

**RESEARCH ARTICLE****Improvement of crop productivity in saline soils through application of saline-tolerant rhizosphere bacteria - Current Perspective****M. Vivekanandan*, R. Karthik and A. Leela**

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Manuscript Info**Manuscript History:**Received: 14 May 2015
Final Accepted: 24 June 2015
Published Online: July 2015**Key words:**Saline-tolerant rhizobacteria;
Nitrogen fixation; Rhizosphere;
Phosphate solubilization;
Siderophore formation; ACC
deaminase; Induction of saline
tolerance; Crop productivity
improvement; Saline soil***Corresponding Author****M. Vivekanandan****Email:**
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ecokar5@gmail.com**Abstract**

Worldwide salinity is one of the most severe abiotic stresses limiting crop growth and productivity. Further salt-affected area is fast escalating due to intrusion of saline water on arable land. In view of ever increasing population, it has become necessary to cultivate not only saline soil but also coastal saline to step up crop productivity. Land impoverishment is most often attributed to salt infiltration into the ground water which weakens the plants and lowers yield. Developing salt-tolerant crops is a desired alternative but with little success due to major determinants of genetic traits improving crop productivity have not been fully understood. An alternate strategy to improve crop plants for salt tolerance is to introduce salt-tolerant plant growth promoting rhizobacteria (PGPR) that enhance crop growth in saline soil. It is suggested that root-colonizing bacteria that produce phytohormones may stimulate plant growth and help in nutrient recycling in the rhizosphere microcosm and thus the microbes can alleviate the effects of salinity in the environment. In addition, PGPR might also increase nutrient uptake by plants from soils and thereby reduce the need for fertilizers. The present review focuses on the evaluation of saline - tolerant bacterial strains to stimulate saline tolerance and promote growth of crop plants leading to better productivity in saline soil.

*Copy Right, IJAR, 2015,. All rights reserved***INTRODUCTION**

Several environmental factors drastically affect plant growth development and thereby yield performance of a crop [1, 2]. Salinity, drought and extremes of temperature adversely affect crop productivity worldwide. More than 2.6 billion people in the world rely on agriculture of all the stresses, salinity is one of the most serious environmental problems reducing crop productivity and serves as the major cause for abatement of lands and aquifers earmarked for agriculture purposes. More than 20% of the world- irrigated land is salt – affected with 2500-5000 km² of production is lost every year due to salinity [3]. Salt affects even the development of tiny nodules on plant roots where nitrogen fixation occurs. Nitrogen is a critical element limiting plant growth. In addition to inland salinity, the cultivable areas in the coastal regions are affected by varying degrees of salinity and therefore, normal crop production is hindered [4]. The harmful effects of the presence of salts in the soil result in increased level of ethylene in root, ionic imbalance and hyper osmotic condition in plants [5]. Therefore, there is a need to overcome the effects of salinity to raise crop plants in saline soil. Physical removal of salts from the surface of the

soil or chemical treatment is not only expensive but can not be applied to vast areas for soil reclamation purposes. It has become increasingly important to explore the possibilities of increasing the potential use of these saline lands for increased crop production [6].

Plants are generally divided into four salinity rating groups; sensitive, moderately sensitive, moderately tolerant and tolerant. But majority of crop plants are sensitive to salinity (Fig.1).

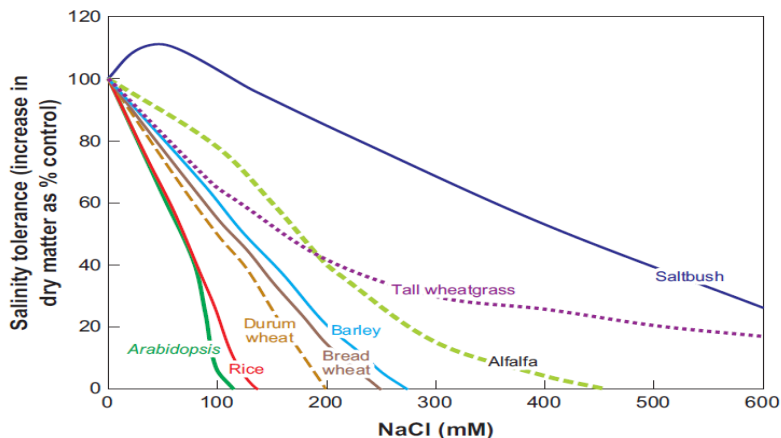


Fig.1 various crops show different sensitivities to different salinity levels [7].

