unclear whether the observed effects are specific or reflect changes in the plant phytohormone status. It also is unclear whether the observed changes depend on the type of the microorganism and the host, their physiological status, and the impact of these changes on the overall fitness of the plant in the field.

Microbial volatile organic compounds (VOCs) are small molecules with low boiling point and high vapor pressure that are produced by many rhizobacteria and diffuse via a gaseous phase. Recent studies suggest a key role of these metabolites in the mediation of mutualistic interactions between plants and their associated microbiomes (Garbeva & Weiskopf, 2020). Microbial VOCs are perceived by plants and exert a multitude of biological effects including growth promotion in the presence of salinity, drought, and other abiotic stresses. For example, Cho et al. (2008) identified 2,3-butanediol as a key contributor to the ability of *P. chlororaphis* O6 to confer tolerance to drought stress in *Arabidopsis*. The effect was abolished in 2,3-butanediol non-producing mutants of O6 and was mediated on the plant side by salicylic acid, nitric oxide, and the synthesis of hydrogen peroxide (Cho, Kim, Anderson, & Kim, 2013). The capacity of *Paraburkholderia phytofirmans* PsJN to confer tolerance to water stress in *Arabidopsis* was mediated by exposure of the plant to volatile metabolites emitted by the bacteria. Further studies revealed that volatile blends released by PsJN contained 2-undecanone, 7-hexanol, and 3-methylbutanol, which collectively provided growth promotion and stress tolerance levels similar to those of the strain itself (Ledger et al., 2016). Similarly, a blend of VOCs released by *Pseudomonas simiae* AU induced tolerance to salinity in soybean (*Glycine max*) (Vaishnav, Kumari, Jain, Varma, & Choudhary, 2015). Although the metabolites involved were not identified, the treatment decreased plant Na<sup>+</sup> levels and increased the content of proline and chlorophyll. Similar results were observed by Zhang, Kim, et al. (2008), who exposed salt-stressed *Arabidopsis* to a mix of VOCs produced by *B. subtilis* GB03.

They observed increased leaf surface area, root mass, and total  $K^+$  content, as well as a reduction in  $Na^+$  content concurrent with tissue-specific changes in expression of the sodium transporter HKT1. Despite some in-depth studies, the effect of bacterial VOCs on plant abiotic stress tolerance remains poorly understood. It has been proposed that plants perceive microbial VOCs as a sign of pathogen attack and prime general defense responses, which provides protection against both biotic and abiotic (i.e., drought) forms of stress (Garbeva & Weiskopf, 2020).

Bacterial exopolysaccharides (EPSs) are complex mixtures of polysaccharides, DNA, proteins, and lipids that have very high water content and protect microorganisms from desiccation (see Section 6) (Chang et al., 2007; Schmid, Sieber, & Rehm, 2015). Hence, in some rhizobacteria the capacity to alleviate water stress has been linked to the formation of rhizosphere biofilms and the production of the hydrating EPS matrix that creates an osmotically stable environment during drought. For example, the inoculation of sunflower seedlings with the EPS-producing strain *Pseudomonas* sp. GAP-P45 significantly increased root-adhering soil, leading to better uptake of water and nutrients and survival under drought stress (Sandhya, Ali, Grover, Reddy, & Venkateswarlu, 2009). The treatment of maize seeds with EPS-producing strains of *Proteus*, *Pseudomonas*, and *Alcaligenes* (along with their exopolysaccharides) promoted plant growth and improved the content of protein, sugar, proline, and antioxidant enzymes (Naseem & Bano, 2014b). Other examples include the inoculation of chickpea with salt-tolerant strains of *Halomonas variabilis* and *Planococcus rifietoensis*, which promoted plant growth and improved the aggregation of rhizosphere soil under high salinity (Qurashi & Sabri, 2012). Similarly, the inoculation of quinoa seeds with halotolerant strains of *Enterobacter* and *Bacillus* improved plant growth as well as osmotic and ionic stress under saline irrigation (Yang et al., 2016). The accumulation of bacterial EPS strongly influences soil moisture by retaining moisture within the polymeric matrix, promoting the formation of soil aggregates and inhibiting evaporation by changing soil water repellency (Guo et al., 2018). It is thought that these changes may benefit drought- and salt-stressed plants by supporting the flow of water, nutrients, and ions to the root system.



## 5. The belowground plant microbiome is shaped by water stress

Drought has a strong impact on the structure of the root microbiome, either by selection of microbial taxa that are tolerant to desiccation or by

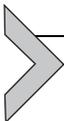
alteration of soil chemistry and rates of diffusion (Naylor & Coleman-Derr, 2018). Bacterial biomass has been seen to decrease in some water stressed conditions (Hueso, García, & Hernández, 2012) but not in others, where it remained the same (Hartmann et al., 2017) or increased (Fuchslueger, Bahn, Fritz, Hasibeder, & Richter, 2014), possibly due to prior exposure of the bacteria to drought. There have been relatively few reports of the impact of drought on bacterial phylogenetic diversity, although this pattern may depend on the context of the drought; alpha diversity in plots exposed to drought for the first time decreased by 40%, but there was no such decrease in soils pre-exposed to the condition (Bouskill et al., 2013). With the use of more refined methods, however, the composition of the soil microbial community is being found to change substantially due to drought. There was a significant enrichment in lineages of Gram-positive bacteria (monoderms) such as *Firmicutes* and *Actinobacteria*, and fewer Gram-negative phyla such as *Proteobacteria* and *Bacteroidetes*, within the root and rhizosphere as compared to the surrounding soil (Naylor & Coleman-Derr, 2018). Differences in results among studies could be dependent upon the conditions at various study sites, as the relative abundance of *Proteobacteria* and *Acidobacteria* has been seen to vary markedly in different studies but shifts in their relative abundance have been small in most cases. In one interesting exception to this generality, German forest ecosystems showed a 300% increase in the family *Micromonosporaceae*, a much greater change than in the rest of the parent phylum *Actinobacteria* (Felsmann et al., 2015).

Alterations in microbial community structure due to drought have been revealed in studies directed at various individual host plant species. Water-stressed conditions changed the overall root microbiome of rice cultured in a greenhouse. Changes in the communities present in the rhizosphere and the endosphere were consistent across cultivars and showed an increase in the abundance of *Actinobacteria* and *Chloroflexi*, and fewer *Deltaproteobacteria* and *Acidobacteria* (Santos-Medellín, Edwards, Liechty, Nguyen, & Sundaresan, 2017). In water-stressed field-grown rice plants, the root microbiome was developmentally immature; it changed considerably during the vegetative phase of plant growth and remained relatively constant afterwards. These results provide insight into the relationship between microbial composition of the root and the growth phase of the host plant (Edwards et al., 2018). Similar results were observed in studies conducted in millet plants in which *Actinobacteria* were enriched on the roots under drought conditions. The enrichment was observed along the entire length of the roots, although the drought response was only expressed in

areas that experienced the water stress (Simmons et al., 2020). In drought-sensitive pepper plants cultivated in desert farms, the main taxa associated with the root system were *Actinobacteria*, *Proteobacteria* and *Firmicutes*, with 61% of the rhizosphere microbial community comprised of *Klebsiella* spp. and 68% of the endosphere microbial community comprised of *Bacillus* spp. (Marasco et al., 2012).

While these studies focused mostly on single host species, other studies with multiple hosts under drought conditions have observed similar results. Research conducted on 18 grass lineages, for example, with wheat, rye, sorghum, and tomato as an outgroup, showed a consistently large increase in communities of *Actinobacteria* across all host groups (Naylor, DeGraaf, Purdom, & Coleman-Derr, 2017). In contrast, another study conducted with sorghum plants showed a substantial decrease in the abundance of *Actinobacteria*, which could have been due to a number of confounding factors including the degree of drought to which the plants were exposed (Naylor & Coleman-Derr, 2018). Analysis of the root microbiomes of 30 angiosperm species in water-stressed conditions showed that the abundance of *Actinobacteria* in the endosphere increased twofold, with a concomitant decrease in the abundance of *Proteobacteria* (Fitzpatrick et al., 2018).

Interestingly, a reverse trend was observed when ecosystems with a history of water limitation were subjected to irrigation. A long-term irrigation experiment in a semi-arid pine forest in the Rhone Valley of Switzerland revealed pronounced shifts in the soil microbiome from oligotrophic to more copiotrophic taxa (Hartmann et al., 2017). The increased soil moisture stimulated plant-derived inputs and increased levels of copiotrophic *Proteobacteria*, which have higher rates of metabolic activity (Fierer et al., 2007), while displacing oligotrophic *Actinobacteria*, *Gemmatimonadetes*, *Acidobacteria*, and *Armatimonadetes* that are more tolerant of water stress. This study indicates that the activity of microbes changes not only because of irrigation, but also due to the difference in efficient carbon use among taxa (Hartmann et al., 2017).



## 6. Microbial responses to water stress in the rhizosphere

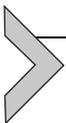
Rhizobacteria exist in environments that regularly experience dramatic changes in water activity ranging from extremely hypotonic, as can occur after a massive rainfall, to extremely hypertonic, such as during a prolonged drought. Bacteria employ diverse physiological mechanisms to cope with deleterious effects of water stress that differ depending on

the plant host and the other bacteria occupying plant-associated habitats. Among rhizosphere pseudomonads, these mechanisms have been studied in considerable detail in *Pseudomonas putida*, which responds to water limitation by producing biofilms and accumulating compatible solutes (Elbein, Pan, Pastuszak, & Carroll, 2003; Fernandez-Aunión et al., 2010; Potts, 1994). Bacterial biofilms are structurally complex cellular assemblages enclosed in an extracellular matrix comprised of proteins, exopolysaccharides, and DNA (Bloemberg, 2007; Borlee et al., 2010; Watt, Hugenholtz, White, & Vinall, 2006; Zachow, Fatehi, Cardinale, Tilcher, & Berg, 2010). The exopolysaccharides (EPSs) can hold up to 10 times their weight in water (Chenu & Roberson, 1996; Roberson & Firestone, 1992) and are a major water-binding agent and reservoir under water-limiting conditions (Sutherland, 2001). *P. putida* has genes for the production of four different EPSs: alginate (*alg*), putida exopolysaccharide a (*pea*), putida exopolysaccharide b (*peb*), and cellulose (*bcs*) (Nilsson et al., 2011; Nielsen, Li, & Halverson, 2011). In saturated biofilms, alginate has a critical role in creating hydrated environments (Chang et al., 2007; Mann & Wozniak, 2012), and its synthesis is upregulated under conditions of water limitation (Nilsson et al., 2011; van de Mortel & Halverson, 2004). The other EPSs also contribute to the formation of rhizosphere biofilms by *P. putida*. Mutant testing has shown that Bcs and Pea contribute to hydration and that Bcs and alginate, but not Pea, contribute to rhizosphere colonization in gnotobiotic assays (Nielsen et al., 2011). A mutant devoid of all known EPS components produced biofilms similar in structure to those of the parental strain in vitro, albeit with much reduced stability (Nilsson et al., 2011). These results suggest that all four EPS components in *P. putida* contribute to biofilm integrity and highlight the importance of the EPS in fitness under environmental stress. Although nearly all pseudomonads are capable of synthesizing alginate, individual species differ markedly in the other forms of EPS they produce (Mann & Wozniak, 2012) and the exact role and relative importance of these molecules in biofilm formation, stability, and stress tolerance must be determined for individual species and strains of bacteria.

Aside from forming biofilms, most rhizobacteria respond to water stress by producing and/or taking up otherwise inert molecules that help them balance osmotic pressure across the cellular membrane without compromising protein folding or other cellular processes. These metabolites, collectively termed compatible solutes, osmolytes, or osmoprotectants, include certain polyols, sugars, amino acids, amino acid derivatives, and peptides (Miller & Wood, 1996). When stressed osmotically, *P. putida* accumulates

the osmolytes glycine betaine (GB), mannitol, glutamate, *N*-acetylglutaminylglutamine amide (NAGGN) and trehalose (Galvao, de Lorenzo, & Canovas, 2006). Genes involved in the synthesis and uptake of these osmolytes have been identified and characterized in *P. putida* and in the related species *P. aeruginosa*, an opportunistic human pathogen (Wargo, 2013) and in *P. syringae*, a plant pathogen (Chen & Beattie, 2008; Freeman et al., 2013; Kurz, Burch, Seip, Lindow, & Gross, 2010). Interestingly, all pseudomonads studied to date can utilize GB as an osmolyte but at the same time they lack genes for the synthesis of this compound (Wargo, 2013). GB and its precursor, choline, are thought to be relatively widespread in plants (McNeil, Nuccio, & Hanson, 1999; Storey & Wyn Jones, 1975), and most pseudomonads can convert choline to GB, and take it and GB into their cells. However, there have been few if any studies of the exact role of GB and other osmolytes in rhizosphere settings.

While there has been considerable interest in characterizing microbial biofilms and osmolytes in recent years, our understanding of physiological responses to water stress in rhizobacteria remains poorly understood. This is due in part to the fact that rhizobacterial populations are phylogenetically complex and the bacteria themselves have large and highly plastic genomes, meaning that stress response traits are highly diverse. Furthermore, because the physiological stress response pathways of most bacteria have never been tested under ecologically relevant conditions (i.e., on plants and in the presence of indigenous microflora), the physiological relevance of water stress response pathways to rhizosphere fitness is still poorly understood. Finally, almost all studies to date have been performed *in vitro* and have not accounted for the exchange of metabolites among rhizobacteria residing in the environment of a water-stressed plant.



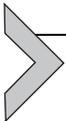
## 7. The role of rhizodeposits in stress-driven selection of the plant microbiome

It has become increasingly clear that stressed plants actively recruit and shape their microbiomes by depositing photosynthetically fixed carbon into the soil directly surrounding plant roots (de la Fuente Canto et al., 2020). In return, plant-associated microorganisms positively influence the fitness of the host in response to stressors associated with global climate change (e.g., drought, salt, temperature, and soil pollution) (Adriaensen et al., 2005; Kawasaki, Watson, & Kertesz, 2012; Lau & Lennon, 2012; Rodriguez et al., 2008). How plants shape the rhizosphere microbiome is

a subject of active research. The differential affinity of rhizobacteria toward host plants is mediated by complex chemical crosstalk between microorganisms and the roots. As much as 40% of the carbon fixed by the plant is released as root exudates and secretions, lysates, and mucilages (Badri & Vivanco, 2009; Curl & Truelove, 1986; Lynch, 1990; Whipps, 1990). The release of these compounds, collectively termed rhizodeposits, is actively controlled in response to the environment, and their composition varies greatly according to the plant species and its physiological condition (De-la-Pena, Lei, Watson, Sumner, & Vivanco, 2008; Lynch, 1990; Nguyen, 2003; Phillips, Fox, King, Bhuvanewari, & Teuber, 2004). That the presence and composition of exudates strongly impact soil microorganisms is consistent with the idea that plants actively select and shape their root microbiota (Zolla, Bakke, et al., 2013).

