Update

Research Focus

# Rhizosphere bacteria help plants tolerate abiotic stress

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**Plant-growth-promoting rhizobacteria (PGPR) are associated with plant roots and augment plant productivity and immunity; however, recent work by several groups shows that PGPR also elicit so-called 'induced systemic tolerance' to salt and drought. As we discuss here, PGPR might also increase nutrient uptake from soils, thus reducing the need for fertilizers and preventing the accumulation of nitrates and phosphates in agricultural soils. A reduction in fertilizer use would lessen the effects of water contamination from fertilizer run-off and lead to savings for farmers.**

## Introduction

Plant-growth-promoting rhizobacteria (PGPR) colonize the rhizosphere of many plant species and confer beneficial effects, such as increased plant growth and reduced susceptibility to diseases caused by plant pathogenic fungi, bacteria, viruses and nematodes [1]. Some PGPR also elicit physical or chemical changes related to plant defense, a process referred to as 'induced systemic resistance' (ISR) [2]. ISR elicited by PGPR has suppressed plant diseases caused by a range of pathogens in both the greenhouse and field [1,2]. However, fewer reports have been published on PGPR as elicitors of tolerance to abiotic stresses, such as drought, salt and nutrient deficiency or excess. The subject of PGPR-elicited tolerance to heavy metals has been reviewed recently [3,4], so it is excluded from this discussion. Here, we propose the term 'induced systemic tolerance' (IST) for PGPR-induced physical and chemical changes in plants that result in enhanced tolerance to abiotic stress, and we review recently published work related to this subject. Biotic stress is excluded from IST because conceptually it is part of biological control and induced resistance.

## Bacterial effects on thirsty plants

Drought stress limits the growth and productivity of crops, particularly in arid and semi-arid areas [5]. Early studies on IST to drought [6] reported that inoculation with the PGPR *Paenibacillus polymyxa* enhanced the drought tolerance of *Arabidopsis thaliana*. RNA differential display on parallel RNA preparations from *P. polymyxa*-treated and untreated plants revealed that mRNA transcriptions of a drought-response gene, *EARLY RESPONSIVE TO DEHYDRATION 15* (*ERD15*), were also augmented. Another PGPR strain, *Achromobacter piechaudii* ARV8, which produces 1-aminocyclopropane-1-carboxylate (ACC) deaminase, conferred IST to drought stress in pepper (*Capsicum annuum* L.) and tomato (*Solanum lycopersicum* L.) plants [7]. Under stress conditions, including drought, the plant hormone ethylene endogenously regulates plant homeostasis and results in reduced root and shoot growth [8]. However, degradation of the ethylene precursor ACC by bacterial ACC deaminase releases plant stress and rescues normal plant growth [8].

Recent efforts to apply these results to greenhouse and field situations include using mixtures of PGPR strains with symbiotic nitrogen-fixing rhizobia [9] or with mycorrhizal fungi [10]. The rhizobia are sensitive to drought stress, resulting in a significant decrease of $N_2$ fixation when faced with low soil-water content. Under drought stress, co-inoculation of bean (*Phaseolus vulgaris* L.) with *Rhizobium tropici* and two strains of *P. polymyxa* resulted in augmented plant height, shoot dry weight and nodule number [9]. Interestingly, the effect on IST and increased nodule number was greater when a mix of two strains of *P. polymyxa* was applied than when one strain was applied, suggesting some synergistic effects from the use of strain mixtures.

Investigations into how drought stress affects plant hormone balance revealed an increase in abscisic acid (ABA) content in the leaves, indicating that the reduction of endogenous cytokinin levels magnifies ABA content, eliciting stomata closure [9,11] (Figure 1). The cytokinin–ABA antagonism might be the result of metabolitic interactions because they share a common biosynthetic origin [11]. It will be interesting to determine whether cytokinin produced by *P. polymyxa* affects ABA signaling of plants or rhizobia-elicited nodulation [6,9].

