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The Effect of Plant Growth Promoting Rhizobacteria in Saline Condition

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Abstract: The increasing frequency of dry periods in many regions of the world and the problems associated with salinity in irrigated areas frequently result in the consecutive occurrence of salinity on cultivated land. Currently, 50% of all irrigation schemes are affected by salinity. In salinity areas, the high levels of NaCl affect the plant development by altering its functional state. Several studies show successfully using the plant growth promoting rhizobacteria (PGPR) to increase the plant resistance against salinity. These studies show the possibility of using PGPRs in order to reduce the undesirable effects of salinity.

Key words: Plant growth promoting rhizobacteria, salinity, stress

INTRODUCTION

Salinity is an important problem to crop production in many parts of the world, especially in irrigated fields of arid and semiarid regions (Schleiff, 2008). The capability of plant to habituate to salt stress and tension comprise of alternations at leaf level which is in regard to physiological, morphological and biological characteristics whereby many plants habituate with the high level of salinity and then the low level of water availability (Cicek and Cakirlar, 2008). Principally, salt stress induces decreases in leaf net CO_2 assimilation rate (Hajlaoui *et al*., 2006). The lack of photosynthesis leads to a high level of decrease of photosynthesis electron order and turn the way of photon energy into the processes which improve the production of Reactive Oxygen Species. (ROS) which are harmful to plant growth due to their detrimental effects on the function of the most sensitive biological macromolecules and membrane (Johnson *et al*., 2003).

Although, the ability of rhizobacteria to occupy the root decreases in saline condition. Brown (1962) associated the useful effect of *Azotobacter chroococcum* on tomato growth to producing plant hormones including IAA and gibberellins.

The first studies on PGPRs, e.g., *Azotobacter paspali* reveal that comparing to plants not inoculated these bacteria can release IAA to the medium and significantly raise the growth and dry weight of the roots of different plant and leaves (Brown, 1974).

In a study, *Azospirillum brasilense*, being able of producing root promoting substances such as indole-3-acetic acid, indole-3-lactic acid, gibberellins and cytokinins, increased the number of lateral roots that were covered by hairy roots and so the absorbing root surface area increased (Tien *et al*., 1979). Therefore, experiments of pure plant hormones reflected that gibberellins increased producing lateral root, cytokinins stimulated hairy root formation but decreased lateral root formation and main root lengthening. Compound application of this hormone made morphology of pearl millet change as the time when the plant inoculates with *A. brasilense* (Tien *et al*., 1979). PGPRs may increase plant operation and growth by controlling the pathogens. Parsaeimehr *et al*. (2009) found that *Pseudomonads fluorescens* strain effectively prevented the activity of *Pythium* sp. and could produce *Pyluteorin* as an antifungal compound. They also stated that simultaneous inoculation of a strain producing antibiotics of *Bacillus* sp. and *Streptomyces griseus* enhanced occupying the roots of alfalfa and soya by *R. meliloti* and *Brady rhizobium*, respectively and increased nitrogen fixation. Some of the PGPRs can increase the plant ability to absorb iron by producing siderphore.

Kloepper and Schroth (1978) noted that through the required plant iron siderphore can stimulate the growth of the plant directly. Neilands and Leong (1986) said that siderphores are molecules with low molecular weight that are able to absorb iron and are made by a lot of PGPRs and can increase iron absorption of plant.

Barbieri and Galli (1993) realized that inoculation of wheat and *A. brasilnese* lead to an increase in the length and number of the lateral roots in a significant fashion more than the non-inoculated *Triticum* spp. According to a study in Canada done on more than 400 bacterial isolates from different plant rhizospheres, 222 isolates

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increased canola's growth. The inoculation of canola with above strains increased canola germination and activity about 5 to 29% in the farm condition (Kloepper and Schroth, 1978).

Kloepper and Schroth (1978) reported a 57% increase of canola seeds yield after its inoculation with *P. putida* and *P. fluorescens* compared to 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 above bacteria significantly increased the root length and plant shoot weight compared to control organism.

Although, there are a lot of phosphate solubilizing bacteria in the soil, their number is not often much more than the plant rhizosphere bacteria to compete with. Therefore the amount of phosphorus released by them can't afford the plant need. So it is necessary to inoculate the plants by means of the micro-organisms in a concentration higher than that founded naturally in the soil, because the dissolved phosphorus should reach an amount to increase the plant yield.

