ELBA BIOFLUX
Extreme Life, Biospeology & Astrobiology
International Journal of the Bioflux Society

The influence of Plant Growth Promoting Rhizobacteria (PGPR) on the reduction of abiotic stresses in crops

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Abstract. Plants are always subjected to biotic and abiotic stresses in the environment which have influences on the growth and development of the plants. Beneficial free-living soil bacteria are usually referred as Plant-Growth Promoting Rhizobacteria or PGPR. Different plant growth-promoting Rhizosphere bacteria, including associative bacteria such as: *Azospirillum*, *Bacillus*, *Pseudomonas* and *Enterobacter* group have been used for their beneficial influences on plants. Typically, PGPRs are associated with plants root and augment plant productivity and immunity; however, recent works showed that PGPRs not have just induced the systemic tolerance to abiotic stress such as salt and drought, but also they have increased the nutrient uptake from soils, and as a result the hazardous accumulation of nitrates and phosphates in the agricultural soils can be reduced by usage of them.
Key Words: plant growth bacteria, abiotic stress, crops.

Rezumat. Plantele sunt întotdeauna supuse unor stresuri biotice şi abiotice din mediul înconjurător, acestea influenţându-le creştera şi dezvoltarea. Bacteriile libere benefice din sol sunt de obicei denumite Rizobacterii Promotoare a Creşterii Plantelor sau RPCP. Diferite bacterii din rizosferă promotoare a creşterii plantelor, incluzând bacterii asociative precum cele din grupul *Azospirillum*, *Bacillus*, *Pseudomonas* şi *Enterobacter* au fost folosite pentru influenţa lor benefică asupra plantelor. De obicei RPCP sunt asociate rădăcinilor plantelor şi cresc productivitatea şi imunitatea plantelor; totuşi, studii recente arată că RPCP nu au indus doar toleranţa sistemică la stres abiotic precum salinitate şi secetă ci au şi crescut preluarea de nutrienţi din sol şi prin urmare acumulările periculoase de nitraţi şi fosfaţi în solurile agricole pot fi reduse cu ajutorul lor.
Cuvinte cheie: bacterii-promotori de creştere, stress abiotic, recoltă.

Introduction. Crops grown in arid and semi-arid regions are often exposed to adverse environmental factors such as drought or high soil salinity. Salinity is an important problem to the crop production in many parts of the world, especially in the irrigated fields of arid and semiarid regions (Schleiff 2008). In several Tunisian regions, the agricultural production is affected by the rarity and the saltiness of irrigation water (Hajlaoui et al 2007). In such areas, the high levels of NaCl affect the plant development by altering its functional state, but considerably the authors have indicated the possible usage of PGPRs in order to reduce the undesirable effects of salinity and drought condition. Several reasons have been presented for this resistance, which are discussed as follows:

A - Synthesis of plant growth regulators and increase of the lateral roots. Initial studies on PGPRs such as *Azotobacter paspali* showed that the bacteria can release indole acetic acid (IAA) to the medium and cause a significant increase in the growth and dry weight of leaves and roots of various plants (Alizadeh & Ordookhani 2011). In another study, *Azospirillum brasilense* showed the ablility to synthetize the root promoter substances such as: indole -3- acetic acid, indole -3- lactic acid, gibberellins and cytokinines which increased the number of lateral roots that were covered by hairy roots. As a result the absorbing root surface area increased. These experiments on the plant hormones also reflected that gibberellins had increased the production of lateral roots and cytokinines stimulated the hairy roots formation but caused a decrease in lateral roots formation and the length of the main roots. Applications of PGPRs considerably changed the morphology of pearl millet (*Pennisetum glaucum*) when the plant was inoculated with *A. brasilensis* (Tien et al 1979). Barbieri et al (1986) reported that inoculation of wheat with *A. brasilense* caused a significant increase in the number and length of lateral roots compared with non-inoculated *Triticum* spp. According to a study conducted in Canada on more than 400 isolated bacteria from different plant rhizospheres authors claimed that 222 isolates had increased the canola's growth. The inoculation of canola (*Brassica napus L.*) with isolated strains had increased the canola germination and activity from 5% to 29% under the farm condition (Kloepper et al 1987a).

Kloepper et al (1987b) reported a 57% increase of canola seeds yield after the inoculation by *P. putida* and *P. fluorescens* compared with non-inoculated plant. The growth-stimulating effect of the *P. putida* strain was assessed under a condition free of microbial mass and the results showed that inoculation of canola with pre-mentioned bacteria had considerably increased the length of the root and the plant shoot weight compared to control. There are also some evidences based on the ability of rhizobacteria to produce and release growth regulators like auxin.