Co-inoculation of lettuce (*Lactuca sativa* L.) with PGPR *Pseudomonas mendocina* and arbuscular mycorrhizal fungi (*Glomus intraradices* or *G. mosseae*) augmented an antioxidant catalase under severe drought conditions, suggesting that they can be used in inoculants to alleviate the oxidative damage elicited by drought [10] (Figure 1).

## Help from bacteria for salty plants

Soil salinity in arid regions is frequently an important limiting factor for cultivating agricultural crops. Although many technologies have been implicated in the improvement of salt tolerance, only PGPR-elicited plant tolerance against salt stress has been previously studied. In one study [12] the ethylene content in tomato seedlings exposed to high salt was reduced by application of *Achromobacter piechaudii,* indicating that bacterial ACC dea-

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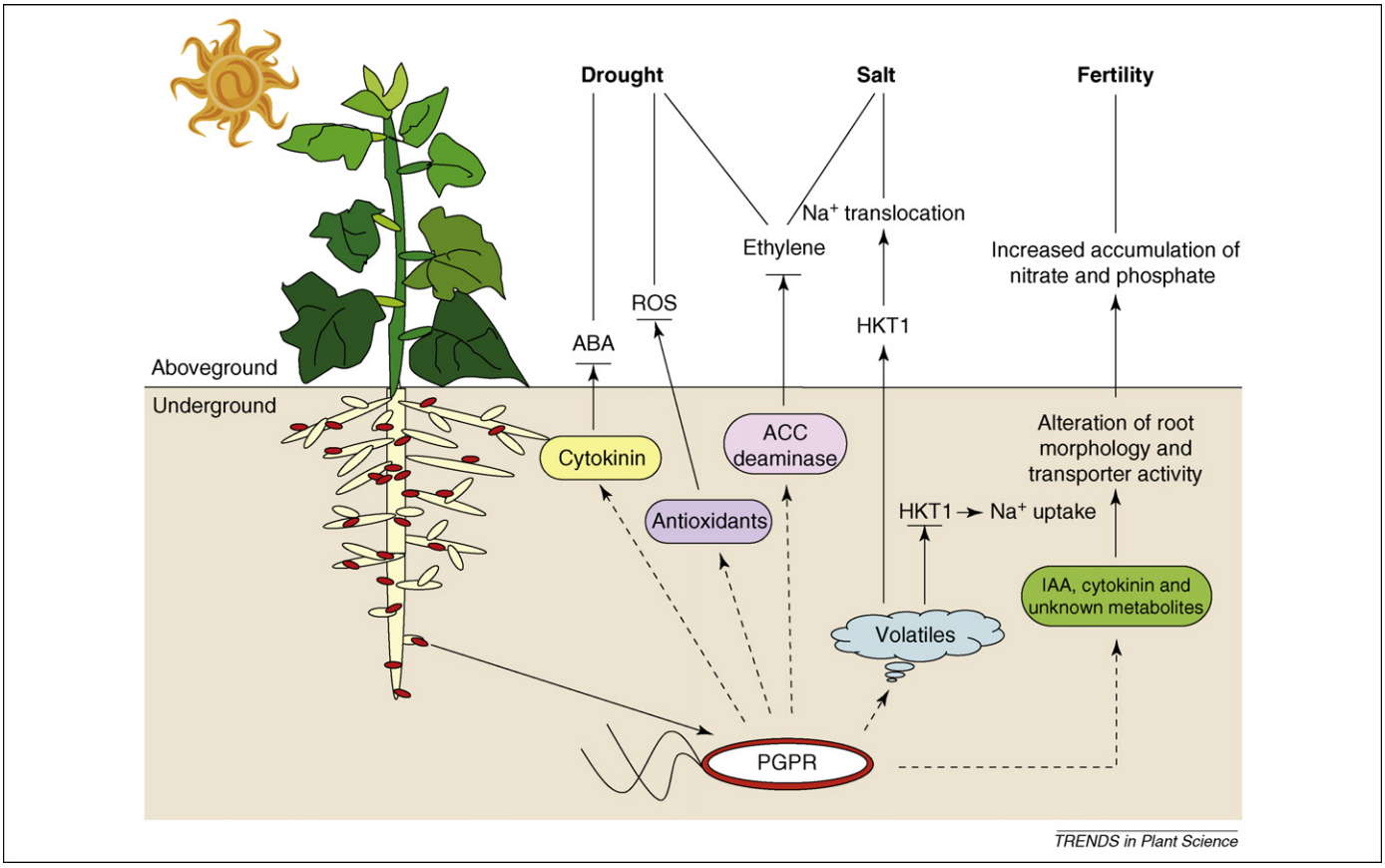