The utilization of salt-affected land for agriculture has become necessary to meet the rise in food demand by the burgeoning populace. High salinity affects plant growth through osmotic effects, toxicity of salt ions and changes in the physiological and chemical properties of the soil. It also suppresses the phosphorus uptake by plant roots and reduces the available phosphorus by sorption process and thereby low availability of Ca-P minerals. Since phosphorus is a critical nutrient limiting plant growth, the adverse effects on plant growth in saline soil are multiplied. Consequently, the yields and profits of agriculture production in saline soil are reduced drastically [8]. In this respect, the use of chemical fertilizers is the most common approach to improve soil fertility. But, the available phosphorus in the chemical fertilizers is rapidly fixed to the unavailable forms especially in saline soil and accounts for low phosphorus efficiency [9]. However, Phosphate solubilizing bacteria (PSB) are known to improve the solubilization of fixed soil phosphorus and result in increased crop yields [10].

Solution to the problem

Different strategies are being employed to maximize plant growth under salinity. Developing salt-tolerant crops has been a much desired scientific goal but with little success to date, as only a few major-determinants of genetic traits of salt tolerance have been identified [11, 7]. While important physiological insights about the mechanism of salt tolerance in plants have been gained, the transfer of such knowledge into crop improvement has been limited. Therefore, an alternative strategy to improve crop salt tolerance may be to introduce salt-tolerant microbes that enhance crop growth. There is a need to identify efficient strains of saline tolerant nitrogen fixing bacteria to support the growth of crop plants in the saline soil. Moreover, biological nitrogen fixation is an inexpensive source of nitrogen for higher yields in non-leguminous crops. Many workers recommend the introduction of salt-tolerant strain of *Azotobacter* [12]. A strain of *Azotobacter* which showed maximal N₂ fixation at 30% NaCl was isolated by Blinkov (1963).

The solution lies with using phytoremediation (i.e. using halotolerant plants) or bioremediation (using the salt-tolerant bacteria) for reclamation of salt-affected soils on a large scale. The bacteria obtained from saline environment include *Flavobacterium*, *Azospirillum*, *Alcaligenes*, *Acetobacterium* and *Pseudomonas* [14]. This approach may succeed, where it has been proved difficult to develop salt-tolerant germplasm. One possible strategy to counteract the adverse effect of salinity is to isolate salt-tolerant rhizobacteria from halophilic environment, where the soils are severely affected by high pH characterized by high Na⁺ and other toxic ions in the soil [15]. The identification and exploitation of soil microorganisms (especially mycorrhizae, fungi and rhizosphere bacteria) that interact with plants by alleviating stress, opens up new alternatives for pyramiding strategies against salinity.

This review evaluates the beneficial effects soil biota on plant response to saline stress with reference to phytohormonal signaling that interacts with key physiological process to improve plant tolerance to osmotic and toxic components of salinity. The review also discusses the evaluation of bacterial strains to stimulate salinity tolerance and promotion of plant growth and identification of the bacterium using phenotypical and 16Sr RNA sequence analysis. It is our fond hope that the review might also help to hatch out a device to find out whether use of plant growth promoting rizhobacteria (PGPR) can improve the plants' tolerance in a stressed environment especially salinity [16,17,18].

Rhizosphere region

It is a centre of microbial and nutrient dynamics. Rhizosphere describes the zone of soil surrounding the roots of plant species which release organic substances. Bacteria that are present in the rhizosphere and enhance plant growth are referred to as plant growth promoting rhizobacteria (PGPR). In fact, root exudates in the rhizosphere region contain lot of compounds such as amino acid, organic acids, sugars, vitamins, enzymes and inorganic ions that are congenial for the growth of PGPR [19]. In both natural and man-made agro-ecosystems, interactions between plants and soil microorganisms have a profound effect on adaptation of plants to changing environment and plant growth. Selection of microbes from naturally stressed environment or rhizosphere is considered as a possible measure for improving crop health and thereby plant growth as well [20, 5]. In an era of sustainable agriculture production, the interaction in the rhizosphere by microorganisms with soil and plant plays a vital role in mobilization of nutrients from the limited pool of the soil [21].