Primary root exudates include mixtures of simple and complex sugars, amino acids, polypeptides and proteins, organic, aliphatic and fatty acids, sterols and phenolics (Badri & Vivanco, 2009; Badri, Weir, van der Lelie, & Vivanco, 2009; Nguyen, 2003) that serve as carbon and energy sources for rhizobacteria, and corresponding catabolic pathways in the bacteria are essential for competitive colonization of roots and disease suppression (Kamilova, Validov, Azarova, Mulders, & Lugtenberg, 2005; Lugtenberg, Dekkers, & Bloemberg, 2001; Lugtenberg & Kamilova, 2009). Root exudates also contain signal molecules and secondary metabolites, the role of which is only now beginning to be understood (Bais, Prithiviraj, Jha, Ausubel, & Vivanco, 2005; Bais, Weir, Perry, Gilroy, & Vivanco, 2006; Walker, Bais, Halligan, Stermitz, & Vivanco, 2003). The few analyses of plant-induced gene expression by transcriptional profiling of rhizobacteria *in vitro* (Mark et al., 2005) and in the rhizosphere (Barret et al., 2009; Matilla, Espinosa-Urgel, Rodriguez-Herva, Ramos, & Ramos-Gonzalez, 2007; Ramos-Gonzalez, Campos, & Ramos, 2005; Silby & Levy, 2004) have identified diverse genes that are differentially regulated by exposure to roots or root exudates. Among the bacterial pathways expressed during rhizosphere colonization are those that control the utilization of plant-derived metabolites (Camacho-Carvajal, 2001; Lugtenberg & Kamilova, 2009; Simons et al., 1996, 1997), motility and chemotaxis (de Weert et al., 2002; Lugtenberg & Kamilova, 2009), phase variation (Dekkers, Phoelich, van der Fits, & Lugtenberg, 1998; Sanchez-Contreras et al., 2002; van den Broek, Bloemberg, & Lugtenberg, 2005), outer membrane integrity (de Weert et al., 2006; Lugtenberg & Kamilova, 2009); and the ability to sequester limiting resources (Raaijmakers et al., 1995) and resist

environmental stresses (Miller & Wood, 1996; Sarniguet, Kraus, Henkels, Muehlchen, & Loper, 1995; Schnider-Keel, Lejbolle, Baehler, Haas, & Keel, 2001; van Veen, van Overbeek, & van Elsas, 1997). In its spatial and temporal properties, root colonization resembles biofilm formation, and biofilm-related pathways have been implicated in adhesion to seeds, roots and the process of rhizosphere colonization (Espinosa-Urgel, Salido, & Ramos, 2000; Fuqua, 2010; Hinsa, Espinosa-Urgel, Ramos, & O'Toole, 2003; Martinez-Gil, Yousef-Coronado, & Espinosa-Urgel, 2010; Nielsen et al., 2011; Yousef-Coronado, Travieso, & Espinosa-Urgel, 2008). Importantly, root exudates strongly influence the expression of a wide variety of plant growth promotion and biocontrol genes (Vacheron et al., 2013). Drought stress increases the quantity and changes the composition of root exudates (Boeuf-Tremblay, Plantureux, & Guckert, 1995; Grayston, Campbell, Vaughan, & Jones, 1995; Henry, Doucette, Norton, & Bugbee, 2007; Reid, 1974; Song, Han, Zhu, & Herbert, 2012; Walker, Bais, Grotewold, & Vivanco, 2003), but how these changes influence rhizobacteria is poorly understood.



## **8. Dryland wheat and *Pseudomonas* as a model for studying rhizosphere plant-microbe interaction under water stress**

### **8.1 Inland Pacific Northwest: A unique dryland wheat-producing region**

The Inland Pacific Northwest (IPNW) of the USA includes central and eastern Washington, northeastern Oregon, and northern Idaho. At the heart of this region is the Columbia Plateau, a 90,059-km<sup>2</sup> (34,772 mi<sup>2</sup>) area covered with arid sagebrush steppe and native grasses that extends from eastern and central Washington into northern Oregon (Sleeter, 2012) (Fig. 1). Soils of the IPNW are predominantly derived from windblown sediments called loess (50–70% silt and less than 25% sand) that formed during interglacial floods of the late Pleistocene (15,400–13,100 years ago) and early Holocene (13,100 years ago–present) eras and were distributed by wind erosion (Sweeney, Busacca, & Gaylord, 2005). The region also received volcanic ash from eruptions of the Mount Mazama (ca. 7700 years ago) and Mount St. Helens (40 years ago). The band of ash from the eruption of Mount St. Helens is still visible in the soil profile.

In the drier western parts of the region, soils are characterized by neutral pH, less than 1% organic matter, higher percent sand and lower percent clay



**Fig. 1** Left, the low (150–300 mm annually) precipitation zone of the Columbia Plateau of the Inland PNW (shaded) covers 1.6 million cropland hectares and is the largest contiguous cropping zone in the western U.S. Intermediate (300–450 mm) and high (450–600) precipitation zones are mostly to the east of the low-precipitation zone (left). Dryland wheat fields in April near Ritzville, WA (right).

(Ownley, Duffy, & Weller, 2003). In contrast, in the eastern wet-cold areas, soils are more acidic with greater than 2% organic matter and contain a higher percent clay and less sand. Much of the IPNW is well suited to dry-land crops and this geographic region has been farmed continuously for 135 years (Schillinger & Papendick, 2008). Small grains, especially winter wheat, are the dominant crop in the IPNW, and Washington, Oregon, and Idaho collectively produce over 18% of the U.S. wheat crop (Karimi, Stockle, Higgins, & Nelson, 2018). Five different classes of wheat (i.e., soft white, hard red winter, hard red spring, hard white, durum) are grown in the IPNW, with most of the wheat acreage located in the low and intermediate precipitation areas that receive annually between 250 and 400 mm of precipitation in the form of snow and rain. Soft white wheat is the dominant type of wheat grown and it is primarily exported to Asian markets. The IPNW is unique in that it also produces club wheat, a subclass of soft white wheat characterized by short thick compact club-shaped spikes that is highly adapted to growth under dryland conditions, especially in a wheat-fallow rotation.

The IPNW agroecosystem has six agroclimatic zones that differ in soil depth, annual precipitation, and cumulative growing degree days (Douglas, Rickman, Klepper, & Zuzel, 1992). Zone 1 (cold-moist) includes high elevation areas with over 400 mm of annual precipitation and less than 700 cumulative growing degree days per year. Most of this zone encompasses mountains and wilderness areas, and only 10–15% of it is cultivated. Zone 2 (cool-moist) has 700–1000 growing degree days and a significantly deeper and fertile soil profile than Zone 1. It includes the Palouse region with its deep loess soils that contain considerable amounts of organic matter. In fact, these conditions make the Palouse the most productive wheat growing area in the world. Zone 3 (cool-deep-moderately dry) has moderately deep loess-derived soils with less organic matter than Zones 1 and 2. It marks the transition to annual crop-fallow rotations and occasionally receives 350–400 mm of annual precipitation, which is too little to support an annual crop. Zone 4 (cool-shallow-dry) includes rocky scrubland and rangeland areas with shallow (less than 1 m) loess soils over basalt bedrock. It receives between 250 and 400 mm annual precipitation and has fewer than 1000 growing degree days. Zone 5 (cool-deep-dry) has deep soils and a near optimum growing season, with cool to cold and moist winters and warm and dry summers but insufficient precipitation for annual cropping (150–300 mm annually). Therefore, the alternate winter wheat-summer fallow rotation has been the dominant cropping system since the onset of farming in this

region. About 90% of this zone is cultivated and includes most of the dryland agriculture of Washington State and northern Oregon. It is notable that this low-precipitation part of the IPNW of 1.6 million cropland hectares constitutes the largest contiguous crop production region in the western U.S. (Schillinger & Papendick, 2008) (Fig. 1). Zone 6 (hot-very dry), located in the rain shadow of the Cascade Mountains, has hot summers and receives very low annual precipitation. It is unsuited for growing crops unless they are irrigated.

Wheat farming in the low-precipitation region of the IPNW (Zone 5) is challenging due to adverse environmental problems and pests. In the absence of irrigation, wheat growers are restricted to planting winter wheat every other year in a wheat-summer fallow rotation. Climate change is expected to reduce the amount of water that accumulates in the soil profile during the fallow year. The traditional fallowing requires several passes with tilling equipment and the application of herbicides to eliminate volunteer wheat and weeds that if not controlled, would quickly reduce stored water in the soil profile needed for the next wheat crop (Schillinger, Papendick, Guy, Rasmussen, & van Kessel, 2006). However, these practices exacerbate soil erosion (Schillinger & Papendick, 2008). Conventional tillage turns under crop residues and exposes the topsoil to the action of wind and water. To reduce the environmental costs of conventional tillage, many IPNW wheat growers in all zones are switching to some form of reduced tillage or no-till and direct-seed systems. Conservation tillage and no-till systems help to control wind erosion and airborne particulates, reduce operating costs, retain soil moisture, increase soil organic matter and microbial activity (Cook, 2006; Huggins & Reganold, 2008). No-till fields capture significantly more soil carbon than conventionally tilled fields, thus serving as C sinks for atmospheric CO<sub>2</sub> (Ogle et al., 2019).

## 8.2 Interplay between environmental factors, pathogens and beneficial microorganisms in the rhizosphere of dryland wheat

Major barriers to greater adoption of reduced- or no-till farming by IPNW wheat farmers are yield losses due to diseases caused by soilborne fungal pathogens and parasitic nematodes. Soilborne diseases increase significantly within the first few years of the transition from conventional to no-till and direct seeding, resulting in significantly reduced yields (Schroeder & Paulitz, 2006). The primary diseases of dryland cereals include take-all, caused by *Gaeumannomyces graminis* var. *tritici* (Ggt); root and crown rots caused by

*Fusarium culmorum* and *F. pseudograminearum*, *Rhizoctonia solani* AG-8 and *R. oryzae*; and damping-off caused by a complex of *Pythium* spp. (Paulitz, Smiley, & Cook, 2002). In addition, parasitic nematodes such as root-lesion nematodes (*Pratylenchus* spp.) (Smiley, Yan, & Gourlie, 2014) are now recognized as yield-limiting factors in dryland cropping systems. Among these diseases, take-all is most severe on wheat in irrigated dryland fields and in areas with higher precipitation (>450 mm annually). However, a form of the disease known as “dryland take-all” also occurs. *G. graminis* var. *tritici* hyphae infect and rot the roots, blocking vascular tissues, reducing water uptake and ultimately resulting in stunted or dead plants. A common symptom of take-all is bleached empty spikes (white heads) due to choked off water transport by the pathogen. In addition to take-all, cereals grown in wet-cold parts of the IPNW are also affected by root rot and damping-off caused by a complex of *Pythium* spp. (Paulitz & Adams, 2003). *Pythium* root rot is considered the “common cold” of root diseases because the pathogens are ubiquitous in soils and always cause some level of damage to young roots even when above-ground symptoms are not visible. *Fusarium* crown rot, caused by *Fusarium culmorum* and *F. pseudograminearum*, is a chronic and often severe problem of wheat in non-irrigated dryland fields. Symptoms include lesions on subcrown internodes and premature death of spikes (whiteheads) or the entire plant due to rotting crown tissue and lower stem internodes caused by blocked water transport (Cook & Veseth, 1991). Another serious disease of dryland wheat and barley is *Rhizoctonia* root rot caused by *R. solani* AG-8 and *R. oryzae*. These pathogens attack the root system, causing root rot and stunting that appears as uneven stands in the field (Paulitz et al., 2002). During an acute phase of the disease called “bare patch,” severely stunted or dead plants occur in irregular patches that can range from only a few centimeters in size to tens of meters in diameter (Weller et al., 1986) (Fig. 2). *Rhizoctonia* root rot and bare patch occur primarily in no-till or reduced-till cropping systems (Macnish, 1985; Rovira, 1986) and are greatly reduced by tilling the soil, but the reasons for this are not fully understood. Tillage may break up hyphal networks of the pathogen that develop in undisturbed soil and stubble or increase the suppressive microbial activity of the soil microbiome. *Rhizoctonia* root rot is also exacerbated when wheat is sown after weeds and volunteer wheat from a previous crop are killed by herbicide treatment. *Rhizoctonia* rapidly colonizes the dying plants and if the new crop is planted too soon afterwards, pathogen inoculum that has built up on the dying roots can “bridge” to the new crop, causing severe disease (Smiley, Ogg, & Cook, 1992). Thus, it is



**Fig. 2** Rhizoctonia bare patch of dryland wheat at the Ron Jirava Farm near Ritzville, WA (left). Antagonistic activity of *Pseudomonas* spp. isolated from the rhizosphere of dryland wheat against plant pathogens *G. graminis* var. *tritici* and *R. solani* AG-8 (right).

recommended that farmers wait at least 2 weeks after herbicide application before sowing a new crop, which allows time for the *Rhizoctonia* inoculum on those plants to decline.

Although soilborne pathogens represent a major constraint to wheat production in the IPNW and worldwide, farmers must control these pathogens primarily through management practices. Unlike with foliar pathogens, commercial cultivars lack resistance or tolerance to most necrotrophic soilborne pathogens such as *Pythium* and *Rhizoctonia*. Fungicides are commonly applied to seed, which is effective during the seedling phase but does not protect the root system throughout the entire growing season (Raaijmakers, Paulitz, Steinberg, Alabouvette, & Moenne-Loccoz, 2009). Cultural methods such as conventional tillage or control of the so-called green bridge can have significant environmental costs. The use of microbial communities capable of suppressing pathogens is a sustainable, economical, and environmentally friendly alternative strategy for managing soilborne disease.

Disease-suppressive soils are those in which a susceptible plant is grown in the presence of the pathogen and in an environment favorable for disease to develop, but disease does not, or develops and then declines. Suppressive soils are the best examples of how antagonistic microorganisms within the soil and root microbiome serve as defenders against soilborne pathogens. Such soils owe their activity to a combination of “general” and “specific” suppression (Weller, Raaijmakers, Gardener, & Thomashow, 2002). General suppression occurs in all soils and is the limited ability of the total microflora to restrict pathogen attack through multi-trophic interactions. In contrast, specific suppression, which is less common and much more

powerful than general suppression, is linked to the buildup and activity of individual or distinct groups of microorganisms that inhibit a specific soil-borne pathogen via antibiosis, parasitism, competition, predation, induced resistance, or a combination of mechanisms. A key characteristic of specific suppression (but not general suppression) is transferability, the ability of a small amount of suppressive soil added to a (non-suppressive) conducive soil, to convert it to a suppressive state. Specific suppression is eliminated by heat treatment or fumigation of the soil (Mazzola, 2002; Schlatter, Kinkel, Thomashow, Weller, & Paulitz, 2017). “Induced” specific suppressiveness is initiated and sustained by monoculture of a susceptible crop and is triggered by a severe outbreak of a disease. Other examples of suppressive soils are called “long-standing” because the origin of the specific suppression is not known (Schlatter et al., 2017).

