

Chapter 8

Abiotic Stress Tolerance Induced by Endophytic PGPR

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8.1 Introduction

Plant roots release substantial amounts of compounds into the surrounding soil and as consequence a dense microflora colonize the roots and use the root exudates and lysates to propagate, survive, and disperse along the growing root (Ryan and Delhaize 2001). Among the root-zone microflora, 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 (Kloepper et al. 2004). Some PGPR also elicit physical or chemical changes related to plant defense, a process referred to as “induced systemic resistance” (ISR, Kloepper et al. 2004; Van Loon and Glick 2004). However, few reports have been published on PGPR as elicitors of tolerance to abiotic stresses, such as drought, salt, and nutrient deficiency or excess. Recent work by several groups shows that PGPR also elicit the so-called induced systemic tolerance to salt and drought (Yang et al. 2009 and references included therein).

Although the potential use of PGPR (Kloepper et al. 1991; Bashan and Holguin 1998) as plant growth and yield enhancers (either not under abiotic or biotic stresses) has been known for several decades (Döbereiner et al. 1976; Okon and Labandera-González 1994; Glick et al. 1999), there has been limited success in practical use. A considerable amount of literature has been produced in the meantime relating the understanding of the mechanisms involved in the purported beneficial effects (Dimkpa et al. 2009), but the yield increases obtained by inoculation with PGPR are rather modest. As an example, the effect of long-living spore

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formulations of the *Bacillus* strain FZB24 and FZB42 produced and commercialized in Germany were tested in extended and long-lasting field trials with potatoes under practical farming conditions. Forty-eight field trials performed with FZB24 under standard conditions (250 g/ha) during 1995–1998 resulted in an increase of tuber potatoes yield of 8.3 % as the mean value. Similar experiments conducted in 2002, 2003, and 2004 confirmed that FZB strains in fact enhance productivity of potatoes about 7.5–10 % (mean value of 87 independent trials). Best results were obtained if application of bacilli was combined with use of fungicides. In such cases an increase of tuber yield up to 40 % was obtained (Choudhary and Johri 2009), although the question rises how much of the effect was due to the inoculation.

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 runoff and lead to savings for farmers (Gyaneshwar et al. 2002; Mantelin and Touraine 2004). Some studies tested the hypothesis that PGPR might enable agricultural plants to maintain productivity with reduced rates of fertilizer application. In field-grown maize partial replacement of N fertilization with *Azospirillum* sp., bacterization has been obtained (Fulchieri and Frioni 1994), although under conditions of limited soil fertility. In another field study with *Triticum aestivum* L. (Shaharoon et al. 2008), 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 tomato, 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, the yields for some combinations of PGPR and mycorrhizal fungi with fertilizers at 50 % were greater than the yield of the 100 % fertilizer control without microbes.

8.2 Plants Are Mostly Subjected to Several Stresses That Act as Signals Preparing the Organism to Afford More Stress

Plants growing in natural environments are frequently exposed to different stresses, even in environments where there is no apparent reason. For instance, one of the most common stresses is water restriction during mid-afternoon hours even in humid environments (Granier and Tardieu 1999). Plants respond to such stressful situations by modifying their metabolism in a way that they become more tolerant to that situation but also to other stresses. As an example, the plant hormone abscisic acid (ABA) is produced by dehydrating roots and transported to leaves as a sensitive indicator of degree of water deficits in the soils (Zhang and Davies

1989). Thus leaves from plants growing under water-stress conditions usually show an abrupt rise in ABA level which produce stomata closure and therefore decreasing water losses that prepare the plant to cope with other stresses (Huang and Zhu 2004). Also, grape plants perceiving relatively high levels of UV-B radiation (potentially harmful for the plant's tissues) responded by enhancing ABA levels, which in turn promote increase of polyphenols that filter UV-B at epidermal level, trigger antioxidant enzymatic mechanisms and the sterol-structural defense of membranes (Berli et al. 2010). By consequence, acclimation to relatively high UV-B conditions prepares plants to better afford drought, and, conversely, water-stress situations may promote UV-B tolerance.