Screening of PGPR for Saline tolerance

Many of the recent studies indicate that plants require microbial association for stress tolerance [22, 23, 24]. Rhizosphere of a halotolerant *Suaeda fruticosa* from saline desert was explored for isolation of PGPR from the ecological niche having 4.33% salinity [25]. They isolated from the rhizosphere 85 isolates, out of which 23 could solubilize phosphate and 11 isolates produced IAA. Seven more isolates showed both the traits of phosphate solubilization and IAA production. All the isolates were screened for PGP traits like production of ammonia, siderophore, chitinase, HCN and assessment of antifungal activity of all the screened isolates, *Bacillus licheniformis* A2 showed most prominent PGP traits both in vitro and in vivo for growth promotion of groundnut (*Arachis hypogaea*) under saline condition. Sapsirisopa *et al.* (2009) investigated the effects of three groups of salt-tolerant *Bacillus* inoculums namely *Bacillus megaterium* A12ag, a phosphate solubilizing bacterium (PSB), *B. licheniformis* B2r, an ACC deaminase producing bacterium and a co-inoculum of *B. megaterium* A12 ag and *B. licheniformis* B2r at 1:1 ratio. Their results revealed that the use of salt-tolerant PSB, *B. megaterium* A12 ag could increase the growth of rice and yield components. Islam *et al.* (2013) isolated five salinity-tolerant rhizobial strains (L-19, L-68, L-292, L-304 and L-335) from saline soils and evaluated their performance in lentil (*Lens culinaris*) in saline soil. Strains L-19 and L-304 were found to be effective for nodulation, growth, yield and nitrogen fixation in lentil as well as soil N build up. Rabie *et al.* (2005) indicated that dual inoculation with AM fungi and N-fixer *Azospirillum brasilense* increased N and P nutrition in the treated cowpea plant at different NaCl salinity levels. Higher number of nodules with increased nitrogenase activity was observed in the treated plant.

Effectiveness of PGPR in induction of saline tolerance in plants

For growth promotion and induction of salinity resistance in many crops, the effectiveness of PGPR has been well documented [29]. The available reports point out to the use of PGPR for growth and yield improvement of plants like wheat which is the most important staple crop of the world. Rajput *et al.* (2013) have shown that a strain called SAL-15 isolated from saline soil is capable of protecting plants like wheat against salt stress. After inoculation with the strain, SAL-15, the plant height and biomass significantly increased, as compared to the uninoculated plants. The strain, SAL 15 was identified as *Planococcus rifietoensis* on the basis of 16SrRNA sequence analysis. The bacterium is able to survive high salt concentrations (65g/L) and pH (>9) and can improve plant growth under these conditions. Their results further suggest that the selection and subsequent use of ACC deaminase containing salt-tolerant bacteria having a mixture of PGP activities may improve the growth of plants in saline soils. The study hence recommended the great potential of using the strain, SAL-15 as bacterial inoculant for production of plant biofertilizer for saline soils. Application of PGPR has also been reported to promote growth in many crops like barley, sorghum and tomato [31], cotton [32, 33], maize [34] and rice [35]. The genus, *Planococcus* is known to be present in diverse environments [36, 37, 38]. There have been increasing reports of the presence of this genus in the

rhizosphere of plants like rose and *Salicornia rhizosphere* [39]. *P. halophilus* and *P. rifietoensis* have been reported as halophilic bacteria. *P. rifietoensis* has been known to contain multiple plant growth promoting traits e.g., nitrogen fixation, production of IAA, chitinase, cellulase, lipase and protease [40]. The SAL-15 has an inherent ability to produce IAA as well as solubilization of inorganic phosphate. The ACC deaminase activity of the bacterium helps plants withstand biotic as well as abiotic stress conditions [5, 41]. It is possible that auxin and deaminase activity stimulate root growth in a synchronized manner [42]. In general, SAL-15 inoculation enhanced growth and yield of crop plants by alleviating toxic effects of salinity.