In the IPNW, there are two well-characterized examples of “induced” specific suppression of root pathogens in wheat cropping systems. The first is the decline of take-all disease in fields under irrigation and in the higher rainfall zones (Weller et al., 2007, 2002). When wheat is grown in continuous monoculture, the incidence and severity of take-all increase for several years, and then spontaneously decline, even though inoculum of the pathogen persists and is easily isolated from the roots (Kwak & Weller, 2013). The second example is suppression of Rhizoctonia root rot and bare patch (Yin et al., 2013). Like take-all decline, Rhizoctonia decline requires several years of wheat or barley monoculture, after which the bare patches decrease to negligible levels and yields rebound. The basis of the suppressiveness of take-all and *Rhizoctonia* decline are microbiological; they are transferable to conducive soils and are eliminated by soil pasteurization (Schlatter et al., 2017). Take-all and Rhizoctonia suppressive soils differ in the type of antagonists responsible for pathogen suppression. Take-all decline involves the buildup of populations of bacteria in the *Pseudomonas fluorescens* complex that produce the antibiotic 2,4-diacetylphloroglucinol (DAPG) in the rhizosphere (Schlatter et al., 2017). However, further studies indicate that additional, related microbes may assist in the buildup of DAPG producers in the rhizosphere (M.K. LeTourneau & D.M. Weller, unpublished). Rhizoctonia decline involves multiple antagonistic copiotrophs including members of the *Flavobacteriaceae*, *Oxalobacteraceae*, *Chitinophagaceae*, and *Pseudomonadaceae*. Also implicated is a possible role for *Pedobacter*, *Variovorax*, and nitrogen-fixing *Rhizobium*. Significantly, both of these examples of suppressive soils involve members of the *Pseudomonas fluorescens* complex, which includes numerous highly competitive copiotrophic rhizosphere colonists capable of suppressing a wide range of pathogens.

### 8.3 Soil moisture, population levels and activity of indigenous antibiotic-producing rhizosphere *Pseudomonas*

*Pseudomonas* spp. are ubiquitous *Gammaproteobacteria* that utilize a wide array of organic compounds, produce diverse secondary metabolites and antibiotics, colonize eukaryotic hosts, and inhibit plant and animal pathogens (Moore et al., 2006; Schroth, Hildebrand, & Panopoulos, 2006; Yahr & Parsek, 2006). Multilocus sequence and phylogenomic analyses have divided *Pseudomonas* into 14 lineages and over 180 species (Hesse et al., 2018), and no doubt the number of new species will continue to increase. The *P. fluorescens* lineage contains the largest number of species, comprising 8–10 phylogroups (Garrido-Sanz et al., 2017, 2016; Hesse et al., 2018; Mulet, Lalucat, & Garcia-Valdes, 2010) and a high proportion of strains with plant growth-promoting and biocontrol activity (Zboralski & Filion, 2020). *Pseudomonas* spp. colonizing roots of IPNW wheat belong primarily to the *P. fluorescens* lineage and protect wheat against biotic and abiotic stresses. For example, strains of *P. fluorescens* and *P. brassicacearum*, responsible for take-all decline, belong to the *P. corrugata* phylogroup and are abundant on wheat grown under irrigation or in zones with high precipitation (Mavrodi, Mavrodi, Parejko, Thomashow, & Weller, 2012). They are rarely found on wheat in fields that receive under 380 mm of annual precipitation. They carry the *phlACBDEF* locus that functions in regulation, synthesis, and export of 2,4-diacetylphloroglucinol (DAPG), the antibiotic that has a major role in the suppression of *G. graminis* var. *tritici* (Banger & Thomashow, 1999; Bonsall, Weller, & Thomashow, 1997; Raaijmakers & Weller, 1998). *P. brassicacearum* is especially noteworthy because it is found in all IPNW soils in which take-all has been suppressed and it can be a minor pathogen of both tomato and wheat at doses above  $10^6$  colony-forming units per gram of tissue (Yang, Mavrodi, Mavrodi, Thomashow, & Weller, 2019). Wheat cultivars differ significantly in their response to *P. brassicacearum* and those with the greatest tolerance are the best protected against take-all by the bacterium.

A second group of pseudomonads in the *P. fluorescens* phylogroup colonize dryland wheat and belong to the species *P. synxantha*, *P. orientalis*, and *P. aridus* (Biessy et al., 2019; Parejko, Mavrodi, Mavrodi, Weller, & Thomashow, 2013). They are widespread in low-precipitation wheat-summer fallow (WW-SF) fields of agroclimatic Zone 5 and carry genes for the synthesis of the antibiotic phenazine-1-carboxylic acid (PCA), which is linked with their ability to control take-all (Mavrodi, Mavrodi, Parejko, Bonsall, et al., 2012; Mavrodi et al., 2010; Thomashow & Weller, 1988)

(Fig. 2). Their abundance in dryland wheat also suggests a role for them in the natural suppression of *Rhizoctonia* root rot (Jaaffar, Parejko, Paulitz, Weller, & Thomashow, 2017; Mavrodi, Mavrodi, Parejko, Bonsall, et al., 2012) and *Fusarium* crown rot. Interestingly, a recent metagenome analysis of dryland wheat collected at Washington State University's Lind Dryland Research Station demonstrated that, in addition to PCA-producing *P. fluorescens*-like strains, arid IPNW soils contain an abundance of phenazine-producing bacteria of the *P. chlororaphis* phylogroup (Dar, Thomashow, Weller, & Newman, 2020). That study also revealed that most sampled plants harbored *Streptomyces* spp. with gene clusters predicted to synthesize multiple chemically diverse phenazines. Unlike PCA-producing pseudomonads which favor the plant rhizosphere, these phenazine-producing streptomycetes and other actinobacteria also were abundant in bulk soil.

Mavrodi, Mavrodi, Parejko, Bonsall, et al. (2012) and Mavrodi, Mavrodi, Parejko, Thomashow, et al. (2012) reported that 80–100% of winter wheat plants collected from 61 commercial farm fields in the low precipitation zone were colonized by PCA-producing pseudomonads. These pseudomonads were not only widely distributed throughout the region but also maintained high populations sizes ( $10^5$ – $10^6$  CFU/g of root) and produced copious amounts (up to 1.6  $\mu\text{g/g}$  of root) of PCA in the rhizosphere of wheat and barley (Mavrodi, Mavrodi, Parejko, Bonsall, et al., 2012). Surprisingly, they were scarce or non-detectable in irrigated fields or neighboring higher precipitation areas that received greater than 380 mm of annual precipitation. Thus, soil moisture was the major factor modulating the development of PCA-producing populations on roots of wheat. The abundance of PCA producers was inversely related to annual precipitation (or irrigation), suggesting that these rhizobacteria are adapted to the cereals growing in low precipitation areas. A subsequent 3-year field study that compared microbiomes of wheat growing in adjacent irrigated and dryland plots at the WSU Lind Dryland Research Station supported these results (Mavrodi et al., 2018). That 16S amplicon sequencing analysis revealed that the availability of water in a dryland agroecosystem strongly affected the belowground wheat microbiome, including multiple taxa within *Bacteroidetes* and *Proteobacteria*. Differences in the composition of the rhizosphere microbiomes from irrigated and dryland wheat plots strongly correlated with changes in the water potential and soil pH, whereas seasonal shifts correlated with precipitation and temperature. The study also demonstrated that irrigation of dryland wheat negatively

affected PCA-producing *Pseudomonas*, resulting in reduced population sizes, plant colonization frequencies, and amounts of PCA recovered the roots. These observations confirm the hypothesis that soil moisture acts as a critical environmental factor to influence the dynamics and activity of indigenous *Pseudomonas* rhizobacteria that contribute to the natural suppression of soilborne pathogens of wheat in the IPNW.

#### **8.4 Microbial phenazines contribute to the ability of rhizosphere *Pseudomonas* to alleviate water stress in wheat**

The plant-driven selection of phenazine-producing *Pseudomonas* spp. in response to limited soil moisture and over a wide area of the IPNW suggests that dryland wheat gains benefits other than plant defense from these rhizobacteria. To address this possibility, Yuan, Pan, Boak, Pierson, and Pierson (2020) challenged wheat treated with another phenazine producer, *P. chlororaphis* 30-84, under osmotic stress or water replete conditions. Seedlings colonized with strain 30-84 or its phenazine-deficient and phenazine-overproducing mutant variants exhibited growth promotion under water-replete conditions, but under salt stress, the effect was dependent on the wheat variety and the capacity of the bacteria to produce phenazines. In addition, reactive oxygen species and catalase activity were increased in leaf tissues only when salt-stressed plants were treated with phenazine-producing derivatives of *P. chlororaphis* 30-84. In a related experiment (Mahmoudi, Yu, Liu, Pierson, & Pierson, 2019), seedlings inoculated with strain 30-84 or its phenazine-overproducing variant exhibited less severe water deficit symptoms after a week of water withdrawal than did noninoculated plants or plants treated with a phenazine-deficient mutant. Following a recovery period, plants treated with phenazine producers also had significantly higher survival rates. A second cycle of water withdrawal reduced the survival of control plants to  $\leq 10\%$ , whereas the survival of plants colonized by phenazine producers remained at 50%. In addition, in a 10-day severe water deficit experiment, plants treated with the phenazine-overproducing variant demonstrated threefold better survival than did the control group. Colonization by the phenazine producers resulted in wheat plants with higher relative water content and more robust root systems a greater number of root tips. Together, these findings indicate that phenazine-producers significantly improved the tolerance of wheat to drought stress.

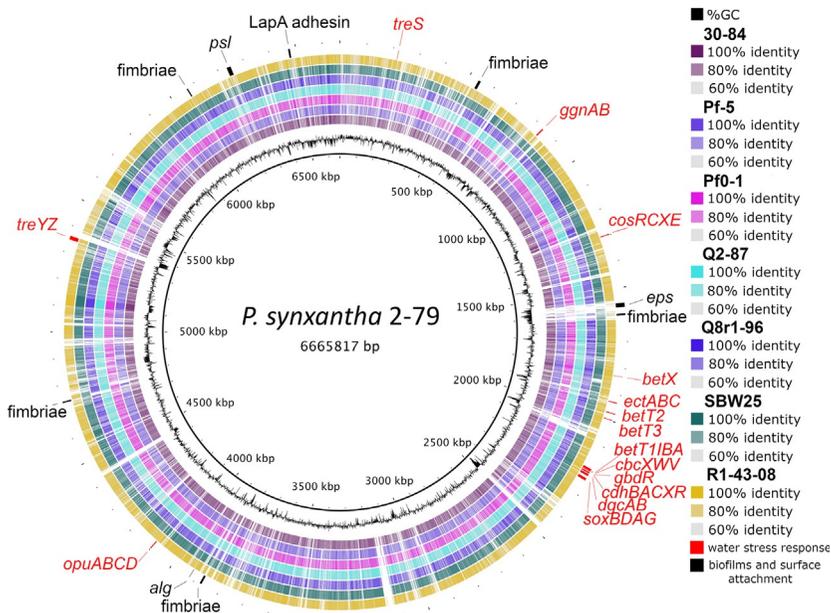
One mechanism underpinning water-deficit avoidance by wheat colonized by phenazine producers may lie in the ability to of the bacteria to

modulate biofilm formation. In *P. chlororaphis* (Maddula, Pierson, & Pierson, 2008) and especially *P. aeruginosa* (Ramos, Dietrich, Price-Whelan, & Newman, 2010) phenazines stimulate biofilm formation by acting as alternative electron acceptors (Dietrich et al., 2013), promoting the release of extracellular DNA (Das & Manefield, 2012), and indirectly affecting levels of the second messenger cyclic di-GMP and production of extracellular polysaccharide (EPS) (Okegbe et al., 2017). Previous studies have demonstrated that the secretion of microbial exopolysaccharides improves soil structure and increases retention of water in the rhizosphere, promoting plant growth (Chang et al., 2007; Naseem & Bano, 2014a). Similar mechanisms may underpin the ability of phenazine-producing strains of *Pseudomonas* to alleviate water stress in dryland wheat. LeTourneau et al. (2018) used a combination of bioreporters, stable isotope probing and nano-scale resolution secondary ion mass spectrometry (NanoSIMS) to investigate the effect of soil moisture in the rhizosphere of wheat on the dynamics of PCA production and formation of biofilms by *P. synxantha* 2-79 (formally *P. fluorescens* 2-79). The study revealed that phenazine gene expression and levels of PCA positively correlated with soil moisture in the rhizosphere of wheat. The presence of PCA affected the turnover of microbial biomass in the rhizosphere and rates of incorporation of bacterial  $^{15}\text{N}$  into wheat roots. Under water-limiting conditions, the phenazine-producing strain *P. synxantha* 2-79 formed more robust biofilms and secreted more EPS than its phenazine-deficient mutant. These results suggest the formation of rhizosphere biofilms and the secretion of extracellular matrices that retain moisture around plant roots play an important role in the ability of phenazine-producing *Pseudomonas* to alleviate water stress in dryland wheat.