Kucey and Janzen (1987) indicated that inoculating *Triticum* spp. with *A. brasilense* increased specific surface area of the root more significantly than the control treatment two weeks after cultivation. This matter increased the nutrient absorption and improved inoculated corn growth rather than non-inoculated one. According to the findings of Grayston and Germida (1991), inoculating canola with *B. cereus* and *B. cereus* 83-6 strains increased the function about 11.5% compared to control. Kennedy *et al*. (1997) examined the effect of different phosphate solubilizing bacteria strains on *Triticum* spp. They reported that phosphate solubilizing bacteria improved phosphorous absorption by means of wheat and increased wheat yield significantly under the condition of phosphorus deficiency. In a pot experiment of this study, researchers said that the dry weight of shoot increased 31.4% and phosphorous absorption of the shoot increased 30.7% compared to non-inoculated treatment. They stated that the phosphate solubilizing ability can't be the only factor of improving wheat growth and yield, although the relation of bacterial ability to dissolve phosphorous and affecting on wheat growth wasn't assessed.

Many researchers reported the effects of PGPRs inoculation on oil turnips growth and yield. The effect of canola inoculation with PGPRs was an increase in canola yield as well as in oil amount, the root system activity and nutrient removal (Xia *et al*., 1990). Doing a two-year farm experiment, Xia *et al*. (1990) showed that inoculating the wheat and *Bacillus cereus* A47 make an increase of 11.4-14.7% in the seeds.

Bar-Ness *et al*. (1992) concluded that bacterial siderphores (Pseudobactin and Ferrioxamine) can't provide required iron for plant. But they make the iron required for plant pathogenic factors out of reach and indirectly increase the plant growth and yield. The researchers found that it is more probable for plants to absorb microbial siderphores of micro-organisms inside the plant tissue or in direct contact to plant, because in these cases the possibility of siderphores absorption of soil clay, their inactivation and leaching would be minimized. Maynard and Hochmuth (1997) showed that the number of lateral roots in maize depends on nutritional status of plant carbon. If the plant can produce more carbohydrates through water and nutrients absorption during the process of photosynthesis, it would produce more lateral roots.

Wang *et al*. (2000) illustrated that siderphore is a good source of iron for plants such as sorghum, peanuts, cotton, cucumber, sunflower and rye and can meet al needs of the plant to iron. They said that the secondary metabolites are not produced in large amounts in normal condition (when superior strains are not inoculated). Among growth promoting bacteria, pseudomonads fluorescent is very important because of being able to produce a broad range of plant growth regulators, iron-absorbing compounds, producing organic acids such as succinic and lactic acid and finally biological control of plant pathogens. The ability of some of the pseudomonas strains to increase solubility of insoluble phosphate sources and non-absorbent organic phosphate puts stress on the need of using them to increase absorbing nutrients especially phosphorous in terms of nutrient shortages (Wang *et al*., 2000). In a greenhouse experiment on tomato done by Gagne *et al*. (1993), an increase of 18.2% was reported in the weight of each plant fruit after inoculating with *P. fluorescens*. Many reports suggest the positive effect of different strains of *Pseudomonads fluorescent* on canola plant.

Marschner and Romhold (1994) reported that plants are able of absorbing siderphores produced by micro-organisms occupying rhizosphere. Another mechanism proposed for siderphores function includes making iron used by plant pathogens out of their reach; this can expose these organisms to iron deficiency and protect the plants against them (Marschner and Romhold, 1994).

Chen *et al*. (1994) separated some plant growth promoting bacteria strains from canola rhizospheres and observed an increase of 11.5% in function rather than the control by means of inoculating 5 strains of them with canola. The above strains could significantly increase the germination rate of vegetative growth of canola. Chen *et al*. (1994) showed that inoculating wheat with plant growth promoting bacteria increased wheat yield from 6.3 to 15% compared to non-inoculated control treatment.

Glick (1995) stated that there are evidences indicating an increase in plant nutrient availability. The mechanisms include nutrient dissolution or chelating agent production such as siderphores. According to Tang *et al* (1994), *E. coli* and *P. putida* bacteria were able to increase the length of canola root in a condition free of microbial mass and they associated this increase to the activity of ACC deaminase enzyme inside the bacteria. The researches of Glick (1995) showed that the length of canola root and stem increased because of inoculation with *P. putida* GR12-2 under the condition of free bacterial mass. There are also some evidences based on the ability of rhizobacteria to produce and release growth regulators including auxin and as well as their effect on root morphology, nutrition and growth of the plant. it is observed in most studies that growth regulators, especially IAA, affect most features of the root system including primary root growth, formation of lateral roots and piliferous (Glick, 1995). Alizadeh and Namazi (2011) showed that micro-organisms able to dissolve inorganic phosphate improved lettuce and maize growth. Noel *et al.* (1996) studied the effect of *R. leguminosarum* bacteria on the plants of Brassicaceae family and *B. napus* cv. wester and *B. campestris* cv. tobin species. The results showed that the effect of inoculating a seed with mentioned bacteria in a condition free of microbial mass is a significant increase in the root length of the above plant seedlings compared to non-inoculated control treatment.