Eighty four halotolerant bacterial strains were obtained from the saline habitats and screened for growth at different NaCl concentrations [43]. All grew well at 5% NaCl but only 25 isolates showed growth at 20% NaCl concentration. Five strains, SL3, SL32, SL35, J8W and PU62 growing well in 20% NaCl concentration were further characterized for multiple plant growth promoting traits such as indole-3-acetic acid (IAA) production, siderophore formation, ACC deaminase activity and P-solubilization. None were positive for HCN production, and PCR amplification of *acds*, the structural gene for ACC deaminase was found negative. 16S rRNA gene sequencing analysis of the five strains revealed that they belong to the two genera, *Bacillus* and *Halobacillus*. Inoculation of the 5 halotolerant bacterial strains to ameliorate salt stress (80, 160 and 320 mM) in wheat seedlings produced a significant increase in root length and biomass in comparison to uninoculated positive controls. *Hallobacillus sp* S13 and *Bacillus halodenitrificans* PU62 showed more than 90% increase in dry weight when compared to uninoculated wheat seedlings at 320 mM NaCl indicating a significant reduction of the deleterious effects of NaCl. Soil salinity in arid zone is an important limiting factor for cultivation of crop plants. Mayak *et al.* (2004) reported that ethylene content in tomato seedlings exposed to high salt was reduced by application of *Achromobacter piechaundii* indicating that bacterial ACC deaminase was functional. *A. piechaundii* increased the growth of tomato seedlings by 66% in the presence of high salt content.

Under salt stress, PGPR have shown positive effects in plants on parameters like germination rate, weight of shoots and roots, economic yield and plant growth [44]. Damodaran *et al.* (2013) identified PGPR traits coupled with saline tolerance in *Bacillus pumilus* and *Bacillus subtilis*. These isolates elicited significantly higher vigour index in tomato seedlings grown in pot culture experiments under saline- sodic soils of pH 9.35 and an EC value of 4.2. A detailed survey of natural population in the rhizosphere of grasses grown in sodic soils was carried out by Damodaran *et al.* (2013) to isolate and identify salt tolerant bacteria that could express PGP traits at high salt concentrations. The bacterial diversity reduces with increase in soil pH. It was reported earlier that soil salinity plays a prominent role in the microbial selection process, as environmental stress leads to reduce bacterial diversity [46]. Damodaran *et al.* (2013) prepared 13 isolates from the rhizosphere of grasses and sorted them into 16 different pure colonies. Majority of the bacterial isolates were identified as *Bacillus spp.* earlier studies indicated that genera such as *Bacillus* and *Pseudomonas* were predominant in saline soils [47]. PGPR activity of the bacteria present in the rhizosphere was found to exert beneficial effects on crop plants. The Halotolerant microorganisms associated with plant roots can alleviate salt stress and lead to fertility improvement in saline soils [48]. Several mechanisms such as production of IAA, activation of solubilization and promotion of the mineral nutrient uptake are believed to be involved in plant growth promotion by PGPR [49]. IAA, the most common auxin, functions as an important signal molecule in the regulation of plant development [50]. Generally plant growth promoting rhizobacteria facilitate the plant growth directly by either assisting in resource acquisition (nitrogen, phosphorus and essential minerals) or modulation plant hormone levels. In addition to increases in general plant growth, PGPR can also promote root development [21] and alter root architecture by the production of phytohormones such as indole acetic acid (IAA) [51]. Induced systemic tolerance to salt stress was observed in a study on *Arabidopsis* using *Bacillus subtilis* GB03 (17). Borneman *et al.* (1996) also observed mitigation of salt stress in wheat seedlings by halotolerant bacteria isolated from saline soil.