### 8.5 Molecular mechanisms behind the adaptation of rhizosphere *Pseudomonas* to dryland wheat

The ongoing research in our groups aims to understand at the molecular level how rhizobacteria maintain physiological activity and tight mutualistic interactions with their plant hosts in dry soils. We use as model *P. synxantha* 2-79, a well-described biocontrol agent that was isolated from a field in Washington State that had been cropped to wheat for 14 consecutive years (Weller & Cook, 1983). This strain produces the antibiotic phenazine-1-carboxylic acid, which underpins its ability to suppress soilborne diseases of wheat, including the take-all pathogen *G. graminis* var. *tritici* (Weller & Cook, 1983), *Pythium aristosporum* (Thomashow & Weller, 1988), and

*R. solani* AG-8 (Jaaffar et al., 2017). 2-79 belongs to a complex of beneficial *P. fluorescens*-like bacteria that flourish in dryland wheat fields of the semi-arid Pacific Northwest, USA (Parejko et al., 2013). The analysis of the *P. synxantha* 2-79 genome has revealed the presence of multiple water stress response pathways that function in (i) de novo synthesis of microbial osmoprotectants, (ii) the uptake and catabolism of plant-derived quaternary amine osmoprotectants and their precursors, and (iii) pathways involved in the aggregation and formation of biofilms. Specifically, the 2-79 genome encodes two routes for the de novo synthesis of the osmoprotectant trehalose (Fig. 3). The first pathway consists of the maltooligosyltrehalose synthase TreY and maltooligosyltrehalose trehalohydrolase TreZ, which



**Fig. 3** Putative water stress response, biofilm and surface attachment pathways predicted in the genome of *P. synxantha* 2-79. Moving from the perimeter toward the center: circle 1, clusters of genes of potential importance for water stress response (red), biofilm formation and surface attachment to plant roots (black); circles 2, blastn comparison against the genome of *Pseudomonas* sp. R1-43-08; circle 3, blastn comparison against *P. fluorescens* SBW25; circle 4, blastn comparison against *P. brassicacearum* Q8r1-96; circle 5, blastn comparison against *P. fluorescens* Q2-87; circle 6, blastn comparison against *P. fluorescens* Pf0-1; circle 7, blastn comparison against *P. protegens* Pf-5; circle 8, blastn comparison against *P. chlororaphis* 30-84; circle 9, percentage G + C in a 2000-bp window. The inner scale designates the coordinates in kilobase pairs.

form trehalose via degradation of cytosolic glucans (Chandra, Chater, & Bornemann, 2011). The second pathway consists of the trehalose synthase TreS, which catalyzes a reversible conversion of maltose to trehalose. The strain also can produce the osmoprotectant *N*-acetyl glutaminyl glutamine amide (NAGGN) through the concerted action of the *N*-acetylating and peptide bond-forming amidotransferase GgnA and the GNAT superfamily *N*-acetyltransferase GgnB.

The 2-79 genome encodes an extensive set of pathways that function in the uptake and catabolism of quaternary amine compounds (QACs). Genome analysis suggested that QACs are taken up by two distinct classes of membrane proteins: (i) ATP-binding cassette (ABC) transporters, which use ATP to actively transport substrates, and (ii) transporters of the betaine-carnitine-choline (BCCT) family that are energized by proton symport. 2-79 has two QAC-specific ABC transporters (CbcWV and OpuC) and three BCCT family transporters (BetT1, BetT2, and BetT3). The genome encodes pathways involved in the catabolism of choline, glycine betaine (GB), carnitine, choline-*O*-sulfate, and sarcosine, as well several regulatory genes (including *gbdR*) that govern the transport and utilization of QACs and detoxification of the catabolic byproducts. Finally, the genome of 2-79 contains multiple genes that are predicted to function in the formation of biofilms. The strain carries a 12-gene *alg* operon that is involved in the synthesis of the capsular exopolysaccharide alginate, as well as a cluster of 11 co-transcribed genes that function in the production of the aggregative exopolysaccharide Psl (Mann & Wozniak, 2012). The genome also contains a 15-gene cluster that encodes multiple polysaccharide biosynthesis and modification enzymes. We preliminarily termed the locus *eps* and included it in our experiments. In addition to 2-79, this locus is present in several other *P. fluorescens* strains but absent from *P. aeruginosa*, *P. syringae*, or *P. putida*. The presence of a putative *O*-antigen ligase suggests that *eps* genes may not be involved in the production of an aggregative exopolysaccharide, but rather function in the modification of *O*-antigen.

We hypothesized that the abundance of beneficial 2-79-like pseudomonads in the rhizosphere of dryland cereals grown across the IPNW reflects the fact that their metabolic make-up is adapted to carbon and nitrogen resources exuded by roots under low moisture conditions. To test this hypothesis, we produced sterile root exudates of *Brachypodium distachyon*, a temperate grass used as a model species for economically important monocot plants that include wheat, barley, rye and oats, and also biofuel grasses (Vogel et al., 2010) (Fig. 4). The metabolomic profiling of root exudates



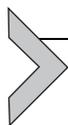
**Fig. 4** Collection of sterile root exudates from hydroponically grown seedlings of the model monocot plant *B. distachyon* Bd21.

revealed the presence of over 140 different plant-derived metabolites, 85 of which were identified as various carbohydrates, sugar alcohols, amino acids, organic, terpenoids, phenolics and heterocyclic compounds. Most importantly, we confirmed that root exudates contain the quaternary ammonium compounds (QACs), trehalose, mannitol, sorbitol, proline, and glutamine, which act as compatible solutes in plants or microorganisms (Yancey, Clark, Hand, Bowlus, & Somero, 1982). We also used Phenotype Microarray PM1 and PM2 plates (Biolog) to profile *P. synxantha* 2-79 for the utilization of 190 different carbon sources. Results of that analysis identified 82 organic compounds that supported the growth of this strain as carbon and/or nitrogen source. We further matched the metabolic profile of 2-79 against the list of plant-derived metabolites from root exudates. Interestingly, 37 carbon and nitrogen sources from the Phenotype Microarray panel were also present in the root exudates and catabolized by *P. synxantha* 2-79.

The transcriptome responses of *P. synxantha* 2-79 to plant exometabolites were further analyzed by subjecting cultures grown in the presence of root exudates to RNA-seq analysis. The exposure to exudates resulted in the induction and repression of, respectively, 77 and 153 genes. Based on the cross-genome comparisons, these differentially expressed genes were almost equally distributed between the core (50%) and non-core (46.9%) parts of the genome, whereas the strain-specific singleton genes constituted on average only 3.1%. The differentially expressed 2-79 genes were also subjected to Blast2Go analysis and Gene Ontology (GO) annotation. Metabolic process, catalytic activity, and membrane were the most common annotation terms

across of the three primary GO term categories (i.e., biological process, molecular function, and cellular component). Within the biological process category, metabolic process, cellular process, localization, response to stimulus, and regulation were over-represented. Within the molecular function category, the most significant proportion was assigned to catalytic activity, binding, and transporter activity categories. Within the cellular component, the majority were assigned to membrane, membrane part, cell, and cell part categories. Genes downregulated in response to root exudates were those involved in the uptake and metabolism of sulfur compounds (sulfonates and taurine) and encoding a prophage. Some notable upregulated pathways function in metal ion homeostasis (iron, copper) and the uptake and/or catabolism of carbohydrates (fructose, xylose, trehalose, arabinose, myo-inositol), phenolics, and quaternary ammonium compounds (QACs), including glycine betaine (GB).

In many species of soil- and water-dwelling bacteria (including *Pseudomonas*) glycine betaine (GB) is derived from choline and functions as a key osmoprotectant (Wargo, 2013). The genome analysis revealed that *P. synxantha* 2-79 does not synthesize choline de novo but is well equipped to import it and to either catabolize or convert it to GB. Our results also suggest that root exudates serve as a primary source of choline for 2-79. We further established that the quaternary amines GB, choline and carnitine efficiently protect 2-79 from water stress. The important role of plant-derived QACs for the survival of 2-79 in a water-stressed rhizosphere was evident from the fact that a mutant devoid of all QAC transporters was less competitive in the colonization of *Brachypodium* than the wild-type strain. Collectively, our results demonstrate the importance of root exudate-derived osmoprotectants for microbial adaptation to the rhizosphere lifestyle and support the idea that the exchange of metabolites between plant roots and microorganisms profoundly affects and shapes the belowground plant microbiome under water stress.



## 9. Conclusions

Current levels of agricultural productivity must increase by 50% by the year 2050 to meet the calorie demands of a projected world population of at least 9 billion people. Climate change is undoubtedly the most important challenge facing farmers globally as we move toward this goal because general circulation models forecast a grim picture of significant changes in temperature, extreme weather events and precipitation patterns. Extremes

in weather already are evident in the form of 100-year floods that now occur every few years, more intense and frequent forest fires, rising sea levels, and record annual temperature extremes. Farmers know the weather is unpredictable, but they traditionally depend on a stable climate that has guided their decisions about what crops and cultivars to plant, planting and harvest dates and management of agricultural pests. The impact of an increasingly unstable climate will be most impactful on dryland cropping systems that comprise over 40% of the world's arable land. Typically, dryland cropping systems receive enough annual precipitation to meet only a quarter to half of the water demands of the crops grown. Rising temperatures and reduced precipitation during the growing season will push significant areas beyond the range of arability without supplemental irrigation, and water itself is already a limited resource. There is a critical need for research over the next 30 years and beyond to meet the challenges of climate change in dryland cropping systems. In particular, a better understanding of complex feedbacks between plants and associated microbes is crucially important for our ability to harness the rhizosphere microbiome for maximizing yields and crop resilience to drought, salinity, and other forms of abiotic and biotic stress. This knowledge will contribute to the development of a more holistic view of agroecosystems, as well as more sustainable farming practices focused on water conservation, biological approaches to control insects, pathogens and weeds, accelerated development of climate-resilient crops, and new technologies that incorporate better management of plant-associated microbiota, especially commensal and beneficial microbes that contribute to crop health.

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## References

- Adriaensen, K., Vralstad, T., Noben, J. P., Vangronsveld, J., & Colpaert, J. V. (2005). Copper-adapted *Suillus luteus*, a symbiotic solution for pines colonizing Cu mine spoils. *Applied and Environmental Microbiology*, *71*, 7279–7284.
- Aloni, R., Aloni, E., Langhans, M., & Ullrich, C. I. (2006). Role of cytokinin and auxin in shaping root architecture: Regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism. *Annals of Botany*, *97*, 883–893.

- Ansary, M. H., Rahmani, H. A., Ardakani, M. R., Paknejad, F., Habibi, D., & Mafakheri, S. (2012). Effect of *Pseudomonas fluorescens* on proline and phytohormonal status of maize (*Zea mays* L.) under water deficit stress. *Annals of Biological Research*, *3*, 1054–1062.
- Armada, E., Roldan, A., & Azcon, R. (2014). Differential activity of autochthonous bacteria in controlling drought stress in native *Lavandula* and *Salvia* plants species under drought conditions in natural arid soil. *Microbial Ecology*, *67*, 410–420.
- Badri, D. V., & Vivanco, J. M. (2009). Regulation and function of root exudates. *Plant, Cell and Environment*, *32*, 666–681.
- Badri, D. V., Weir, T. L., van der Lelie, D., & Vivanco, J. M. (2009). Rhizosphere chemical dialogues: Plant-microbe interactions. *Current Opinion in Biotechnology*, *20*, 642–650.
- Bais, H. P., Prithiviraj, B., Jha, A. K., Ausubel, F. M., & Vivanco, J. M. (2005). Mediation of pathogen resistance by exudation of antimicrobials from roots. *Nature*, *434*, 217–221.
- Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., & Vivanco, J. M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology*, *57*, 233–266.
- Bangera, M. G., & Thomashow, L. S. (1999). Identification and characterization of a gene cluster for synthesis of the polyketide antibiotic 2,4-diacetylphloroglucinol from *Pseudomonas fluorescens* Q2-87. *Journal of Bacteriology*, *181*, 3155–3163.
- Barret, M., Frey-Klett, P., Guillermin-Erckelboudt, A. Y., Boutin, M., Guernec, G., & Sarniguet, A. (2009). Effect of wheat roots infected with the pathogenic fungus *Gaeumannomyces graminis* var. *tritici* on gene expression of the biocontrol bacterium *Pseudomonas fluorescens* Pf29Arp. *Molecular Plant-Microbe Interactions*, *22*, 1611–1623.
- Basu, S., Ramegowda, V., Kumar, A., & Pereira, A. (2016). Plant adaptation to drought stress. *F1000Research*, *5*, 1554.
- Belimov, A. A., Dodd, I. C., Safronova, V. I., Dumova, V. A., Shaposhnikov, A. I., Ladatko, A. G., et al. (2014). Abscisic acid metabolizing rhizobacteria decrease ABA concentrations in planta and alter plant growth. *Plant Physiology and Biochemistry*, *74*, 84–91.
- Biessy, A., Novinscak, A., Blom, J., Leger, G., Thomashow, L. S., Cazorla, F. M., et al. (2019). Diversity of phytobeneficial traits revealed by whole-genome analysis of worldwide-isolated phenazine-producing *Pseudomonas* spp. *Environmental Microbiology*, *21*, 437–455.
- Bloembergen, G. V. (2007). Microscopic analysis of plant-bacterium interactions using auto fluorescent proteins. *European Journal of Plant Pathology*, *119*, 301–309.
- Boeuf-Tremblay, V., Plantureux, S., & Guckert, A. (1995). Influence of mechanical impedance on root exudation of maize seedlings at two development stages. *Plant and Soil*, *172*, 279–287.
- Bohnert, H. J., Nelson, D. E., & Jensen, R. G. (1995). Adaptations to environmental stresses. *Plant Cell*, *7*, 1099–1111.
- Bonsall, R. F., Weller, D. M., & Thomashow, L. S. (1997). Quantification of 2,4-diacetylphloroglucinol produced by fluorescent *Pseudomonas* spp. in vitro and in the rhizosphere of wheat. *Applied and Environmental Microbiology*, *63*, 951–955.
- Borlee, B. R., Goldman, A. D., Murakami, K., Samudrala, R., Wozniak, D. J., & Parsek, M. R. (2010). *Pseudomonas aeruginosa* uses a cyclic-di-GMP-regulated adhesin to reinforce the biofilm extracellular matrix. *Molecular Microbiology*, *75*, 827–842.
- Bota, J., Medrano, H., & Flexas, J. (2004). Is photosynthesis limited by decreased Rubisco activity and RuBP content under progressive water stress? *New Phytologist*, *162*, 671–681.
- Bouskill, N. J., Lim, H. C., Borglin, S., Salve, R., Wood, T. E., Silver, W. L., et al. (2013). Pre-exposure to drought increases the resistance of tropical forest soil bacterial communities to extended drought. *ISME Journal*, *7*(2), 384–394. <https://doi.org/10.1038/ismej.2>.