8.3 Beneficial Bacteria Are Present in Plants and They Often Have the Capability to Stimulate the Host's Growth and Yield

In nature, beneficial endophytic bacteria play a fundamental role in plant adaptation to the environment (Hallman et al. 1997). They can pre-sensitize plant cell metabolism, so that upon exposure to stress primed plants are able to respond more quickly and efficiently than non-primed individuals (Compant et al. 2005). Moreover, microorganisms from the rhizosphere or tissues of a specific plant may be better adapted to that plant and environment conditions, and they may therefore provide better control of diseases than organisms originally belonging from other rhizosphere (Cook 1993). Since PGPR were characterized as beneficial for plants, genera like *Azospirillum*, *Herbaspirillum*, *Bacillus*, *Burkholderia*, *Pseudomonas*, *Gluconacetobacter*, and others have been tested to improve growth and yield in different crops (Egamberdiyeva and Höflich 2004). Oliveira et al. (2002) and Muthukumarasamy et al. (2006) found that *Gluconacetobacter* and *Herbaspirillum* improved N uptake and increased biomass in sugar cane. *Burkholderia phytofirmans* is able to colonize several parts of grapevines cv. Chardonnay (Compant et al. 2005), to increase root and shoot dry weight, and to induce growth of secondary roots (Ait Barka et al. 2000; Compant et al. 2005). Among the PGPR *Azospirillum* sp. may be considered the most important genus for improving plant growth or crop yield worldwide, under a variety of environmental and soil conditions (Bashan and de-Bashan 2010). The main visible effects of inoculations with *Azospirillum* sp. and other PGPR are in the plant root system. *Azospirillum* sp. can promote root elongation (Levanony and Bashan 1989; Dobbelaere et al. 1999), formation of lateral and adventitious roots (Creus et al. 2005; Molina-Favero et al. 2008), root hairs (Hadas and Okon 1987; Fulchieri et al. 1993), and branching of root hairs (Jain and Patriquin 1985), which increase the root area active in water and nutrient uptake. It has also been proposed that some PGPR increase the plant tolerance against abiotic stresses such as drought, salinity, and metal toxicity (Creus et al. 1997; Mayak et al. 2004; Cohen et al. 2009). In

addition to their usefulness in agriculture, they possess potential in solving environmental problems by improving growth of desert plants and by reducing pollution through phytoremediation that decontaminates soils and waters (de-Bashan et al. 2011).

Among the mechanisms that explain the beneficial effects on plant growth and yield promotion by *Azospirillum* sp. is the production of phytohormones (Costacurta and Vanderleyden 1995; Bastián et al. 1998; Bloemberg and Lugtenberg 2001; Bottini et al. 2004), mainly the auxin indol-3-acetic acid (IAA; Crozier et al. 1988; Patten and Glick 2002), gibberellins (GAs; Bottini et al. 1989, 2004; Fulchieri et al. 1993), and cytokinins (Arkipova et al. 2005). Results of several researches indicated that the most important mechanism in plant-growth promotion by PGPR is production of phytohormones and/or enhancement of phytohormones synthesis by the plant tissues [for reviews see Bottini et al. (2004), Spaepen et al. (2007), Bashan and de-Bashan (2010)]. Also production of the stress-related hormones, salicylic acid (De Meyer and Höfte 1997; Forchetti et al. 2010), ABA (Cohen et al. 2008, 2009) and jasmonic acid (Forchetti et al. 2010; Piccoli et al. 2011), and of the signaling molecule nitric oxide (Creus et al. 2005), have also been indicated as possible players in the game. Complementary, impairment in ethylene production via 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity may also be involved in plant growth promotion by PGPR (Belimov et al. 2009).