Zhang *et al.* (1997) identified some strains of the PGPRs that increased root development and nitrogen fixation of legume plants in a temperature lower than optimal condition in the root zone. According to the results of Hall *et al.* (1996) experiments conducted on different plant seeds including canola, lettuce, tomato, wheat, barley and wild oat, aforesaid plant seeds were inoculated with *P. putida* GR12-2 and the mutant that had no ACC-Deaminase enzyme activity. Inoculating with *P. putida* strain increased length of the seedlings and root, whereas mutant strain having no ACC-deaminase enzyme had no effect on increasing length of seedlings and root. Khalid *et al.* (2004) studied the strain potential of *Pseudomonas* bacteria in a farm experiment to increase the wheat yield and stated that in inoculation treatment with the above bacteria, yield of seed, straw, tillering, grain nitrogen concentration and total nitrogen uptake increased significantly compa red to uninoculated control treatment.

Germida and Walley (1996) studied the effect of PS. *Cepacia* RSS, R 85, PS. Aeruginosa R80, PS. Fluorescens R92 and PS. Putida R104 strains on spring wheat growth and yield in two areas. They reported that root distribution and length increased significantly. The R92 and R85 increased significantly the dry weight of the root in 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 northern Utah and found that seedling emergence increased 6-8% compared to uninoculated control treatment.

Glick *et al.* (1997a) 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 it to ammonium and alpha ketobutyrate and seeps out much more ACC to balance plant ACC and the external growth environment. Thus the amount of plant ACC will diminish and as a result the amount of produced ethylene will also decrease.

De Freitas and Germida (1992) during their experiments found that a number of *Bcillus* sp. and *Xanthomonas maltophilia* isolates separated from canola rhizosphere that had a positive effect on the plant growth had no effect on the amount of host plant phosphorus. They said that other mechanisms other than solubilizing phosphorus mechanism can cause the plant growth to improve or the phosphorus concentration doesn't increase because of the dilution effect and growth improvement. Javed and Arshad (1997) studied the ability of 38 strains of growth promoting bacteria to produce IAA and cultivated the seeds of two wheat types (Inglab and Lu-2bs) after inoculating with these strains in optimal condition of farm. The results showed that inoculation treatment compared to uninoculate control treatment make an increase of 3.5% in the Inglab type yield and 28% in Lu-2bs type yield. They also stated that the number of tillerings, straw weight and one thousand seeds weight increased significantly in both types.

Glick *et al.* (1997b) studied the primary extension of canola seedlings under the condition of cold and salt stress in treatments inoculated with *P. putida* Gr, the mutant species and treatment without inoculation. They found that the bacterial primary and mutant strain increase the canola growth compared to treatment without inoculation.

De Freitas and Germida (1992), in their studies, separated 111 bacteria 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. Inoculating with *Bcillus thuringiensis* increased the height of shoots and pod yield significantly. Inoculating with *Xanthomonas maltophilia* increased the pod weight, seed yield and plant height. But none of the isolates increased phosphorus absorption.

Cucumber growth in the presence of microbial siderphores made an increase in dry weight of the plant and the amount of chlorophyll. This increase was significant compared to control plant (absence of siderphores) (Ismande, 1998).

Benizri *et al* (2001) showed that *Pseudomonas fluorescens* can produce auxin in the presence of root exudates of maize. They also stated that the strain can change root exudates to secondary metabolites that play an important role in development and yield of the plant.

Cattelan *et al.* (1999), in a screening test of 166 the purified isolates of soil, found that the isolates having the ability of producing ACC deaminase or siderphores or phosphorus solubilizing ability, could increase primary growth of cultivated soya in non-sterile soil. Masalha *et al.* (2000) know it necessary for bacteria to have the ability of producing siderphores in order to provide required plant iron. They said that siderphores can help the iron absorption to increase.

Salamone (2000) found that the growth promoting effect of *P. fluorescens* strain C-20-18 on the wheat and radish yield is because of producing cytokinin hormone.

In another study, Penrose and Glick, 2003 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 and this indicates the inhibiting role of ethylene in root growth.

Grichko and Glick (2001) studied the effect of four plant growth promoting rhizobacterial strain including *E. cloacae* 4W4, *E. colacae* 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 tomato with them 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, Belimov *et al.* (2002) reported an increase of 25.5% in the dry weight of canola root because of inoculation with *P. putida* AM2 bacterial strain.