- Bresson, J., Varoquaux, F., Bontpart, T., Touraine, B., & Vile, D. (2013). The PGPR strain *Phyllobacterium brassicacearum* STM 196 induces a reproductive delay and physiological changes that result in improved drought tolerance in *Arabidopsis*. *New Phytologist*, *200*, 558–569.
- Camacho-Carvajal, M. M. (2001). *Molecular characterization of the roles of type 4 pili, NDH-I and PyrR in rhizosphere colonization of Pseudomonas fluorescens WCS365*. Doctoral dissertation University of Leiden.
- Carlson, R., Tugizimana, F., Steenkamp, P. A., Dubery, I. A., Hassen, A. I., & Labuschagne, N. (2020). Rhizobacteria-induced systemic tolerance against drought stress in *Sorghum bicolor* (L.) Moench. *Microbiological Research*, *232*, 126388.
- Castillo, P., Escalante, M., Gallardo, M., Alemanno, S., & Abdala, G. (2013). Effects of bacterial single inoculation and co-inoculation on growth and phytohormone production of sunflower seedlings under water stress. *Acta Physiologiae Plantarum*, *35*, 2299–2309.
- Chandra, G., Chater, K. F., & Bornemann, S. (2011). Unexpected and widespread connections between bacterial glycogen and trehalose metabolism. *Microbiology*, *157*, 1565–1572.
- Chang, W. S., Van De Mortel, M., Nielsen, L., De Guzman, G. N., Li, X., & Halverson, L. J. (2007). Alginate production by *Pseudomonas putida* creates a hydrated microenvironment and contributes to biofilm architecture and stress tolerance under water-limiting conditions. *Journal of Bacteriology*, *189*, 8290–8299.
- Chaves, M. M., Flexas, J., & Pinheiro, C. (2009). Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. *Annals of Botany*, *103*, 551–560.
- Chen, C., & Beattie, G. A. (2008). *Pseudomonas syringae* BetT is a low-affinity choline transporter that is responsible for superior osmoprotection by choline over glycine betaine. *Journal of Bacteriology*, *190*, 2717–2725.
- Cheng, Z., Park, E., & Glick, B. R. (2007). 1-Aminocyclopropane-1-carboxylate deaminase from *Pseudomonas putida* UW4 facilitates the growth of canola in the presence of salt. *Canadian Journal of Microbiology*, *53*, 912–918.
- Chenu, C., & Roberson, E. B. (1996). Diffusion of glucose in microbial extracellular polysaccharide as affected by water potential. *Soil Biology and Biochemistry*, *28*, 877–884.
- Cho, S. M., Kang, B. R., Han, S. H., Anderson, A. J., Park, J. Y., Lee, Y. H., et al. (2008). 2R,3R-butanediol, a bacterial volatile produced by *Pseudomonas chlororaphis* O6, is involved in induction of systemic tolerance to drought in *Arabidopsis thaliana*. *Molecular Plant-Microbe Interactions*, *21*, 1067–1075.
- Cho, S. M., Kim, Y. H., Anderson, A. J., & Kim, Y. C. (2013). Nitric oxide and hydrogen peroxide production are involved in systemic drought tolerance induced by 2R,3R-butanediol in *Arabidopsis thaliana*. *Plant Pathology Journal*, *29*, 427–434.
- Cohen, A. C., Bottini, R., & Piccoli, P. N. (2008). *Azospirillum brasilense* Sp 245 produces ABA in chemically-defined culture medium and increases ABA content in *Arabidopsis* plants. *Plant Growth Regulation*, *54*, 97–103.
- Cohen, A. C., Travaglia, C. N., Bottini, R., & Piccoli, P. N. (2009). Participation of abscisic acid and gibberellins produced by endophytic *Azospirillum* in the alleviation of drought effects in maize. *Botany*, *87*, 455–462.
- Cohen, A. C., Bottini, R., Pontin, M., Berli, F. J., Moreno, D., Boccanlandro, H., et al. (2015). *Azospirillum brasilense* ameliorates the response of *Arabidopsis thaliana* to drought mainly via enhancement of ABA levels. *Physiologia Plantarum*, *153*, 79–90.
- Coleman-Derr, D., & Tringe, S. G. (2014). Building the crops of tomorrow: Advantages of symbiont-based approaches to improving abiotic stress tolerance. *Frontiers in Microbiology*, *5*(283). doi:org/10.3389/fmicb.2014.00283.
- Cook, R. J. (2006). Toward cropping systems that enhance productivity and sustainability. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 18389–18394.

- Cook, R. J., & Veseth, R. J. (1991). *Wheat health management*. St. Paul: APS Press.
- Couee, I., Sulmon, C., Gouesbet, G., & El Amrani, A. (2005). Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. *Journal of Experimental Botany*, *57*, 449–459.
- Cui, G., Zhang, Y., Zhang, W., Lang, D., Zhang, X., Li, Z., et al. (2019). Response of carbon and nitrogen metabolism and secondary metabolites to drought stress and salt stress in plants. *Journal of Plant Biology*, *62*, 387–399.
- Curl, E. A., & Truelove, B. (1986). *The rhizosphere*. Berlin: Springer-Verlag.
- Dar, D., Thomashow, L. S., Weller, D. M., & Newman, D. K. (2020). Global landscape of phenazine biosynthesis and biodegradation reveals species-specific colonization patterns in agricultural soils and crop microbiomes. *eLife*, *9*, e59726.
- Das, T., & Manefield, M. (2012). Pyocyanin promotes extracellular DNA release in *Pseudomonas aeruginosa*. *PLoS One*, *7*, e46718.
- de la Fuente Canto, C., Simonin, M., King, E., Moulin, L., Bennett, M. J., Castrillo, G., et al. (2020). An extended root phenotype: The rhizosphere, its formation and impacts on plant fitness. *The Plant Journal*, *103*, 951–964.
- De Vries, F. T., Griffiths, R. I., Knight, C. G., Nicolitch, O., & Williams, A. (2020). Harnessing rhizosphere microbiomes for drought-resilient crop production. *Science*, *368*, 270–274.
- de Weert, S., Vermeiren, H., Mulders, I. H., Kuiper, I., Hendrickx, N., Bloemberg, G. V., et al. (2002). Flagella-driven chemotaxis towards exudate components is an important trait for tomato root colonization by *Pseudomonas fluorescens*. *Molecular Plant-Microbe Interactions*, *15*, 1173–1180.
- de Weert, S., Dekkers, L., Bitter, W., Tuinman, S., Wijffes, A., van Boxtel, R., et al. (2006). The two-component colR/S system of *Pseudomonas fluorescens* WCS365 plays a role in rhizosphere competence through maintaining the structure and function of the outer membrane. *FEMS Microbiology Ecology*, *58*, 205–213.
- De-la-Pena, C., Lei, Z., Watson, B. S., Sumner, L. W., & Vivanco, J. M. (2008). Root-microbe communication through protein secretion. *Journal of Biological Chemistry*, *283*, 25247–25255.
- Dekkers, L. C., Phoelich, C. C., van der Fits, L., & Lugtenberg, B. J. (1998). A site-specific recombinase is required for competitive root colonization by *Pseudomonas fluorescens* WCS365. *Proceedings of the National Academy of Sciences of the United States of America*, *95*, 7051–7056.
- Dietrich, L. E., Okegbe, C., Price-Whelan, A., Sakhtah, H., Hunter, R. C., & Newman, D. K. (2013). Bacterial community morphogenesis is intimately linked to the intracellular redox state. *Journal of Bacteriology*, *195*, 1371–1380.
- Dimkpa, C., Weinand, T., & Asch, F. (2009). Plant-rhizobacteria interactions alleviate abiotic stress conditions. *Plant, Cell and Environment*, *32*, 1682–1694.
- Dinnyeny, J. R. (2019). Developmental responses to water and salinity in root systems. *Annual Review of Cell and Developmental Biology*, *35*, 239–257.
- Dodd, I. C., Jiang, F., Teijeiro, R. G., Belimov, A. A., & Hartung, W. (2009). The rhizosphere bacterium *Variovorax paradoxus* 5C-2 containing ACC deaminase does not increase systemic ABA signaling in maize (*Zea mays* L.). *Plant Signaling & Behavior*, *4*, 519–521.
- Douglas, C. L. J., Rickman, R. W., Klepper, B. L., & Zuzel, J. F. (1992). Agroclimatic zones for dryland winter wheat producing areas of Idaho, Washington, and Oregon. *Northwest Science*, *66*, 26–34.
- Dubois, M., Van den Broeck, L., & Inze, D. (2018). The pivotal role of ethylene in plant growth. *Trends in Plant Science*, *23*, 311–323.
- Duca, D., Lorv, J., Patten, C. L., Rose, D., & Glick, B. R. (2014). Indole-3-acetic acid in plant-microbe interactions. *Antonie Van Leeuwenhoek*, *106*, 85–125.

- Edwards, J. A., Santos-Medellín, C. M., Liechty, Z. S., Nguyen, B., Lurie, E., Eason, S., et al. (2018). Compositional shifts in root-associated bacterial and archaeal microbiota track the plant life cycle in field-grown rice. *PLoS Biology*, *16*(2), e2003862. <https://doi.org/10.1371/journal.pbio.2003862>.
- Elbein, A. D., Pan, Y. T., Pastuszak, I., & Carroll, D. (2003). New insights on trehalose: A multifunctional molecule. *Glycobiology*, *13*, 17R–27R.
- Eldhuset, T. D., Nagy, N. E., Volařík, D., Børja, I., Gebauer, R., Yakovlev, I. A., et al. (2013). Drought affects tracheid structure, dehydrin expression, and above- and below-ground growth in 5-year-old Norway spruce. *Plant and Soil*, *366*, 305–320.
- Espinosa-Urgel, M., Salido, A., & Ramos, J. L. (2000). Genetic analysis of functions involved in adhesion of *Pseudomonas putida* to seeds. *Journal of Bacteriology*, *182*, 2363–2369.
- Estevez-Geffriaud, V., Vicente, R., Vergara-Diaz, O., Narvaez Reinaldo, J. J., & Trillas, M. I. (2020). Application of *Trichoderma asperellum* T34 on maize (*Zea mays*) seeds protects against drought stress. *Planta*, *252*, 8.
- Fan, X., Hu, H., Huang, G., Huang, F., Li, Y., & Palta, J. (2015). Soil inoculation with *Burkholderia* sp. LD-11 has positive effect on water-use efficiency in inbred lines of maize. *Plant and Soil*, *390*, 337–349.
- FAO. (2004). *Carbon sequestration in dryland soils*. *World soil resources reports 102*. Food and Agriculture Organization of the United Nations. <http://www.fao.org/3/y5738e/y5738e00.htm>.
- Felsmann, K., Baudis, M., Gimbel, K., Kayler, Z. E., Ellerbrock, R., Bruehlheide, H., et al. (2015). Soil bacterial community structure responses to precipitation reduction and forest management in forest ecosystems across Germany. *PLoS One*. doi:10:e0122539.
- Fernandez-Aunon, C., Hamouda, T. B., Iglesias-Guerra, F., Argandona, M., Reina-Bueno, M., Nieto, J. J., et al. (2010). Biosynthesis of compatible solutes in rhizobial strains isolated from *Phaseolus vulgaris* nodules in Tunisian fields. *BMC Microbiology*, *10*, 192.
- Fierer, N., Breitbart, M., Nulton, J., Salamon, P., Lozupone, C., Jones, R., et al. (2007). Metagenomic and small-subunit rRNA analyses reveal the genetic diversity of bacteria, archaea, fungi, and viruses in soil. *Applied and Environmental Microbiology*, *73*(21), 7059–7066. <https://doi.org/10.1128/AEM.00358-07>.
- Finkel, O. M., Castrillo, G., Herrera Paredes, S., Salas Gonzalez, I., & Dangel, J. L. (2017). Understanding and exploiting plant beneficial microbes. *Current Opinion in Plant Biology*, *38*, 155–163.
- Fitzpatrick, C. R., Copeland, J., Wang, P. W., Guttman, D. S., Kotanen, P. M., & Johnson, M. (2018). Assembly and ecological function of the root microbiome across angiosperm plant species. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(6), E1157–E1165. <https://doi.org/10.1073/pnas.1717617115>.
- Flexas, J., & Medrano, H. (2002). Drought-inhibition of photosynthesis in C3 plants: Stomatal and non-stomatal limitations revisited. *Annals of Botany*, *89*, 183–189.
- Freeman, B. C., Chen, C., Yu, X., Nielsen, L., Peterson, K., & Beattie, G. A. (2013). Physiological and transcriptional responses to osmotic stress of two *Pseudomonas syringae* strains that differ in epiphytic fitness and osmotolerance. *Journal of Bacteriology*, *195*, 4742–4752.
- Fuchslueger, L., Bahn, M., Fritz, K., Hasibeder, R., & Richter, A. (2014). Experimental drought reduces the transfer of recently fixed plant carbon to soil microbes and alters the bacterial community composition in a mountain meadow. *New Phytologist*, *201*, 916–927.
- Fuqua, C. (2010). Passing the baton between laps: Adhesion and cohesion in *Pseudomonas putida* biofilms. *Molecular Microbiology*, *77*, 533–536.
- Galvao, T. C., de Lorenzo, V., & Canovas, D. (2006). Uncoupling of choline-O-sulphate utilization from osmoprotection in *Pseudomonas putida*. *Molecular Microbiology*, *62*, 1643–1654.

- Garbeva, P., & Weiszkopf, L. (2020). Airborne medicine: Bacterial volatiles and their influence on plant health. *New Phytologist*, *226*, 32–43.
- García Teijeiro, R., Belimov, A. A., & Dodd, I. C. (2020). Microbial inoculum development for ameliorating crop drought stress: A case study of *Variovorax paradoxus* 5C-2. *New Biotechnology*, *56*, 103–113.
- Garrido-Sanz, D., Meier-Kolthoff, J. P., Göker, M., Martín, M., Rivilla, R., & Redondo-Nieto, M. (2016). Genomic and genetic diversity within the *Pseudomonas fluorescens* complex. *PLoS One*, *11*, e0150183.
- Garrido-Sanz, D., Arrebola, E., Martínez-Granero, F., García-Mendez, S., Muriel, C., Blanco-Romero, E., et al. (2017). Classification of isolates from the *Pseudomonas fluorescens* complex into phylogenomic groups based in group-specific markers. *Frontiers in Microbiology*, *8*, 413.
- Glick, B. R. (2012). Plant growth-promoting bacteria: Mechanisms and applications. *Scientifica*, *2012*, 963401.
- Glick, B. R. (2014). Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microbiological Research*, *169*, 30–39.
- Glick, B. R., Todorovic, B., Czarny, J., Cheng, Z., Duan, J., & McConkey, B. (2007). Promotion of plant growth by bacterial ACC deaminase. *Critical Reviews in Plant Sciences*, *26*, 227–242.
- Goicoechea, N., Antolín, M. C., & Sanchez-Diaz, M. (1997). Gas exchange is related to the hormone balance in mycorrhizal or nitrogen-fixing alfalfa subjected to drought. *Physiologia Plantarum*, *100*, 989–997.
- Grayston, S. J., Campbell, C. D., Vaughan, D., & Jones, D. (1995). Influence of root exudate heterogeneity on microbial diversity in the rhizosphere. *Journal of Experimental Botany*, *46*, 27.
- Guo, Y.-S., Furrer, J. M., Kadilak, A. L., Hinestroza, H. F., Gage, D. J., Cho, Y. K., et al. (2018). Bacterial extracellular polymeric substances amplify water content variability at the pore scale. *Frontiers in Environmental Science*, *6*, 93.
- Gupta, A., Rico-Medina, A., & Cano-Delgado, A. I. (2020). The physiology of plant responses to drought. *Science*, *368*, 266–269.
- Gururani, M. A., Upadhyaya, C. P., Baskar, V., Venkatesh, J., Nookaraju, A., & Park, S. W. (2013). Plant growth-promoting rhizobacteria enhance abiotic stress tolerance in *Solanum tuberosum* through inducing changes in the expression of ROS-scavenging enzymes and improved photosynthetic performance. *Journal of Plant Growth Regulation*, *32*, 245–258.
- Hansen, N. C., Allen, B. L., Anapalli, S., Blackshaw, R. E., Lyon, D. J., & Machado, S. (2016). Dryland agriculture in North America. In M. Farooq, & K. H. M. Siddique (Eds.), *Innovations in dryland agriculture* (pp. 415–442). Cham: Springer International Publishing.
- Hartmann, M., Brunner, I., Hagedorn, F., Bardgett, R. D., Stierli, B., Herzog, C., et al. (2017). A decade of irrigation transforms the soil microbiome of a semi-arid pine forest. *Molecular Ecology*, *26*, 1190–1206. <https://doi.org/10.1111/mec.13995>.
- Hasanuzzaman, M., Nahar, K., Bhuiyan, T. F., Anee, T. I., Inafuku, M., Oku, H., et al. (2017). Salicylic acid: An all-rounder in regulating abiotic stress responses in plants. In M. El-Esawi (Ed.), *Phytohormones—Signaling mechanisms and crosstalk in plant development and stress responses* (pp. 31–75). London: IntechOpen.
- Hasegawa, S., Poling, S. M., Mayer, V. P., & Bennett, R. D. (1984). Metabolism of abscisic acid: Bacterial conversion to dehydrovomifoliol and vomifoliol dehydrogenase activity. *Phytochemistry*, *23*, 2769–2771.
- Helena, M., & Carvalho, C. (2008). Drought stress and reactive oxygen species: Production, scavenging and signaling. *Plant Signaling & Behavior*, *3*, 156–165.