The applied PGPR can also sometimes act as bio-control agents Success of the PGPR application requires survival of the introduced strain in the new soil and its capacity of competing with the better adapted native microflora [20]. For instance, *P. extremorientalis* TSAU20 and *P. chlororaphis* TSAU13 proved their capacity to increase the growth of various crops under saline conditions [52, 22].

How to amend salt stress?

There are many instances of PGPR improving the growth of vegetables and crops under abiotic stress conditions (Salinity/Drought) [52, 53, 54]. *Azospirillum brasilense*, an osmotolerant strain restored growth of wheat in saline soil [55]. Egamberdieva (2012) isolated *Pseudomonas chlororaphis* (TSAU13), a salt tolerant bacterium. Application of *P. chlororaphis* to tomato and cucumber promoted growth and fruit yield in saline soil and also

reduced the incidence of disease by *Fusarium solani*. Egamberdieva *et al.* (2013) also observed alleviation of salt stress in *Galega officinalis* by *Rhizobium galea* and root colonizing *Pseudomonas extremorientalis* Basha and Vivekanandan (2000) isolated a salt tolerant rhizobial strain from tannery sludge which successfully nodulated vicia and cowpea in saline soils (250 mM NaCl). Silini-Cherif *et al.* (2012) identified a nitrogen fixing bacterium, *Pantose agglomerans* from the wheat rhizosphere, which can tolerate a salinity level of 100 to 400 mM and its application increased IAA production, siderophore formation and solubilization of phosphate. Mapelli *et al.* (2013) isolated halotolerant bacteria from the rhizosphere of *Salicornia*, resistant to high temperature, high osmoticum and salinity stress. Twenty *Halomonas* strains selected from the collection promoted plant growth under high salinity. These *Halomonas* strains are included in the biofertilizer formulates to sustain crop productivity in degraded and arid zones.

Egamberdieva *et al.* (2013) Identified beneficial salt-resistant bacteria that help plants grow better, causing no harm to men. These bacteria are found around the roots of plants and they belonged to the *Pseudomonas* family, in particular, *Pseudomonas extremorientalis* [43]. They are salt resistant and grow closer to roots. *Pseudomonas* produce antibiotics that plants use to defend themselves against fungi and trigger the rooting process as well and produce nodulation promoting factors, thus giving the vegetation better chances to fix nitrogen and grow bigger. As an exchange for these favours, plants secrete exudates useful for the bacteria. Crops treated with these bacterial fertilizers give yields 12-15% higher than normal in tomato and cucumber. These results indicate that halotolerant bacteria isolated from saline environments have the potential to enhance plant growth under saline stress and therefore, they would be most appropriate as bioinoculants under such conditions.

Amelioration of salinity effect on phosphorus uptake by plants

High salinity affects plant growth through (i) osmotic effects, (ii) toxicity of salt ions and (iii) changes in the physical and chemical properties of the soil. It also suppresses the phosphorus uptake by plant roots and reduces the available phosphorus by sorption processes. Since phosphorus is a critical nutrient limiting plant growth, the adverse effects on plant growth in saline soil are multiplied. Consequently, the yields and profits of agricultural production in saline soil are reduced drastically [8]. In this respect, the applied chemical fertilizer is rapidly fixed to the unavailable forms especially in saline soil and accounts for low phosphorus efficiency [9]. Phosphate solubilizing bacteria (PSB) are known to improve solubilization of fixed soil phosphorus by the production of organic acids and acid phosphatase, and thereby increased crop yields [10]. Therefore, the PSB- based biofertilizers appear to be a more effective choice than the chemical fertilizers and are also friendly to the environment. The accomplishments of PSB in promoting plant growth are well documented [60, 61, 62, 63]. Karlidag *et al.* (2013) pointed out that application of PGPR (*Bacillus subtilis*, *B. atrophaeus* and *B. sphaerica*) inoculation could alleviate the deleterious effects of salt stress on the growth and yield of strawberry under saline condition. They obtained higher concentration of nitrogen, potassium, phosphorus, calcium, magnesium, sulfur, manganese, copper and iron with the exception of sodium and chlorine. Key factors for the successful applications of microbial inoculants are their abilities to survive and outcompete the often well adapted native microflora and colonize in the rhizosphere region [2].