According to the studies of Belimov *et al.* (2002), an increase in the length of canola seedlings root because of inoculating with *Pseudomonas* spp. and *Rhodococcus* spp. in the sterile condition was observed. 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 one of the reasons for improving plant yield as an increase in water absorption of the plant due to root growth improvement in inoculation treatments. Ashraf and Harris (2004) decreased the sodium absorption in plants and increased the plant yield by inoculating wheat by means of the external polysaccharide-producing bacteria. Wheat inoculation with these bacteria increased the root dry weight about 149 to 522%. This increase was about 85 to 281% in shoots. They showed that the external produced polysaccharides prevented sodium absorption by plant root. Studying the effect of inoculating bacteria containing ACC deaminase enzyme on wheat growth and yield, Ahmed *et al.* (2004) 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 inoculating with bacteria containing ACC deaminase and claimed that enzyme activity differes in different isolates and the strains whose ACC deaminase enzyme activity is more would be more effective. Woitke *et al.* (2004) reported that inoculating tomato seeds with Bacillus subtilis, the gram-positive bacteria, didn't have any significant effect on tomato yield cultivated in saline condition and the yield significantly decreased in high salinity treatment (7.4 dS m^{-1}) and nutrient uptake didn't followed 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 inoculating with plant growth promoting bacteria.

Studying ten pseudomonas strains having the phosphate solubilizing ability and also producing ACC deaminase, Poonguzhali *et al.* (2005) found that the strains increased the growth and dry weight of root but had no effect on phosphate absorption by plant. They concluded that stimulating plant growth happened due to mechanisms other than phosphate sulubilizing mechanisms. Plant growth promoting rhizobacteria improve plant growth in saline condition through different mechanisms, one of which is gene expression. One of the important promoters for HKT1 gene expression were plant growth promoting bacteria. HTK transporter (high affinity K^+ transporter) is responsible for transporting sodium in addition to potassium transporting in organic plants including wheat and rice. Ribosomal 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 regulating mechanism of HKT1 hasn't known yet but nutrient transporters type HKT were found in plant species such as rice and wheat (Horie *et al.*, 1967).

Gravel *et al.* (2007) studied the effect of five bacteria including *P. fluorescens*, *P. fluorescens* subgroup, *P. putida* subgroup 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* increased tomato yield under saline condition in hydroponic environment. They attributed the increase to the ability of the bacteria to produce IAA. Shaharoona *et al.* (2006) by studying the role of ACC deaminase enzyme-producing bacteria found that ACC 50 *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* (2006) studied the effect of bacterial strains containing ACC deaminase enzyme in different soil salinity (4, 8, 12 dS m^{-1}) on canola yield. They mentioned that *P. syringae* and P.sp improved canola yield and growth in salinity of 12 dS m^{-1}. the ratio of K/Na and chlorophyll increased. Kumar *et al.* (2001) by studying the effect of four strains of PGPRs said that *P. fluorescens* TDK1 had the greatest influence on peanut yield to decrease the 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.

Root occupancy: Successful root occupancy and presence of sufficient amount of rhizobacteria are necessary in plant rhizospheres to establish their beneficial effects (Alizadeh and Namazi, 2011).

Previous studies showed that rhizobacteria attacked the plant in the first level of root occupancy because of the chemotaxis exuded from its seed or root. Ahmed *et al.* (2004) found that mutans of the strains lacking felagella have less ability to occupy potato root. So, it seems that stimulating the bacteria plays an important role in root occupancy process.

Lack of sufficient occupancy may be because of changes in root exudates during plant life cycle that decrease the number of pseudomonas in the next plant growth phase (Miller *et al*, 1989).

Vessey (2003) through studying root occupancy in clover and pea by means of two plant growth promoting bacteria observed that two bacterial strains occupied clover root as a tractor much better. They said that lack of sufficient occupancy of pea root tip may be related to rapid growth of root tip or nitrogen restriction in root zone. The effect of non-living factors including pH and phosphorus on root occupancy was evaluated by Bauske *et al.* (1997). They stated that aromatic compounds in plants and seed-surface pH affect the growth and occupancy of cotton root by means of plant growth promoting rhizobacteria.

Dekkers *et al.* (1998) showed that the encoding gene of NADH dehydrogenase plays an important role in root occupancy by means of bacteria. Another gene required for effective root occupancy is SSS. Also, there is a hypothesis that a two-part system containing COIR and COIS plays an important role in the ability of root occupancy by *P. fluorescens.*

Root occupancy which is a complex process is affected by different factors such as bacterial species, root exudates, living and non-living factors. Miller *et al.* (2001) illustrated that rpos gene in essential to occupy plant root by *P. putida* in a competitive environment. The study of temperature effect on root occupancy showed that effective root occupancy was much more in lower soil temperature (5°C) compared to higher temperature (25°C) (Benizri and Amiaud, 2005).

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