- Henry, A., Doucette, W., Norton, J., & Bugbee, B. (2007). Changes in crested wheatgrass root exudation caused by flood, drought, and nutrient stress. *Journal of Environmental Quality*, *36*, 904–912.
- Herrera-Vasquez, A., Salinas, P., & Holuigue, L. (2015). Salicylic acid and reactive oxygen species interplay in the transcriptional control of defense genes expression. *Frontiers in Plant Science*, *6*, 171.
- Hesse, C., Schulz, F., Bull, C. T., Shaffer, B. T., Yan, Q., Shapiro, N., et al. (2018). Genome-based evolutionary history of *Pseudomonas* spp. *Environmental Microbiology*, *20*, 2142–2159.
- Hinsa, S. M., Espinosa-Urgel, M., Ramos, J. L., & O’Toole, G. A. (2003). Transition from reversible to irreversible attachment during biofilm formation by *Pseudomonas fluorescens* WCS365 requires an ABC transporter and a large secreted protein. *Molecular Microbiology*, *49*, 905–918.
- Hossain, M. A., Munemasa, S., Uraji, M., Nakamura, Y., Mori, I. C., & Murata, Y. (2011). Involvement of endogenous abscisic acid in methyl jasmonate-induced stomatal closure in *Arabidopsis*. *Plant Physiology*, *156*, 430–438.
- Hu, H., & Xiong, L. (2014). Genetic engineering and breeding of drought-resistant crops. *Annual Review of Plant Biology*, *65*, 715–741.
- Hueso, S., García, C., & Hernández, T. (2012). Severe drought conditions modify the microbial community structure, size and activity in amended and unamended soils. *Soil Biology and Biochemistry*, *50*, 167–173.
- Huggins, D. R., & Reganold, J. P. (2008). No-till: How farmers are saving the soil by parking their plows. *Scientific American*, *96*, 77–81.
- Jaaffar, A. K. M., Parejko, J. A., Paulitz, T. C., Weller, D. M., & Thomashow, L. S. (2017). Sensitivity of *Rhizoctonia* isolates to phenazine-1-carboxylic acid and biological control by phenazine-producing *Pseudomonas* spp. *Phytopathology*, *107*, 692–703.
- Jagendorf, A. T., & Takabe, T. (2001). Inducers of glycine betaine synthesis in barley. *Plant Physiology*, *127*, 1827–1835.
- Jayne, B., & Quigley, M. (2014). Influence of arbuscular mycorrhiza on growth and reproductive response of plants under water deficit: A meta-analysis. *Mycorrhiza*, *24*, 109–119.
- Jochum, M. D., McWilliams, K. L., Borrego, E. J., Kolomiets, M. V., Niu, G., Pierson, E. A., et al. (2019). Bioprospecting plant growth-promoting rhizobacteria that mitigate drought stress in grasses. *Frontiers in Microbiology*, *10*, 2106.
- Jochum, M. D., McWilliams, K. L., Pierson, E. A., & Jo, Y. K. (2019). Host-mediated microbiome engineering (HMME) of drought tolerance in the wheat rhizosphere. *PLoS One*, *14*, e0225933.
- Kamilova, F., Validov, S., Azarova, T., Mulders, I., & Lugtenberg, B. (2005). Enrichment for enhanced competitive plant root tip colonizers selects for a new class of biocontrol bacteria. *Environmental Microbiology*, *7*, 1809–1817.
- Karimi, T., Stockle, C. O., Higgins, S., & Nelson, R. (2018). Climate change and dryland wheat systems in the US Pacific Northwest. *Agricultural Systems*, *159*, 144–156.
- Kawasaki, A., Watson, E. R., & Kertesz, M. A. (2012). Indirect effects of polycyclic aromatic hydrocarbon contamination on microbial communities in legume and grass rhizospheres. *Plant and Soil*, *358*, 169–182.
- Kazan, K. (2015). Diverse roles of jasmonates and ethylene in abiotic stress tolerance. *Trends in Plant Science*, *20*, 219–229.
- Kirkegaard, J. A., Hunt, J. R., McBeath, T. M., Lilley, J. M., Moore, A., Verburg, K., et al. (2014). Improving water productivity in the Australian grains industry—A nationally coordinated approach. *Crop & Pasture Science*, *65*, 583–601.
- Kudoyarova, G., Arkhipova, T., Korshunova, T., Bakaeva, M., Loginov, O., & Dodd, I. C. (2019). Phytohormone mediation of interactions between plants and non-symbiotic growth promoting bacteria under edaphic stresses. *Frontiers in Plant Science*, *10*, 1368.

- Kuiper, I., Lagendijk, E. L., Bloemberg, G. V., & Lugtenberg, B. J. (2004). Rhizoremediation: A beneficial plant-microbe interaction. *Molecular Plant-Microbe Interactions*, *17*, 6–15.
- Kurz, M., Burch, A. Y., Seip, B., Lindow, S. E., & Gross, H. (2010). Genome-driven investigation of compatible solute biosynthesis pathways of *Pseudomonas syringae* pv. *syringae* and their contribution to water stress tolerance. *Applied and Environmental Microbiology*, *76*, 5452–5462.
- Kwak, Y. S., & Weller, D. M. (2013). Take-all of wheat and natural disease suppression: A review. *Plant Pathology Journal*, *29*, 125–135.
- Lau, J. A., & Lennon, J. T. (2012). Rapid responses of soil microorganisms improve plant fitness in novel environments. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, 14058–14062.
- Ledger, T., Rojas, S., Timmermann, T., Pinedo, I., Poupin, M. J., Garrido, T., et al. (2016). Volatile-mediated effects predominate in *Paraburkholderia phytofirmans* growth promotion and salt stress tolerance of *Arabidopsis thaliana*. *Frontiers in Microbiology*, *7*, e1838.
- Lesk, C., Rowhani, P., & Ramankutty, N. (2016). Influence of extreme weather disasters on global crop production. *Nature*, *529*, 84–87.
- LeTourneau, M. K., Marshall, M. J., Cliff, J. B., Bonsall, R. F., Dohnalkova, A. C., Mavrodi, D. V., et al. (2018). Phenazine-1-carboxylic acid and soil moisture influence biofilm development and turnover of rhizobacterial biomass on wheat root surfaces. *Environmental Microbiology*, *20*, 2178–2194.
- Lim, J. H., & Kim, S. D. (2013). Induction of drought stress resistance by multi-functional PGPR *Bacillus licheniformis* K11 in pepper. *Plant Pathology Journal*, *29*, 201–208.
- Liu, Y., Song, Q., Li, D., Yang, X., & Li, D. (2017). Multifunctional roles of plant dehydrins in response to environmental stresses. *Frontiers in Plant Science*, *8*, 1018.
- Lugtenberg, B., & Kamilova, F. (2009). Plant-growth-promoting rhizobacteria. *Annual Review of Microbiology*, *63*, 541–556.
- Lugtenberg, B. J., Dekkers, L., & Bloemberg, G. V. (2001). Molecular determinants of rhizosphere colonization by *Pseudomonas*. *Annual Review of Phytopathology*, *39*, 461–490.
- Lundberg, D. S., Lebeis, S. L., Paredes, S. H., Yourstone, S., Gehring, J., Malfatti, S., et al. (2012). Defining the core *Arabidopsis thaliana* root microbiome. *Nature*, *488*, 86–90.
- Lynch, J. M. (1990). Microbial metabolites. In J. M. Lynch (Ed.), *The rhizosphere* (pp. 177–206). Chichester: John Wiley & Sons.
- Macnish, G. C. (1985). Methods of reducing *Rhizoctonia* patch of cereals in Western Australia. *Plant Pathology*, *34*, 175–181.
- Maddula, V. S., Pierson, E. A., & Pierson, L. S. (2008). Altering the ratio of phenazines in *Pseudomonas chlororaphis* (aureofaciens) strain 30-84: Effects on biofilm formation and pathogen inhibition. *Journal of Bacteriology*, *190*, 2759–2766.
- Mahmoudi, T. R., Yu, J. M., Liu, S., Pierson, L. S., & Pierson, E. A. (2019). Drought-stress tolerance in wheat seedlings conferred by phenazine-producing rhizobacteria. *Frontiers in Microbiology*, *10*, 1590.
- Mann, E. E., & Wozniak, D. J. (2012). *Pseudomonas* biofilm matrix composition and niche biology. *FEMS Microbiology Reviews*, *36*, 893–916.
- Marasco, R., Rolli, E., Ettoumi, B., Vigani, G., Mapelli, F., Borin, S., et al. (2012). A drought resistance-promoting microbiome is selected by root system under desert farming. *PLoS One*, *7*, e48479.
- Mark, G. L., Dow, J. M., Kiely, P. D., Higgins, H., Haynes, J., Baysse, C., et al. (2005). Transcriptome profiling of bacterial responses to root exudates identifies genes involved in microbe-plant interactions. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 17454–17459.
- Martinez-Gil, M., Yousef-Coronado, F., & Espinosa-Urgel, M. (2010). LapF, the second largest *Pseudomonas putida* protein, contributes to plant root colonization and determines biofilm architecture. *Molecular Microbiology*, *77*, 549–561.

- Matilla, M. A., Espinosa-Urgel, M., Rodriguez-Herva, J. J., Ramos, J. L., & Ramos-Gonzalez, M. I. (2007). Genomic analysis reveals the major driving forces of bacterial life in the rhizosphere. *Genome Biology*, *8*, R179.
- Mavrodi, D. V., Peever, T. L., Mavrodi, O. V., Parejko, J. A., Raaijmakers, J. M., Lemanceau, P., et al. (2010). Diversity and evolution of the phenazine biosynthesis pathway. *Applied and Environmental Microbiology*, *76*, 866–879.
- Mavrodi, O. V., Mavrodi, D. V., Parejko, J. A., Thomashow, L. S., & Weller, D. M. (2012). Irrigation differentially impacts populations of indigenous antibiotic-producing *Pseudomonas* spp. in the rhizosphere of wheat. *Applied and Environmental Microbiology*, *78*, 3214–3220.
- Mavrodi, D. V., Mavrodi, O. V., Parejko, J. A., Bonsall, R. F., Kwak, Y. S., Paulitz, T. C., et al. (2012). Accumulation of the antibiotic phenazine-1-carboxylic acid in the rhizosphere of dryland cereals. *Applied and Environmental Microbiology*, *78*, 804–812.
- Mavrodi, D. V., Mavrodi, O. V., Elbourne, L. D. H., Tetu, S., Bonsall, R. F., Parejko, J., et al. (2018). Long-term irrigation affects the dynamics and activity of the wheat rhizosphere microbiome. *Frontiers in Plant Science*, *9*, 345.
- Mayak, S., Tirosh, T., & Glick, B. R. (2004). Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. *Plant Science*, *166*, 525–530.
- Mazzola, M. (2002). Mechanisms of natural soil suppressiveness to soilborne diseases. *Antonie Van Leeuwenhoek*, *81*, 557–564.
- McNeil, S. D., Nuccio, M. L., & Hanson, A. D. (1999). Betaines and related osmoprotectants. Targets for metabolic engineering of stress resistance. *Plant Physiology*, *120*, 945–950.
- Miller, K. J., & Wood, J. M. (1996). Osmoadaptation by rhizosphere bacteria. *Annual Review of Microbiology*, *50*, 101–136.
- Miller, G., Suzuki, N., Ciftci-Yilmaz, S., & Mittler, R. (2010). Reactive oxygen species homeostasis and signaling during drought and salinity stresses. *Plant, Cell and Environment*, *33*, 453–467.
- Moore, E. R. B., Tindall, B. J., Martins Dos Santos, V. A. P., Pieper, D. H., Ramos, J.-L., & Palleroni, N. J. (2006). Nonmedical *Pseudomonas*. In M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer, & E. Stackebrandt (Eds.), *The prokaryotes* (pp. 646–703). New York: Springer.
- Morgan, P. W., & Drew, M. C. (1997). Ethylene and plant responses to stress. *Physiologia Plantarum*, *100*, 620–630.
- Moshelion, M., Halperin, O., Wallach, R., Oren, R., & Way, D. A. (2015). Role of aquaporins in determining transpiration and photosynthesis in water-stressed plants: Crop water-use efficiency, growth and yield. *Plant, Cell and Environment*, *38*, 1785–1793.
- Mulet, M., Lalucat, J., & Garcia-Valdes, E. (2010). DNA sequence-based analysis of the *Pseudomonas* species. *Environmental Microbiology*, *12*, 1513–1530.
- Nakabayashi, R., Mori, T., & Saito, K. (2014). Alternation of flavonoid accumulation under drought stress in *Arabidopsis thaliana*. *Plant Signaling & Behavior*, *9*, e29518.
- Nakabayashi, R., Yonekura-Sakakibara, K., Urano, K., Suzuki, M., Yamada, Y., Nishizawa, T., et al. (2014). Enhancement of oxidative and drought tolerance in *Arabidopsis* by overaccumulation of antioxidant flavonoids. *Plant Journal*, *77*, 367–379.
- Nascimento, F. X., Rossi, M. J., Soares, C. R., McConkey, B. J., & Glick, B. R. (2014). New insights into 1-aminocyclopropane-1-carboxylate (ACC) deaminase phylogeny, evolution and ecological significance. *PLoS One*, *9*, e99168.
- Naseem, H., & Bano, A. (2014a). Drought-tolerant plant growth promoting *Bacillus* spp.: Effect on growth, osmolytes, and antioxidant status of maize under drought stress. *Journal of Plant Interactions*, *6*, 1–14.
- Naseem, H., & Bano, A. (2014b). Role of plant growth-promoting rhizobacteria and their exopolysaccharide in drought tolerance in maize. *Journal of Plant Interactions*, *9*, 689–701.