Considering the numerous interactions that exist among the different hormonal signaling pathways in plants, it is difficult to assess which of these pathways is the primary target of PGPR. It is known that many signals affect root architecture and branching, conspicuously plant hormones that regulate initiation and growth of lateral roots (Nibau et al. 2008). As an example, the formation of lateral roots which is an important postembryonic event that is vital for the growth of plants is primarily regulated by auxins (Casimiro et al. 2003), and PGPR are well-known auxin producers (Patten and Glick 2002). More likely, the rhizobacteria alters not just a single but several hormonal pathways, even in a pleiotropic manner, which could account for the different morphological changes observed, for example lateral root elongation and root hair development (Fulchieri et al. 1993; Dobbelaere and Okon 2007). Also, lateral root primordial emergence is repressed by limiting water supply (Deak and Malamy 2005); therefore PGPR-produced ABA may account for plant's growth under water restriction (Cohen et al. 2008, 2009). However, De Smet et al. (2006) observed that *Arabidopsis* seedlings grown on medium containing exogenous ABA did not form clearly visible lateral roots, and roots of *aba2-1* plants (deficient in ABA) had a larger number of lateral roots that grew longer than those of the wild type (Deak and Malamy 2005). Notwithstanding, *Azospirillum* sp. increased the number of lateral roots and root's fresh weight in *Arabidopsis aba2-1* plants suggesting the effects may be in turn mediated by IAA and GAs (Cohen, Bottini, Pontin, Berli, Moreno, Travaglia, Boccalandro, Piccoli, unpublished results), which is sustained by the fact that *Azospirillum* reversed the dwarf phenotype *dx* and *dy* in rice mutants (Cassán et al. 2001a, b). Indeed, it has been proven that *Azospirillum* sp. produce both IAA (Crozier et al. 1988) and GAs

(Bottini et al. 1989). For instance, elicitation of *Arabidopsis thaliana* growth promotion by PGPR involved signaling of cytokinins, brassinosteroids, auxin, salicylic acid, and GAs (Ryu et al. 2003, 2005, 2007). López-Bucio et al. (2007) found that the bacteria *Bacillus megaterium* is able to increase lateral root number and root hair length in *A. thaliana* plants through auxin- and ethylene-independent mechanisms. It has also been found that *Bacillus* sp. modulates the root-system architecture in *A. thaliana* through the emission of volatiles (Gutiérrez-Luna et al. 2010).

A. piechaudii ARV8 confer tolerance to water stress in tomatoes and peppers and therefore can promote growth under such situation (Mayak et al. 2004). It has also been reported that *Bacillus pumilus* and *Bacillus licheniformis* increased leaf area in dwarfed alder seedlings (Gutiérrez-Mañero et al. 2001) and in *Arabidopsis* (Ryu et al. 2007), and *Azospirillum lipoferum* USA 5b augmented leaf area in both well-watered and water-stressed individuals (Cohen et al. 2009). Zhang et al. (2008) reported that *Bacillus subtilis* strain GB03 can stimulate growth of *Arabidopsis* by the emission of organic compounds and increase photosynthesis through modulation of ABA signaling (Xie et al. 2009). Increased chlorophyll, and consequently, enhanced photosynthesis, is a known response of plant to inoculation with several PGPR (Deka and Dileep 2002), including *Azospirillum* sp. (Bashan et al. 2006).

Yield increases ranging from 10 to 20 % with PGPR applications have also been documented for several agricultural crops (Kloepper et al. 1991). Increases in seed yield had also been observed in lettuce inoculated with *Bacillus* sp. (Arkhipova et al. 2005). Wheat grain yield was increased by up to 30 % (Okon and Labandera-González 1994) by inoculation with *Azospirillum brasilense*, and partial replacement of N fertilization with *Azospirillum* sp. bacterization has been obtained in maize (Fulchieri and Frioni 1994).

8.4 PGPR Enhance Stress Tolerance in Plants Subjected to Abiotic Stresses

Drought is one of the main stressful environmental conditions that reduce crop yield worldwide. It has been shown that diurnal water stress is a condition normally found in most species growing in temperate climates during the noon and afternoon hours, even though the soil water status may be at field capacity. This temporary stress might then affect the growth rate (Granier and Tardieu 1999). In fact, mild water deficits that cause reduction of the plant tissues turgidity (equivalent to a reduction of 10–15 % in the plant water content) result in large changes in growth and metabolism. The plant's tolerance to water stress results from both morphological adaptation and responses at biochemical and genetic levels. The central response to water deficits however is the increase in ABA biosynthesis and/or a decrease in ABA breakdown (Bray 2002). In plants experiencing drought, it is assumed that ABA acts as the signal that prepares the plant to resist the water