Generally, authors have reported that the growth regulators, especially IAA, have the potential to affect the most of the features of the root system including primary root growth, formation of lateral and piliferous roots (Glick 1995). Germida & Walley (1996) studied the effect of *P. cepacia* RSS, R 85, *P. aeruginosa* R80, *P. fluorescens* R92, and *P. putida* R104 strains on spring wheat growth and yield in two areas. They reported that root distribution and length were significantly increased and R92 and R85 caused an increase in the root dry weight at the region of 5-15 cm. Kropp et al (1996) studied the effect of inoculation with *Pseudomonas chlororaphis* 2E3 strain on the spring wheat growth and yield in the northern Utah. The authors have reported that seedling emergence increased 6-8% compared to uninoculated control. Gil et al (2001) showed that *Pseudomonas fluorescens* can produce auxin in the presence of root exudates of *Arabidopsis*. They also stated that the strain can influence the production of secondary metabolites that play an important role in development and yield of the plant. Salmeron (1990) found that the growth promoting effect of *P. fluorescens* strain C-20-18 on the wheat and radish yield is due to cytokinine hormone production.

In another study, Khalid et al (2004) offered evidences that imply production of four types of gibberellines by *Bacillus licheniformis* and *Bacillus pumilus*. They also claimed that the amount of gibberellines in rhizosphere environment of *Bacillus pumilus* treatments was higher than in control. Glick (1995) studied the effect of five bacteria including *P. fluorescens*, *P. fluorescens* sub-group, *P. putida* sub-group B strain 1, *P. marginalis*, and *P. syringae* strain 1 and three fungi on tomato growth and yield in saline condition of hydroponic environment. They stated that *P. putida* has increased tomato yield under saline condition in hydroponic environment. They attributed the increase to the ability of the bacteria to produce IAA.

B - Increased nutrient absorption. Kucey et al (1989) indicated that the inoculation of *Triticum spp.* with *A. brasilense* had significantly increased some specific area of the root compared to control two weeks after the cultivation. As a consequence it increased the nutrient absorption and improved the corn growth compared with non-inoculated ones. Domey & Lippman (1989) examined the effect of different phosphate solubilizing bacteria strains on *Triticum spp*. They reported that phosphate solubilizing bacteria improved phosphorus absorption by wheat and significantly increased wheat yield under the condition of phosphorus deficiency. Researchers also claimed that the dry weight of shoot increased 31.4% and phosphorus absorption of the shoot increased 30.7% compared to non-inoculated treatment. Many researchers reported there is a significant relationship between PGPRs, oil turnips growth and yield. In this regard the effect of canola inoculation with PGPRs showed an increase in canola yield, in oil amount, the root system activity and nutrient removal (Xie et al 1999). By doing a two-year farm experiment, Xie et al (1999) also showed that inoculation of wheat with *Bacillus cereus* A47 determined an increase of 11.4% - 14.7% in the seeds. Mainard & Pellerin (1992) showed that the number of lateral roots in maize depends on nutritional status of organic carbon and, as a matter of fact, if the plant can produce more carbohydrates through water and nutrients absorption during the process of photosynthesis, it would produce more lateral roots. The ability of some of the *Pseudomonas* strains to increase solubility of insoluble phosphate sources and non-absorbent organic phosphate emphasizes the need of using them to increase the absorbing of nutrients, especially phosphorus, in terms of nutrient shortages (Defreitas et al 1997). It is also reported that a number of *Bacillus spp.* and *Xanthomonas maltophilia* separated from canola rhizosphere had a positive effect on the plant growth and considerably no effect on the amount of plant phosphorus. Authors claimed that other mechanisms than solubilizing phosphorus mechanism can cause the improvement of plant growth or the phosphorus concentration not to increase because of the dilution effect of growth improvement. Javed & Arshad (1997) studied the ability of 38 strains of growth promoting bacteria according to IAA production showed that the inoculated treatment compared to uninoculated control treatment showed 3.5% increase in one wheat type and 28% in the other type, based on their yield. The authors also stated that the number of tillerings, straw weight, and one thousand seeds weight had increased significantly in both types. Glick et al (1997a) studied the primary extension of canola seedlings under the condition of cold and salt stress in treatments inoculated with *P. putida*, with mutant species and treatment without inoculation. They found that the bacterial primary and mutant strains increase the canola growth compared to treatment without inoculation.