**Figure 1**. IST elicited by PGPR against drought, salt and fertility stresses underground (root) and aboveground (shoot). Broken arrows indicate bioactive compounds secreted by PGPR; solid arrows indicate plant compounds affected by bacterial components. Some PGPR strains, indicated in red on the plant roots, produce cytokinin and antioxidants such as catalase, which result in ABA accumulation and ROS degradation, respectively [9,10]. Degradation of the ethylene precursor ACC by bacterial ACC deaminase releases plant stress and rescues normal plant growth under drought and salt stresses [10,12]. The volatiles emitted by PGPR downregulate *hkt1* expression in roots but upregulate it in shoot tissues, orchestrating lower $Na^+$ levels and recirculation of $Na^+$ in the whole plant under high salt conditions [13]. Production by PGPR of IAA or unknown determinants can increase root length, root surface area and the number of root tips, leading to enhanced uptake of nitrate and phosphorous [16–18]. Abbreviations: ABA, abscisic acid; ACC, 1-aminocyclopropane-1-carboxylate; HKT1, high-affinity $K^+$ transporter 1; IAA, indole acetic acid; IST, induced systemic tolerance; PGPR, plant-growth-promoting rhizobacteria; ROS, reactive oxygen species.

minase was functional. *A. piechaudii*, which produces ACC, increased the growth of tomato seedlings by as much as 66% in the presence of high salt contents. IST to salt stress was also noted in a new study with *Arabidopsis* [13] using *Bacillus subtilis* GB03, a species that has previously been used as a commercial biological control agent. Interestingly, some of the volatile organic compounds (VOCs) that are emitted from *B. subtilis* GB03 [14] (Figure 1) are bacterial determinants involved in IST.

Among the 600 *Arabidopsis* genes isolated by transcriptome analysis, transcriptional expression of *HIGH-AFFINITY $K^+$ TRANSPORTER 1* (*HKT1*), which controls $Na^+$ import in roots, was decreased. HKT1 has been shown to adjust $Na^+$ and $K^+$ levels differentially, depending on the plant tissue. Exposure of an *athkt1* mutant to bacterial VOCs not only resulted in typical salt-stress phenotypes, such as stunting, but also led to the inhibition of seedling growth. Transcriptional validation revealed that bacterial VOCs downregulated HKT1 expression in roots, but upregulated it in shoot tissues, thereby orchestrating lower $Na^+$ levels in the whole plant. Furthermore, there is no difference in IST to salt stress in the $Na^+$-export mutant *salt overly sensitive3* (*sos3*), suggesting that HKT1 functions in shoots to retrieve $Na^+$ from the xylem, thereby facilitating shoot-to-root $Na^+$ recirculation. Overall, plant perception of bacterial VOC causes a tissue-specific regulation of HKT1 that controls $Na^+$ homeostasis under salt stress.

## Bacterial help with fertility and nutrient uptake

Another abiotic stress that plants face is obtaining adequate soil nutrients. Although soil fertilization is typically required for agricultural production, it can cause nitrate and phosphate accumulation that eventually contaminates surface and ground waters. Phosphate run-off is associated with eutrophication of surface waters, resulting in increased fish mortality [15]; in addition, nitrogen run-off from US agriculture into the Mississippi River is linked to oxygen starvation in the Gulf of Mexico, creating 'dead zones' where shrimp and fish populations are greatly reduced [15]. These environmental impacts of fertilization can be attributed, in part, to low uptake efficiency by crops. For example, phosphorous is highly reactive with iron, aluminium and calcium in soils, which can result in precipitation of up to 90% of the soil phosphorous [16], thus making it largely unavailable to plants.