deficit, mainly by controlling stomata closure and water loss (Zhang and Outlaw 2001). Also, there is evidence suggesting that ABA plays a role in root branching, improving the plant water uptake capacity (De Smet et al. 2006), and it has been demonstrated that ABA sprayed onto leaves promotes vegetative growth in *Ilex paraguariensis* plants by alleviating diurnal water stress (Sansberro et al. 2004). In wheat and soybean, applications of ABA increase leaf carotenoid content and favor the allocation of carbohydrates into grains (Travaglia et al. 2007, 2009). ABA treatments also augment yield in wheat cultivated under moderate water restriction (Travaglia et al. 2010) and enhance fruit set (and so yield) in grapevines (Quiroga et al. 2009). Otherwise, gibberellin A₃ (GA₃) and ABA treatments promote carbon allocation in roots and berries of grapevines (Moreno et al. 2011).

ABA has been characterized by full scan mass spectrometry as a by-product of chemically defined growth cultures of *A. brasilense* Sp 245. ABA production by the bacteria increased when NaCl was added to the culture medium, and ABA levels were enhanced in *A. thaliana* seedlings inoculated with *A. brasilense* Sp 245 (Cohen et al. 2008). As well, *A. lipoferum* inoculated to 45-day-old maize plants increased ABA levels and reversed the effects of applied inhibitors of ABA and GAs synthesis, fluridone, and prohexadione-Ca, respectively, on the hormone levels and the plant's drought tolerance. That is, ABA and GA₃ contributed to water-stress alleviation of maize plants by *A. lipoferum* (Cohen et al. 2009). Although reports on the effects of plant hormones produced by PGPR on plants are abundant, information regarding the mechanism involved in ABA effects (including *A. thaliana* as a model) after bacterization with PGPR under drought conditions is scarce. Cho et al. (2012) proposed an ABA-independent stomata closure in Arabidopsis bacterized with *Pseudomonas chlororaphis* but without further information regarding the mechanism involved. In a recent work (Cohen, Bottini, Pontin, Berli, Moreno, Travaglia, Bocalandro, Piccoli, unpublished results), Arabidopsis was used as a model system to further analyze the physiological basis by which *A. brasilense* Sp 245 affects the plant's response to water restriction. In an agar-grown system inoculation with *A. brasilense* of Arabidopsis wild-type Col-0, the mutant *aba2-1* and the transgenic pGL2::GUS genotypes during early growth increased both the aerial part and roots through modifications in root architecture, that is, increase in lateral root number and length (both main and lateral roots). Inoculation with *A. brasilense* also increased photosynthetic and photoprotective pigments and retarded water loss in *A. thaliana* wild-type plants and augmented ABA levels in both the wild-type and *aba2-1* mutant plants; that is, *A. brasilense* has the ability to produce ABA in vivo and restore the wild-type phenotype. Also, inoculation with *A. brasilense* and application of GA₃ increased leaf trichomes, and *Azospirillum*, IAA, and GA₃ increased the number of lateral roots in transgenic pGL2::GUS plants. Furthermore, *Azospirillum* increased growth, survival, seed yield, and ABA and proline levels and decreased stomatal conductance in Arabidopsis plants subjected or not to drought. In a recent study (Salomon, Cohen, Bottini, Gil, Moreno, Piccoli, unpublished results), several bacteria strains were isolated and characterized from roots and rhizosphere of *Vitis vinifera* cv. Malbec. Two of them, *P. fluorescens* and *B. licheniformis*, elicitate

production of defense-related terpenes purportedly involved in protection of dehydrated membranes (Beckett et al. 2012).

Salinity is an important stress that hinders crop yield in many parts of the world, mainly via enhanced biosynthesis of ethylene that inhibits root growth (Feng and Barker 1992). To overcome the ethylene-induced root inhibition is a requirement for successful production, and studies have shown that ethylene level in plants is regulated by ACC deaminase, which is present in some PGPR (Belimov et al. 2009; Nadeem et al. 2010). However, the effects of salinity are far more complicated than the simple augmentation of ethylene synthesis, and a main component in salt effects is drought, so phytohormones provided (or their synthesis being induced) by PGPR may be essential in the stress alleviation (Creus et al. 1997; Mayak et al. 2004; Cohen et al. 2008, 2009; Piccoli et al. 2011). As well, salinity increases oxidative stress so any mechanism that collaborates in avoiding such oxidative situation may help the plant defense (Grassmann et al. 2002). Apart, PGPR are identified that stimulate plant roots to excrete organic acids that chelate Na excess in the soil solution as a mechanism that protects plants against salinity (Li et al. 2007). Production of siderophores by PGPR, that is, substances that increase nutrient uptake under mineral shortage (Bagg and Neilands 1987), may in turn favor heavy (or excess of) metals sequestration, in a sort of homeostatic balance for the rhizospheric environment (Khan et al. 2009).