Defreitas & Germida (1997), in their studies, separated 111 bacterial strains from plant rhizospheres in the farm and identified a set of nine plant growth promoting bacteria as phosphate solubilizing bacteria in the laboratory. The identified phosphate solubilizing bacteria were tested from the aspect of their effect on canola yield and growth. Inoculation with *Bacillus thuringiensis* increased the height of shoots and pod yield significantly. Inoculation with *Xanthomonas maltophilia* increased the pod weight, seed yield and plant height. None of the isolates increased phosphorus absorption. Andrade et al (1997) showed that inoculation pea with *Pseudomonas fluorescens* F113 that produces 2,4 diacetylphoroglucinol antibiotics significantly increased the nodulation on the plant root by *Rhizobium leguminosarum* bacteria. Ashraf et al (2004) decreased the sodium absorption in plants and increased the plant yield by inoculating wheat with external polysaccharide-producing bacteria. Wheat inoculation with these bacteria caused an increase in the root dry weight (149-522%). The recorded increase was from 85 to 281% in shoots and they also reported that the external produced polysaccharides prevented sodium absorption by plant root. Hall et al (1996) experiments were conducted based on different plant seeds including canola, lettuce, tomato, wheat, barley and wild oat, aforesaid and their seed inoculation by *P. putida* GR12-2 and mutant which lacks ACC - deaminase enzyme activity. Their results revealed that inoculation by *P. putida* strain had increased the length of the seedlings and root, whereas mutant strain having no ACC - deaminase enzyme activity had no influence on increase of seedlings and root length. Glick et al (1997b) offered a model for reducing the plant ethylene concentration by means of plant growth promoting rhizobacteria based on the ACC - deaminase enzyme. According to this model, ACC produced by the plant seeps slightly out. Plant growth promoting rhizobacteria having the ACC deaminase enzyme causes ACC to decrease out of the seed through ACC absorption and hydrolyzing of it to ammonium and alpha ketobutyrate and seeps out much more ACC to balance plant ACC with external growth environment. Thus, the amount of plant ACC will diminish and as a result the amount of produced ethylene will also decrease. Penrose et al (2001) used canola plant, chemical inhibitor of ethylene synthesis, bacterial strain having the ACC deaminase enzyme and strains lacking the enzyme in order to study the theory of ethylene reduction model of plant and the role of ethylene in root growth inhibition. The results showed that, in the presence of ethylene inhibitors or strains containing ACC enzyme, root growth decreased. This result indicates the inhibiting role of ethylene in root growth. Grichko & Glick (2001) studied the effect of four plant growth promoting rhizobacterial strain including *E. cloacae* 4W4, *E. cloacae* GAL2, *P. putida* ATCC, and *P. putida* ATCC 17399/PKK415 on plant growth and yield to reduce the flooding stress reduction. The first three strains contained ACC deaminase enzyme and inoculating them to tomato increased the plant resistance to flooding stress However, the strains lacking the enzyme couldn't make any tolerance to flooding stress in tomato plant. In another research, Belimove et al (2002) reported an increase of 25.5% in the dry weight of canola root due to inoculation with *P. putida* AM2 bacterial strain. According to the studies of Belimove et al (2002), the length of canola seedlings root increased as a consequence of inoculation with *Pseudomonas spp*. and *Rhodococcus spp.* in sterile conditions. In this experiment, all the strains had ACC deaminase enzyme activity and in this case had similar effect on increasing root length. Bacilio et al (2004) stated that using *gfp*- tagged *Azospirillum lipoferum* can reduce negative effects of salinity on wheat. Results showed that dry weight of root and leaves as well as height of inoculated plant increased. They considered as one of the reasons for improving plant yield the increase in water absorption of the plant due to root growth improvement in inoculation treatments. Glick et al (1998) found that those bacteria containing the enzyme significantly improve the straw, seed yield, root weight, root length, number of tillers, and phosphorus, nitrogen, and potassium absorption in straw and seed, compared to control. They considered all these effects due to reduced level of ethylene of the plant because of inoculation with bacteria containing ACC deaminase and claimed that enzyme activity differes in different isolates and the strains whose ACC deaminase enzyme activity is higher would be more effective. Woitke et al (2004) reported that inoculation of tomato seeds with *Bacillus subtilis*, a Gram-positive bacteria, didn't have any significant effect on tomato yield on plants cultivated in saline condition and the yield significantly decreased in high salinity treatment (7.4 ds/m) and nutrient uptake didn't follow any specific process. Many studies have been conducted on the yield-increasing bacteria (YIB) in China. The results of these studies show an increase in the yield of wheat (8.5%-16%), rice (8.1-16%), maize (6-11%), beans(7-16%), sugar beet (15-20%), sorghum (5-10%), sweet potato (15-19%), linen (6-13%), oily turnip (16-18%), peanut (10-15%), and vegetables (13-35%) because of inoculation with plant growth promoting bacteria (Zahir et al 2004). Shaharoona et al (2007), by studying the role of ACC deaminase enzyme-producing bacteria, found that ACC50 *P. fluorescens* was the most effective isolate among the 5 studied isolates. They said that ACC deaminase enzyme is a good parameter for choosing plant growth promoting bacteria. Nadeem et al (2007) studied the effect of bacterial strains containing ACC deaminase enzyme at different soil salinity levels (4, 8, 12 dS/m) on canola yield. They mentioned that *Pseudomonas syringae* and *Pseudomonas spp*. improved canola yield and growth in salinity of 12 dS/m. The ratio of K/Na and the chlorophyll content were considerably increased. Shanmugam et al (2002), by studying the effect of four strains of PGPRs, reported that *P. fluorescens* TDK1 had the greatest influence on peanut yield by decrease of salinity effects. They considered it as a result of ACC deaminase enzyme in the strain, although in some cases plant inoculation with a growth promoting bacteria wasn't able to improve yield and absorption of nutrients in saline condition. Zhang et al (1996) identified some strains of the PGPRs that increased root development and nitrogen fixation of legume plants in a temperature lower than optimal condition at the root level. Orhan et al (2006) studied, in a farm experiment, the strain potential of *Pseudomonas* bacteria to increase the raspberry yield. They stated that in inoculation treatment with the above bacteria, yield of seed, straw, grain nitrogen concentration, and total nitrogen uptake increased significantly compared to uninoculated control treatment. Roosta (1998) collected 52 samples of soil around the root zone of maize, wheat, and some kinds of forage gramineae from four provinces including Tehran, Semnan, Qazvin, and Fars, separated 23 strains of these bacteria using collected soil samples and root segments, identified them based on microscopic observations, studied nitrogenase enzyme activity by doing biochemical tests, and finally provided source of inoculums from ten strains having more nitrigenase activity. Azaizeh (1995) germinated hybrid maize seeds through greenhouse experiment under sterile conditions and inoculated them with the inoculum source of ten native strains and three foreign strains of *Azospirillum* bacteria while transferring plantlets into pots. The results showed that in most cases inoculation increased the plant height, dry weight of shoots and roots, root branching, capillary fibers mass, root system spreading, and the ratio of root dry weight to shoot dry weight.