However, little information is available on the use of halotolerant PSB to enhance crop yields under saline conditions. To reach this goal, the efficient halotolerant phosphate solubilizing bacteria that can solubilize the insoluble phosphate in the wide ranges of salinity and temperature were screened to evaluate their effects on tomato grown invitro and under saline conditions. Chookietwattana *et al.* (2012) selected eighty-four halotolerant bacterial strains and screened for the efficient halotolerant phosphate-solubilizing bacterium (PSB). A *Bacillus megaterium* A12 was selected as the efficient halotolerant PSB because it demonstrated the highest phosphate solubilization activity under saline conditions. The tomato (*Lycopersicon esculentum* Mill) seeds which were inoculated with the bacterium significantly increased the germination percentage and germination index especially at NaCl concentration between 30 and 90 mM and increased the seedling dry weight at NaCl upto 120 mM. Their results suggest that the halotolerant PSB may be used to alleviate the effects of salts and provide great potential for use as biofertilizers in the arid and salt-affected areas. However, the effect of halotolerant PSB on germination percentage and root length was less marked at 0 mM NaCl. An enhancement of tomato growth under saline conditions might be a result of increasing availability of phosphorus from microbial activity which was then taken up by plants for the emergence and development of seedlings since only the insoluble phosphate was applied as a source of nutrients to the control tomato seeds. These results are in agreement with the study of Awad *et al.* (1990) the SEM observations confirmed in vitro binding of *B. megaterium* A12 ag to tomato root surface and root hairs.

Possible mechanism of salinity tolerance in plants

The physiological and molecular mechanisms of tolerance to osmotic and ionic components of salinity stress are reviewed at the cellular, organ and whole plant-level [7]. Hypothetical relationships between salinity tolerance and leaf Na^+ concentration for three different species, denoted by a, b, and c for rice, durum wheat, and barley (Fig.2.) Within most species, there is a negative correlation between salinity tolerance and shoot Na^+ concentration, as in rice and durum wheat and, with less conviction, in barley. A larger intercept on the x-axis indicates an increased tolerance to the osmotic pressure of the soil solution or to high internal concentrations of Na^+ or Cl^- .

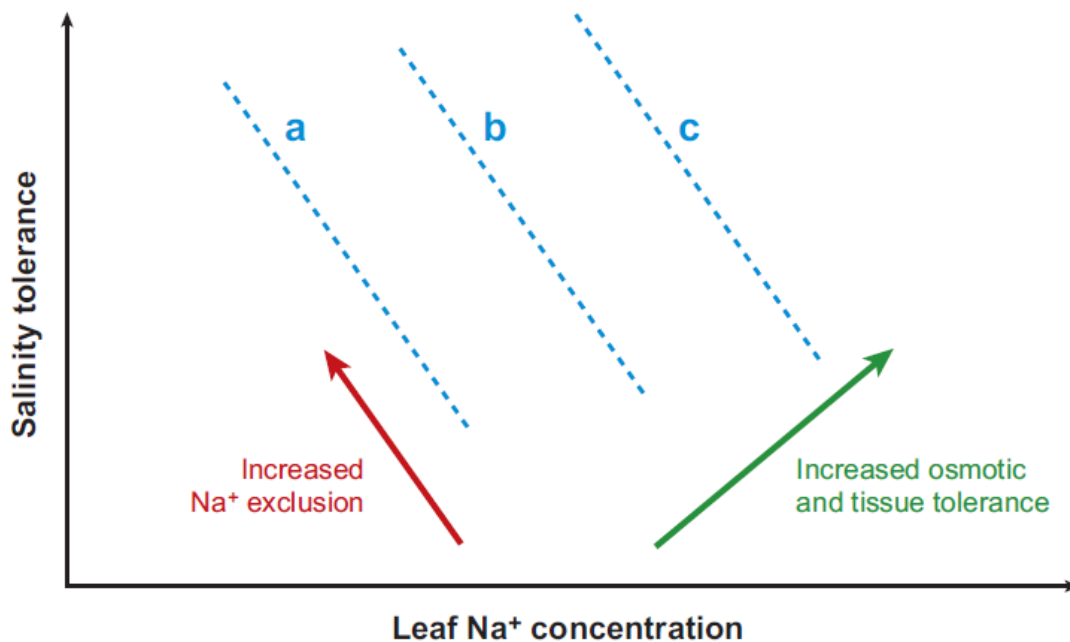


Fig.2 salinity tolerance and leaf Na^+ concentration in rice (a), durum wheat (b), and barley (c) [7].