PGPR have promise as components in approaches for maintaining adequate plant nutrition and reducing the negative environmental effects of fertilizers. Plant growth promotion by some PGPR has been associated with the solubilization and increased uptake of phosphate [16].

PGPR have also been reported to affect nitrate uptake by plants [17,18].

In addition to causing increases in general plant growth, some PGPR promote root development [17] and alter root architecture by the production of phytohormones such as indole acetic acid (IAA) [19] (Figure 1), resulting in increased root surface area and numbers of root tips. Such stimulation of roots can aid plant defense against pathogens and can also relate to IST. Given that root tips and root surfaces are sites of nutrient uptake, it is likely that one mechanism by which PGPR lead to increased nutrient uptake is via stimulation of root development. It has also been suggested that PGPR increase plant uptake of mineral ions via stimulation of the proton pump ATPase [17], although experimental evidence for this is lacking.

Owing to the environmental problems discussed above and the increasing prices of fertilizers, there is a push from farmers worldwide to reduce fertilizer levels below those recommended for optimum yields; however, such reductions would represent an abiotic stress on plants. Hence, several studies are now testing the hypothesis that PGPR might enable agricultural plants to maintain productivity with reduced rates of fertilizer application, and the preliminary results are promising. For example, in one field study with wheat (*Triticum aestivum* L.) [20], the yield for plants that were given 75% of the recommended amount of N-P-K fertilizer plus a PGPR strain was equivalent to the yield for plants that were given the full amount of fertilizer but without PGPR. In another study on tomato [21], the dry weight of tomato transplants grown in the greenhouse was significantly greater with two PGPR strains and 75% fertilizer than with the full amount of fertilizer and without PGPR; after transplanting to the field, yields with some combinations of PGPR and mycorrhizal fungi at 50% recommended field fertilization were greater than the yield of the 100% fertilizer control without microbes.

Another current hypothesis is that PGPR, used as components of integrated nutrient management systems, can help reduce the build up of nutrients in fertilized soils. Support for this hypothesis was presented in a recent report [18] of a three-year field study on maize that evaluated PGPR with and without mycorrhizal fungi, manure and inorganic fertilizer, as well as with and without tillage. Significant increases in grain yield from microbial treatments were accompanied by increased nitrogen content per gram of grain tissue and removal of significantly higher amounts of nitrogen, phosphorous and potassium. Therefore, within the tested nutrient management system, PGPR contributed significantly to reducing nutrient build up in the soil. Many current studies are underway that will further define the utility of PGPR in nutrient management strategies aimed at reducing fertilizer application rates and nutrient runoff from agricultural sources.

## Perspectives

PGPR-elicited IST can aid the growth of crops in environmentally unfavorable conditions. More investigations into the mechanisms by which PGPR elicit tolerance to specific stress factors should improve the use of IST in agriculture by enabling the optimization of microbial mixtures for the production of specific bacterial determinants (e.g. cytokinin, antioxidants, ACC deaminase, VOCs and IAA).

Improved plant nutrition with PGPR might or might not be due to IST as defined here. For example, if increased nutrient content in plants results from enhanced nutrient uptake, IST is operable because physical or chemical changes in the plant caused by PGPR are ultimately responsible, as occurs when PGPR stimulate root development. However, PGPR could increase nutrient availability without directly affecting plants. Although this would also result in greater nutrient levels in plants, it would not be explained by IST. Future investigations into each case where PGPR affect plant nutrition will elucidate this point.

The field of PGPR-elicited ISR should now focus on two directions. First, more studies are needed to demonstrate that PGPR cause a range of crops to be tolerant to various environmental stresses. In addition, studies are needed to elucidate the signal transduction pathways that result from treatment of plants with PGPR under stress conditions. Only then will the full benefits of PGPR be understood.

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