- Naveed, M., Mitter, B., Reichenauer, T. G., Wieczorek, K., & Sessitsch, A. (2014). Increased drought stress resilience of maize through endophytic colonization by *Burkholderia phytofirmans* PsJN and *Enterobacter* sp. FD 17. *Environmental and Experimental Botany*, *97*, 30–39.
- Naylor, D., & Coleman-Derr, D. (2018). Drought stress and root-associated bacterial communities. *Frontiers in Plant Science*, *8*, 2223. <https://doi.org/10.3389/fpls.2017.02223>.
- Naylor, D., DeGraaf, S., Purdom, S., & Coleman-Derr, D. (2017). Drought and host selection influence bacterial community dynamics in the grass root microbiome. *The ISME Journal*, *11*(12), 2691–2704. <https://doi.org/10.1038/ismej.2017.118>.
- Ngumbi, E., & Kloepper, J. (2016). Bacterial-mediated drought tolerance: Current and future prospects. *Applied Soil Ecology*, *105*, 109–125.
- Nguyen, C. (2003). Rhizodeposition of organic C by plants: Mechanisms and controls. *Agronomy*, *23*, 375–396.
- Ni, X. L., Gui, M. Y., Tan, L. L., Zhu, Q., Liu, W. Z., & Li, C. X. (2018). Programmed cell death and aerenchyma formation in water-logged sunflower stems and its promotion by ethylene and ROS. *Frontiers in Plant Science*, *9*, 1928.
- Nielsen, L., Li, X., & Halverson, L. J. (2011). Cell-cell and cell-surface interactions mediated by cellulose and a novel exopolysaccharide contribute to *Pseudomonas putida* biofilm formation and fitness under water-limiting conditions. *Environmental Microbiology*, *13*, 1342–1356.
- Nilsson, M., Chiang, W. C., Fazli, M., Gjermansen, M., Givskov, M., & Tolker-Nielsen, T. (2011). Influence of putative exopolysaccharide genes on *Pseudomonas putida* KT2440 biofilm stability. *Environmental Microbiology*, *13*, 1357–1369.
- Niu, X., Song, L., Xiao, Y., & Ge, W. (2018). Drought-tolerant plant growth-promoting rhizobacteria associated with foxtail millet in a semi-arid and their potential in alleviating drought stress. *Frontiers in Microbiology*, *8*, 1–11.
- Ogle, S. M., Alsaker, C., Baldock, J., Bernoux, M., Breidt, F. J., McConkey, B., et al. (2019). Climate and soil characteristics determine where no-till management can store carbon in soils and mitigate greenhouse gas emissions. *Scientific Reports*, *9*, 11665.
- Okegbe, C., Fields, B. L., Cole, S. J., Beierschmitt, C., Morgan, C. J., Price-Whelan, A., et al. (2017). Electron-shuttling antibiotics structure bacterial communities by modulating cellular levels of c-di-GMP. *Proceedings of the National Academy of Sciences of the United States of America*, *114*, E5236–E5245.
- Ownley, B. H., Duffy, B. K., & Weller, D. M. (2003). Identification and manipulation of soil properties to improve the biological control performance of phenazine-producing *Pseudomonas fluorescens*. *Applied and Environmental Microbiology*, *69*, 3333–3343.
- Pardo-Hernandez, M., Lopez-Delacalle, M., & Rivero, R. M. (2020). ROS and NO regulation by melatonin under abiotic stress in plants. *Antioxidants (Basel)*, *9*, 1078.
- Parejko, J. A., Mavrodi, D. V., Mavrodi, O. V., Weller, D. M., & Thomashow, L. S. (2013). Taxonomy and distribution of phenazine-producing *Pseudomonas* spp. in dryland agroecosystem of the Inland Pacific Northwest (U.S.). *Applied and Environmental Microbiology*, *79*, 3887–3891.
- Patten, C. L., & Glick, B. R. (1996). Bacterial biosynthesis of indole-3-acetic acid. *Canadian Journal of Microbiology*, *42*, 207–220.
- Patten, C. L., & Glick, B. R. (2002). Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. *Applied and Environmental Microbiology*, *68*, 3795–3801.
- Paulitz, T. C., & Adams, K. (2003). Composition and distribution of *Pythium* communities in wheat fields in eastern Washington state. *Phytopathology*, *93*, 867–873.
- Paulitz, T. C., Smiley, R. W., & Cook, R. J. (2002). Insights into the prevalence and management of soilborne cereal pathogens under direct seeding in the Pacific Northwest, USA. *Canadian Journal of Plant Pathology*, *24*, 416–428.

- Phillips, D. A., Fox, T. C., King, M. D., Bhuvanewari, T. V., & Teuber, L. R. (2004). Microbial products trigger amino acid exudation from plant roots. *Plant Physiology*, *136*, 2887–2894.
- Potts, M. (1994). Desiccation tolerance of prokaryotes. *Microbiology Reviews*, *58*, 755–805.
- Qurashi, A. W., & Sabri, A. N. (2012). Bacterial exopolysaccharide and biofilm formation stimulate chickpea growth and soil aggregation under salt stress. *Brazilian Journal of Microbiology*, *43*, 1183–1191.
- Raaijmakers, J. M., & Weller, D. M. (1998). Natural plant protection by 2,4-diacetylphloroglucinol-producing *Pseudomonas* spp. in take-all decline soils. *Molecular Plant-Microbe Interactions*, *11*, 144–152.
- Raaijmakers, J. M., Vandersluis, I., Koster, M., Bakker, P. A. H. M., Weisbeek, P. J., & Schippers, B. (1995). Utilization of heterologous siderophores and rhizosphere competence of fluorescent *Pseudomonas* spp. *Canadian Journal of Microbiology*, *41*, 126–135.
- Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C., & Moenne-Loccoz, Y. (2009). The rhizosphere: A playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil*, *321*, 341–361.
- Ramos, I., Dietrich, L. E., Price-Whelan, A., & Newman, D. K. (2010). Phenazines affect biofilm formation by *Pseudomonas aeruginosa* in similar ways at various scales. *Research in Microbiology*, *161*, 187–191.
- Ramos-Gonzalez, M. I., Campos, M. J., & Ramos, J. L. (2005). Analysis of *Pseudomonas putida* KT2440 gene expression in the maize rhizosphere: In vivo expression technology capture and identification of root-activated promoters. *Journal of Bacteriology*, *187*, 4033–4041.
- Reid, C. P. (1974). Assimilation, distribution, and root exudation of  $^{14}\text{C}$  by ponderosa pine seedlings under induced water stress. *Plant Physiology*, *54*, 44–49.
- Reynolds, J. F., Smith, D. M., Lambin, E. F., Turner, B. L., Mortimore, M., Batterbury, S. P., et al. (2007). Global desertification: Building a science for dryland development. *Science*, *316*, 847–851.
- Roberson, E. B., & Firestone, M. K. (1992). Relationship between desiccation and exopolysaccharide production in a soil *Pseudomonas* sp. *Applied and Environmental Microbiology*, *58*, 1284–1291.
- Rodriguez, R. J., Henson, J., Van Volkenburgh, E., Hoy, M., Wright, L., Beckwith, F., et al. (2008). Stress tolerance in plants via habitat-adapted symbiosis. *ISME Journal*, *2*, 404–416.
- Rodriguez, S. J., Suarez, R., Caballero, M. J., & Itturiaga, G. (2009). Trehalose accumulation in *Azospirillum brasilense* improves drought tolerance and biomass in maize plants. *FEMS Microbiology Letters*, *296*, 52–59.
- Rodriguez, D., de Voil, P., & Power, B. (2016). Modelling dryland agricultural systems. In M. Farooq, & K. H. M. Siddique (Eds.), *Innovations in dryland agriculture* (pp. 239–256). Cham: Springer.
- Rolli, E., Marasco, R., Vigani, G., Ettoumi, B., Mapelli, F., Deangelis, M. L., et al. (2015). Improved plant resistance to drought is promoted by the root-associated microbiome as a water stress-dependent trait. *Environmental Microbiology*, *17*, 316–331.
- Rovira, A. D. (1986). Influence of crop rotation and tillage on *Rhizoctonia* bare patch of wheat. *Phytopathology*, *76*, 669–673.
- Rubin, R. L., van Groenigen, K. J., & Hungate, B. A. (2017). Plant growth promoting rhizobacteria are more effective under drought: A meta-analysis. *Plant and Soil*, *416*, 309–323.
- Salomon, M. V., Bottini, R., de Souza Filho, G. A., Cohen, A. C., Moreno, D., Gil, M., et al. (2014). Bacteria isolated from roots and rhizosphere of *Vitis vinifera* retard water losses, induce abscisic acid accumulation and synthesis of defense-related terpenes in vitro cultured grapevine. *Physiologia Plantarum*, *151*, 359–374.

- Sanchez-Contreras, M., Martin, M., Villacieros, M., O’Gara, F., Bonilla, I., & Rivilla, R. (2002). Phenotypic selection and phase variation occur during alfalfa root colonization by *Pseudomonas fluorescens* F113. *Journal of Bacteriology*, *184*, 1587–1596.
- Sandhya, V., Ali, S. K. Z., Grover, M., Reddy, G., & Venkateswarlu, B. (2009). Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing *Pseudomonas putida* strain GAP-P45. *Biology and Fertility of Soils*, *46*, 17–26.
- Santos-Medellín, C., Edwards, J., Liechty, Z., Nguyen, B., & Sundaresan, V. (2017). Drought stress results in a compartment-specific restructuring of the rice root-associated microbiomes. *Mbio*, *8*(4), e00764–17. <https://doi.org/10.1128/mBio.00764-17>.
- Sarniguet, A., Kraus, J., Henkels, M. D., Muehlchen, A. M., & Loper, J. E. (1995). The sigma factor  $\sigma$  affects antibiotic production and biological control activity of *Pseudomonas fluorescens* Pf-5. *Proceedings of the National Academy of Sciences of the United States of America*, *92*, 12255–12259.
- Schillinger, W. F., & Papendick, R. I. (2008). Then and now: 125 years of dryland wheat farming in the Inland Pacific Northwest. *Agronomy Journal*, *100*, S166–S182.
- Schillinger, W. F., Papendick, R. I., Guy, S. O., Rasmussen, P. E., & van Kessel, C. (2006). Dryland cropping in the western United States. In G. A. Peterson, P. W. Unger, & W. A. Payne (Eds.), *Dryland agriculture. Agronomy monograph No. 23* (2nd ed., pp. 365–393). Madison: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America.
- Schillinger, W. F., Kennedy, A. C., & Young, D. L. (2007). Eight years of annual no-till cropping in Washington’s winter wheat–summer fallow region. *Agriculture, Ecosystems, and Environment*, *120*, 345–358.
- Schlatter, D., Kinkel, L., Thomashow, L., Weller, D., & Paulitz, T. (2017). Disease suppressive soils: New insights from the soil microbiome. *Phytopathology*, *107*, 1284–1297.
- Schmid, J., Sieber, V., & Rehm, B. (2015). Bacterial exopolysaccharides: Biosynthesis pathways and engineering strategies. *Frontiers in Microbiology*, *6*, 496.
- Schnider-Keel, U., Lejbolle, K. B., Baehler, E., Haas, D., & Keel, C. (2001). The sigma factor AlgU (AlgT) controls exopolysaccharide production and tolerance towards desiccation and osmotic stress in the biocontrol agent *Pseudomonas fluorescens* CHA0. *Applied and Environmental Microbiology*, *67*, 5683–5693.
- Schroeder, K. L., & Paulitz, T. C. (2006). Root diseases of wheat and barley during the transition from conventional tillage to direct seeding. *Plant Disease*, *90*, 1247–1253.
- Schroth, M. N., Hildebrand, D. C., & Panopoulos, N. J. (2006). Phytopathogenic pseudomonads and related plant-associated pseudomonads. In M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer, & E. Stackebrandt (Eds.), *The prokaryotes* (pp. 714–740). New York: Springer.
- Seifikalhor, M., Aliniaiefard, S., Hassani, B., Niknam, V., & Lastochkina, O. (2019). Diverse role of gamma-aminobutyric acid in dynamic plant cell responses. *Plant Cell Reports*, *38*, 847–867.
- Shahzad, R., Khan, A. L., Bilal, S., Waqas, M., Kang, S. M., & Lee, I. J. (2017). Inoculation of abscisic acid-producing endophytic bacteria enhances salinity stress tolerance in *Oryza sativa*. *Environmental and Experimental Botany*, *136*, 68–77.
- Shelp, B. J., Bozzo, G. G., Zarei, A., Simpson, J. P., Trobacher, C. P., & Allan, W. L. (2012). Strategies and tools for studying the metabolism and function of  $\gamma$ -aminobutyrate in plants. II. Integrated analysis. *Botany*, *90*, 781–793.
- Silby, M. W., & Levy, S. B. (2004). Use of in vivo expression technology to identify genes important in growth and survival of *Pseudomonas fluorescens* Pf0-1 in soil: Discovery of expressed sequences with novel genetic organization. *Journal of Bacteriology*, *186*, 7411–7419.
- Simmons, T., Styer, A. B., Pierroz, G., Gonçalves, A. P., Pasricha, R., Hazra, A. B., et al. (2020). Drought drives spatial variation in the millet root microbiome. *Frontiers in Plant Science*, *11*(599). <https://doi.org/10.3389/fpls.2020.00599>.