Plant adaptations to salinity are of three types: Osmotic stress tolerance, Na^+ and Cl^- exclusion and the tolerance of tissue to the accumulated Na^+ and Cl^- . Our understanding of the role of HKT (high affinity K^+ transporter) gene is in the infant stage of development. Salinity is caused due to various types of salts namely chlorides of sodium, calcium and magnesium and to a lesser extent sulfates and carbonates [67]. Of these, NaCl is the most soluble and wide spread salt. All plants have evolved a mechanism to regulate its accumulation and to select against it in favor of other nutrients such as K^+ and NO_3^- . In most plants, Na^+ and Cl^- are effectively excluded by roots, while water is taken up from the soil. Whereas in halophytes, the natural flora of highly saline soils are able to maintain this exclusion at higher salinities than glycophytes [68]. In certain other plants, there is an increased accumulation of compatible solutes. Generally if Na^+ and Cl^- are sequestered in the vacuole of a cell, organic solutes (sucrose, proline and glycine betaine) that are compatible with metabolic activity accumulate in the cytosol and organelles to balance the osmotic pressure of the ions in the vacuole [69, 70].

Future Perspectives

Some of the recent investigations on PGPR- induction of saline tolerance in plants [71] are no doubt promising but still more work is needed on the following:

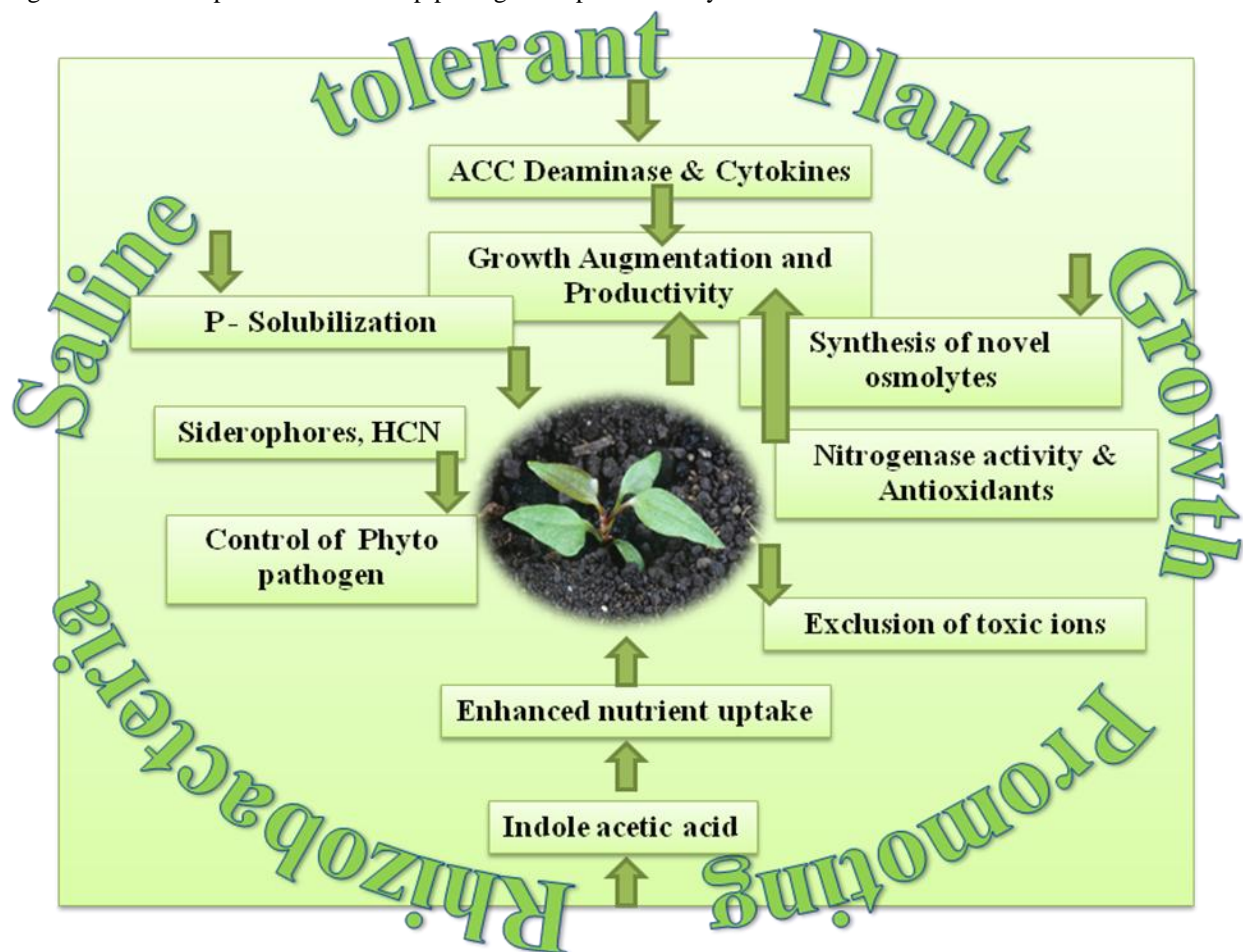
1. In most of the studies, only NaCl salinity has been used. But in reality salinity is caused by a combination of several salts
2. Therefore, there is a need to perform studies using artificial saline water in all *in vitro* assays
3. The paradigms of applicability of these beneficial bacteria in different agro ecosystems may be tried directly in saline soil employing the most popular crops in the locality