Also, other studies have provided new insights into the phytoremediation of metal-contaminated soils by PGPR (Zhuang et al. 2007). A metal-tolerant PGPR, *Enterobacter* sp., was able to enhance extraction of Ni, Zn, and Cr in *Brassica juncea*, along with plant growth increase by IAA production and with ACC deaminase activity (Kumar et al. 2008). A similar capacity was found for two strains of a metal resistant strain of *Pseudomonas* on the plant growth and the uptake of Ni, Cu, and Zn by *Ricinus communis* (Rajkumar and Freitas 2008) and for several PGPR in *Brassica* spp. (Ma et al. 2009). In the hyperaccumulating plant, *Sedum alfredii*, inoculation with *Burkholderia cepacia* enhanced plant growth submitted to excess of Zn, Pb, and Cd, in correlation with stimulation of organic acid production by the plant roots (Li et al. 2007). Co-inoculation of lupines with a mix of metal-resistant PGPR (including *Bradyrhizobium* sp., *Pseudomonas* sp., and *Ochrobactrum cytisi*) improved plant biomass and a decrease in metal accumulation due to a protective effect on the rhizosphere (Dary et al. 2010). The secretion of organic acids appears to be a functional metal resistance mechanism that chelates the metal ions extracellularly, reducing their uptake and subsequent impacts on root physiological processes (Li et al. 2007). In fact, nutrient-solubilizing activity of PGPR has been associated with the release of organic acids and a drop in the pH that may have the additional effect of metal quelation (Dastager et al. 2010). In other words, the mechanisms involved in metal detoxification appear to be the same as per salinity, that is, metal quelation, extrusion pumps, and ACC deaminase activity, with other side effects like nutrient solubilization.

Regarding extreme temperatures, detrimental situations are mostly related with water restriction, and few reports deal specifically with the subject. In this respect,

B. phytofirmans has been reported as enhancing plant resistance to low temperatures (Ait Barka et al. 2002, 2006).

Solar ultraviolet-B radiation (UV-B, wavelength range 280–315 nm), even in relatively small amounts, is potentially harmful for plants (Frohnmeier and Staiger 2003). The syntheses promoted by UV-B of terpenic compounds (Gil et al. 2012) that have the capacity of repelling the attack and propagation of pathogens (Vögeli and Chappell 1988; Escoriza et al. 2013) are in turn enhanced by PGPR (Salomon, Cohen, Bottini, Gil, Moreno, Piccoli, unpublished results). Therefore PGPR prepare the plant tissues to afford UV-B damage. As well, considering that biosynthesis of membrane-related sterols is enhanced by ABA (Berli et al. 2010), an additional benefit of bacterial-produced ABA may be the increase in sterols that are associated with stability and integrity of membranes, and attenuation of oxidative damage.

8.5 Prospective

Most of the studies analyzed the plant/PGPR interaction based on one plant species interacting with one bacterial strain (or a few strains of the same species) under controlled conditions looking for some specific mechanism, which is not the case in nature where a plant population is interrelating with a whole bunch of microorganism of both deleterious or beneficial characteristics along with many environmental signals (some stressful like water restriction, some trophic like photosynthetic light, some just signaling other factors like light quality that indicates neighbors, etc.). In other words, the interaction of so many factors produces a “holistic” response by the plant in which some of these factors may be predominant (like water availability), whereas some others are not (like the relative presence of PGPR). Therefore, no general mechanisms can account for improvement in growth and yield of crops. That is, one may expect a noticeable beneficial effect of PGPR in precise situations in which bacterization becomes effective in alleviating specific environmental treats. In this regard, future research should be directed in addressing specific questions posed for definite conditions in order to develop technologies effective in abiotic stress tolerance induced by endophytic PGPR.

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