- Simons, M., van der Bij, A. J., Brand, I., de Weger, L. A., Wijffelman, C. A., & Lugtenberg, B. J. (1996). Gnotobiotic system for studying rhizosphere colonization by plant growth-promoting *Pseudomonas* bacteria. *Molecular Plant-Microbe Interactions*, *9*, 600–607.
- Simons, M., van der Bij, A. J., Brand, J., de Weger, L. A., Wijffelman, D. A., & Lugtenberg, B. J. J. (1997). Amino acid synthesis is necessary for tomato root colonization by *Pseudomonas fluorescens* strain WCS365. *Molecular Plant-Microbe Interactions*, *10*, 102–106.
- Sleeter, B. M. (2012). Columbia Plateau ecoregion. In B. M. Sleeter, T. S. Wilson, & W. Acevedo (Eds.), *Status and trends of land change in the Western United States: 1973 to 2000* (pp. 229–236). Reston: U.S. Geological Survey.
- Smiley, R. W., Ogg, A. G., & Cook, R. J. (1992). Influence of glyphosate on *Rhizoctonia* root rot, growth, and yield of barley. *Plant Disease*, *76*, 937–942.
- Smiley, R. W., Yan, G. P., & Gourlie, J. A. (2014). Selected Pacific Northwest crops as hosts of *Pratylenchus neglectus* and *P. thornei*. *Plant Disease*, *98*, 1341–1348.
- Song, F. B., Han, X. Y., Zhu, X. C., & Herbert, S. J. (2012). Response to water stress of soil enzymes and root exudates from drought and non-drought tolerant corn hybrids at different growth stages. *Canadian Journal of Soil Science*, *92*, 501–507.
- Spaepen, S., & Vanderleyden, J. (2011). Auxin and plant-microbe interactions. *Cold Spring Harbor Perspectives in Biology*, *3*, a001438.
- Spaepen, S., Vanderleyden, J., & Remans, R. (2007). Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiology Reviews*, *31*, 425–448.
- Stewart, B. A., & Peterson, G. A. (2015). Managing green water in dryland agriculture. *Agronomy Journal*, *107*, 1544–1553.
- Stockle, C. O., Nelson, R. L., Higgins, S., Brunner, J., Grove, G., Boydston, R., et al. (2010). Assessment of climate change impact on Eastern Washington agriculture. *Climatic Change*, *102*, 77–102.
- Storey, R., & Wyn Jones, R. G. (1975). Betaine and choline levels in plants and their relationship to NaCl stress. *Plant Science Letters*, *4*, 161–168.
- Suarez, R., Wong, A., Ramirez, M., Barraza, A., del Carmen Orozco, M., Cevallos, M. A., et al. (2008). Improvement of drought tolerance and grain yield in common bean by over expressing trehalose-6-phosphate synthase in rhizobia. *Molecular Plant-Microbe Interactions*, *21*, 958–966.
- Sutherland, I. (2001). Biofilm exopolysaccharides: A strong and sticky framework. *Microbiology*, *147*, 3–9.
- Sweeney, M. R., Busacca, A. J., & Gaylord, D. R. (2005). Topographic and climatic influences on accelerated loess accumulation since the last glacial maximum in the Palouse, Pacific Northwest, USA. *Quaternary Research*, *63*, 261–273.
- Tanaka, Y., Sano, T., Tamaoki, M., Nakajima, N., Kondo, N., & Hasezawa, S. (2005). Ethylene inhibits abscisic acid-induced stomatal closure in *Arabidopsis*. *Plant Physiology*, *138*, 2337–2343.
- The Intergovernmental Panel on Climate Change (IPCC). (2007). Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. In S. Solomon, D. Qin, M. Manning, Z. H. Chen, M. Marquis, K. B. Averyt, et al. (Eds.), *Climate change 2007: The physical science basis*. Cambridge: Cambridge University Press. <https://www.ipcc.ch/report/ar4/wg1/>.
- Thomashow, L. S., & Weller, D. M. (1988). Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis* var. *tritici*. *Journal of Bacteriology*, *170*, 3499–3508.
- Timmusk, S., Abd El-Daim, I. A., Copolovici, L., Tanilas, T., Kannaste, A., Behers, L., et al. (2014). Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: Enhanced biomass production and reduced emissions of stress volatiles. *PLoS One*, *9*, e96086.

- Tiwari, S., Lata, C., Chauhan, P. S., & Nautiyal, C. S. (2016). *Pseudomonas putida* attunes morphophysiological, biochemical and molecular responses in *Cicer arietinum* L. during drought stress and recovery. *Plant Physiology and Biochemistry*, *99*, 108–117.
- Tsai, H. J., Shao, K. H., Chan, M. T., Cheng, C. P., Yeh, K. W., Oelmüller, R., et al. (2020). *Piriformospora indica* symbiosis improves water stress tolerance of rice through regulating stomata behavior and ROS scavenging systems. *Plant Signaling & Behavior*, *15*, 1722447.
- Turner, N. C. (2018). Turgor maintenance by osmotic adjustment: 40 years of progress. *Journal of Experimental Botany*, *69*, 3223–3233.
- Vacheron, J., Desbrosses, G., Bouffaud, M. L., Touraine, B., Moenne-Loccoz, Y., Müller, D., et al. (2013). Plant growth-promoting rhizobacteria and root system functioning. *Frontiers in Plant Science*, *4*, 356.
- Vaishnav, A., Kumari, S., Jain, S., Varma, A., & Choudhary, D. K. (2015). Putative bacterial volatile-mediated growth in soybean (*Glycine max* L. Merrill) and expression of induced proteins under salt stress. *Journal of Applied Microbiology*, *199*, 539–551.
- van de Mortel, M., & Halverson, L. J. (2004). Cell envelope components contributing to biofilm growth and survival of *Pseudomonas putida* in low-water-content habitats. *Molecular Plant-Microbe Interactions*, *52*, 735–750.
- van den Broek, D., Bloemberg, G. V., & Lugtenberg, B. (2005). The role of phenotypic variation in rhizosphere *Pseudomonas* bacteria. *Environmental Microbiology*, *7*, 1686–1697.
- van Veen, J. A., van Overbeek, L. S., & van Elsas, J. D. (1997). Fate and activity of microorganisms introduced into soil. *Microbiology and Molecular Biology Reviews*, *61*, 121–135.
- Vardharajula, S., Zulfikar Ali, S., Grover, M., Reddy, G., & Bandi, V. (2011). Drought-tolerant plant growth promoting *Bacillus* spp., effect on growth, osmolytes, and antioxidant status of maize under drought stress. *Journal of Plant Interactions*, *6*, 1–14.
- Verma, G., Srivastava, D., Tiwari, P., & Chakrabarty, D. (2019). ROS modulation in crop plants under drought stress. In M. Hasanuzzaman, V. Fotopoulos, K. Nahar, & M. Fujita (Eds.), *Reactive oxygen, nitrogen and sulfur species in plants: Production, metabolism, signaling and defense mechanisms* (pp. 311–336). Hoboken: John Wiley & Sons.
- Vishwakarma, K., Upadhyay, N., Kumar, N., Yadav, G., Singh, J., Mishra, R. K., et al. (2017). Abscisic acid signaling and abiotic stress tolerance in plants: A review on current knowledge and future prospects. *Frontiers in Plant Science*, *8*, 161.
- Vogel, J. P., Garvin, D. F., Mockler, T. C., Schmutz, J., Rokhsar, D., Bevan, M. W., et al. (2010). Genome sequencing and analysis of the model grass *Brachypodium distachyon*. *Nature*, *463*, 763–768.
- Vos, R., & Bellu, L. G. (2019). Global trends and challenges to food and agriculture into the 21st century. In C. Campanhola, & S. Pandey (Eds.), *Sustainable food and agriculture: An integrated approach* (pp. 11–30). London: Academic Press.
- Vurukonda, S. S., Vardharajula, S., Shrivastava, M., & Sk, Z. A. (2016). Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. *Microbiological Research*, *184*, 13–24.
- Walker, T. S., Bais, H. P., Halligan, K. M., Stermitz, F. R., & Vivanco, J. M. (2003). Metabolic profiling of root exudates of *Arabidopsis thaliana*. *Journal of Agricultural and Food Chemistry*, *51*, 2548–2554.
- Walker, T. S., Bais, H. P., Grotewold, E., & Vivanco, J. M. (2003). Root exudation and rhizosphere biology. *Plant Physiology*, *132*, 44–51.
- Wang, C., Yang, W., Wang, C., Gu, C., Niu, D., Liu, H., et al. (2012). Induction of drought tolerance in cucumber plants by a consortium of three plant growth-promoting rhizobacterium strains. *PLoS One*, *7*, e52565.
- Wang, J., Song, L., Gong, X., Xu, J., & Li, M. (2020). Functions of jasmonic acid in plant regulation and response to abiotic stress. *International Journal of Molecular Sciences*, *21*, 1446.
- Wargo, M. J. (2013). Homeostasis and catabolism of choline and glycine betaine: Lessons from *Pseudomonas aeruginosa*. *Applied and Environmental Microbiology*, *79*, 2112–2120.

- Watt, M., Hugenholtz, P., White, R., & Vinall, K. (2006). Numbers and locations of native bacteria on field-grown wheat roots quantified by fluorescence in situ hybridization (FISH). *Environmental Microbiology*, *8*, 871–884.
- Weller, D. M., & Cook, R. J. (1983). Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. *Phytopathology*, *73*, 463–467.
- Weller, D. M., Cook, R. J., Macnish, G., Bassett, E. N., Powelson, R. L., & Petersen, R. R. (1986). *Rhizoctonia* root rot of small grains favored by reduced tillage in the Pacific Northwest. *Plant Disease*, *70*, 70–73.
- Weller, D. M., Raaijmakers, J. M., Gardener, B. B. M., & Thomashow, L. S. (2002). Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual Review of Phytopathology*, *40*, 309–348.
- Weller, D. M., Landa, B. B., Mavrodi, O. V., Schroeder, K. L., De La Fuente, L., Blouin Bankhead, S., et al. (2007). Role of 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* spp. in plant defense. *Plant Biology*, *9*, 4–20.
- Whipps, J. M. (1990). Carbon economy. In J. M. Lynch (Ed.), *The rhizosphere* (pp. 59–97). Chichester: John Wiley & Sons.
- Whitbread, A. M., Davoren, C., Gupta, V. V. S. R., Llewellyn, R., & Roget, D. (2015). Long-term cropping system studies support intensive and responsive cropping systems in the low rainfall Australian Mallee. *Crop & Pasture Science*, *66*, 553–565.
- Williams, A., & de Vries, F. T. (2020). Plant root exudation under drought: Implications for ecosystem functioning. *New Phytologist*, *225*, 1899–1905.
- Xie, H., Pasternak, J. J., & Glick, B. R. (1996). Isolation and characterization of mutants of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2 that over-produce indoleacetic acid. *Current Microbiology*, *32*, 67–71.
- Yahr, T. L., & Parsek, M. R. (2006). *Pseudomonas aeruginosa*. In M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer, & E. Stackebrandt (Eds.), *The prokaryotes* (pp. 704–713). New York: Springer.
- Yancey, P. H., Clark, M. E., Hand, S. C., Bowlus, R. D., & Somero, G. N. (1982). Living with water stress: Evolution of osmolyte systems. *Science*, *217*, 1214–1222.
- Yang, A., Akhtar, S. S., Iqbal, S., Amjad, M., Naveed, M., Zahir, Z. A., et al. (2016). Enhancing salt tolerance in quinoa by halotolerant bacterial inoculation. *Functional Plant Biology*, *43*, 632–642.
- Yang, M. M., Mavrodi, D., Mavrodi, O., Thomashow, L., & Weller, D. M. (2019). Exploring the pathogenicity of *Pseudomonas brassicacearum* Q8r1-96 and other strains of the *Pseudomonas fluorescens* complex on tomato. *Plant Disease*, *104*, 1026–1031.
- Yin, C., Hulbert, S., Schroeder, K., Mavrodi, O., Mavrodi, D., Schillinger, W., et al. (2013). The role of bacterial communities in the natural suppression of *Rhizoctonia* bare patch of wheat. *Applied and Environmental Microbiology*, *79*, 7428–7438.
- Yong, B., Xie, H., Li, Z., Li, Y. P., Zhang, Y., Nie, G., et al. (2017). Exogenous application of GABA improves PEG-Induced drought tolerance positively associated with GABA-shunt, polyamines, and proline metabolism in white clover. *Frontiers in Physiology*, *8*, 1107.
- You, J., & Chan, Z. (2015). ROS regulation during abiotic stress responses in crop plants. *Frontiers in Plant Science*, *6*, 1092.
- Yousef-Coronado, F., Travieso, M. L., & Espinosa-Urgel, M. (2008). Different, overlapping mechanisms for colonization of abiotic and plant surfaces by *Pseudomonas putida*. *FEMS Microbiology Letters*, *288*, 118–124.
- Yuan, P., Pan, H., Boak, E. N., Pierson, L. S., & Pierson, E. A. (2020). Phenazine-producing rhizobacteria promote plant growth and reduce redox and osmotic stress in wheat seedlings under saline conditions. *Frontiers in Plant Science*, *11*, 575314.

- Zachow, C., Fatehi, J., Cardinale, M., Tilcher, R., & Berg, G. (2010). Strain-specific colonization pattern of *Rhizoctonia* antagonists in the root system of sugar beet. *FEMS Microbiology Ecology*, *74*, 124–135.
- Zahir, Z. A., Ghani, U., Naveed, M., Nadeem, S. M., & Asghar, H. N. (2009). Comparative effectiveness of *Pseudomonas* and *Serratia* sp. containing ACC-deaminase for improving growth and yield of wheat (*Triticum aestivum* L.) under salt-stressed conditions. *Archives of Microbiology*, *191*, 415–424.
- Zboralski, A., & Filion, M. (2020). Genetic factors involved in rhizosphere colonization by phytobeneficial *Pseudomonas* spp. *Computational and Structural Biotechnology Journal*, *18*, 3539–3554.
- Zhang, H., Xie, X., Kim, M. S., Korniyev, D. A., Holaday, S., & Pare, P. W. (2008). Soil bacteria augment *Arabidopsis* photosynthesis by decreasing glucose sensing and abscisic acid levels in planta. *Plant Journal*, *56*, 264–273.
- Zhang, H., Kim, M. S., Sun, Y., Dowd, S. E., Shi, H., & Pare, P. W. (2008). Soil bacteria confer plant salt tolerance by tissue-specific regulation of the sodium transporter HKT1. *Molecular Plant-Microbe Interactions*, *21*, 737–744.
- Zhang, H., Murzello, C., Sun, Y., Kim, M. S., Xie, X., Jeter, R. M., et al. (2010). Choline and osmotic-stress tolerance induced in *Arabidopsis* by the soil microbe *Bacillus subtilis* (GB03). *Molecular Plant-Microbe Interactions*, *23*, 1097–1104.
- Zolla, G., Badri, D. V., Bakker, M. G., Manter, D. K., & Vivanco, J. M. (2013). Soil microbiomes vary in their ability to confer drought tolerance to *Arabidopsis*. *Applied Soil Ecology*, *68*, 1–9.
- Zolla, G., Bakker, M. G., Badri, D. V., Chaparro, J. M., Sheflin, A. M., Manter, D. K., et al. (2013). Understanding root-microbiome interactions. In F. J. de Bruijn (Ed.), *Molecular microbial ecology of the rhizosphere* (pp. 745–754). Hoboken: John Wiley & Sons.