4. The coastal saline land also needs to be cultivated to step up crop production to satisfy the burgeoning population.
5. Further research on understanding the mechanisms of PGPR- mediated phytostimulation may pave way to find out more competent rhizobacterial strains to work under diverse agro eco systems.
6. Studies on induction of saline tolerance in many popular vegetable crops such as tomato (*Lycopersicon esculentum*), okra (*Abelmoschus esculentus*), leafy, root and tuberous vegetables employing PGPR are very much needed. This would also facilitate marginal farmers to raise vegetables for house-hold purposes in saline garden soil in and around their locality.

Conclusion

Salinity is a serious environmental issue, as it limits crop growth and drastically reduces productivity. In view of ever increasing global population, we are more constrained than ever before to augment crop productivity. Therefore, in addition to arable land, saline land needs to be cultivated for increased yield output. Besides, the plant-beneficial rhizobacteria may decrease the global dependence on hazardous agrichemicals which destabilize the agro-eco systems. This review accentuates the perception of the rhizosphere and plant growth promoting rhizobacteria in saline environment. In fact, diverse symbiotic (*Rhizobium*, *Bradyrhizobium* and *Mesorhizobium*) and non-symbiotic (*Pseudomonas*, *Bacillus*, *Azotobacter*, *Azospirillum* and *Azomonas*) rhizobacteria are now being used worldwide as bioinoculants to promote plant growth and development under various stresses like salinity [5,72]. Plant function is explained by the operation of genes in cells and tissues to regulate plant growth in coordination with environmental constraints. Developing salt tolerant crops is still in the pipeline and therefore, the only viable alternative seems to be the use of salt tolerant biofertilizers to promote growth and productivity of crop plants in saline soil (Fig: 3)

Fig: 3 Schematic representation of crop plant growth promotion by PGPR in saline soil



Acknowledgement

One of the authors, M. Vivekanandan is thankful to the University of Grants Commission, New Delhi, India for the award of Emeritus Fellowship to carry out this work.

The authors acknowledge all the necessary help and encouragement provided by Prof. M. Krishnan and Prof. R. BabuRajendran, Head, Department of Environmental Biotechnology School of Environmental sciences, Bharathidasan University, Tiruchirappalli – 620 024 in carrying out this work.

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