C - Gene expression. Poonguzhali et al (2005) found that, the strains caused an increase in growth and dry weight of root but had no significant effect on phosphate absorption by plant. They concluded that stimulating plant growth happened due to mechanisms other than phosphate sulubilizing mechanisms. Typically, plant growth promoting rhizobacteria improves the plant growth in saline condition through different mechanisms, one of which is gene expression. Considerably, one of the important promoters for HKT1 gene expression under the saline condition is the plant growth promoting bacteria activity. HTK transporter (high affinity K^+ transporter) is responsible for transporting sodium in addition to potassium transporting in organic plants including tomato (Mayak et al 2004). Rhizobium bacteria reduce the aggregation of Na^+ in all parts of the plant, so that the evidences show reduction in Na^+ level in roots and shoots. It is not possible that the decrease in Na^+ would be related to activation of Na^+ excretion because there was no disturbance in the direction of SOS that regulate Na^+ excretion. In plants inoculated with PGPRs, the ratio of K^+/Na^+ increased in root but this ratio didn't change very much in shoots. The regulator mechanism of HKT1 isn't known yet but nutrient transporters type HKT were found in plant species such as rice and wheat (Bacilio et al 2004; Cramer et al 1985).

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Received: 02 December 2011. Accepted: 12 December 2011 Published: 12 December 2011.
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How to cite this article:
Alizadeh O., Parsaeimehr A., 2011 The influence of Plant Growth Promoting Rhizobacteria (PGPR) on the reduction of abiotic stresses in crops. ELBA Bioflux **3**(2):93-99.