

**FUNCTIONAL ANALYSIS AND MOLECULAR
CHARACTERIZATION OF SALT-TOLERANT
MICROORGANISMS OF AGRICULTURAL
IMPORTANCE**

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1. INTRODUCTION

Adverse environmental factors of multifarious types affect plant growth, metabolism and ultimately the final yield of plants. An increased incidence of abiotic and biotic stresses that impact productivity of principal crops is being witnessed all over the world. Among the different environmental factors, salinity, drought, temperature, flooding, pollutants and radiation are the major abiotic factors limiting the productivity of crops. Salinity in soil or water is one of the major abiotic stresses (Liu *et al.*, 2011) and can severely limit the productivity of approximately 6% of the world's land and 20% of the irrigated land and negatively impacts agricultural yield worldwide (Munns, 2005).

The costs associated with soil salinity are potentially enormous and the effects of salinity may impact heavily on agriculture, biodiversity and the environment. In arid and semi-arid ecosystems, salinization is a major threat to the productivity of agricultural land (Sumner, 1995). In India, over 6.73 m ha of the total of 40 m ha of irrigated farmlands have been affected by salinity (Singh *et al.*, 2010 and Anonymous, 2011). The growth of many agriculturally important crops in arid and semiarid regions is affected adversely because of high soluble salt content, resulting in high osmotic pressure of soil solution and/ or high level of exchangeable sodium leading to poor soil physical conditions and low availability of essential nutrients. The accumulated ions are Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Cl^- , CO_3^{2-} and HCO_3^- .

Intensive agricultural practices with an aim to maximize the crop productivity at an unprecedented rate had led to exploitation of the technologies and strategies which are not in favor of the sustainability of soil health (Fox *et al.*, 2007). The excessive and ill-advised application of agro-chemicals (fertilizers and pesticides) in agricultural fields is posing grave threats to the soil health and fertility (Yu *et al.*, 2009). The progressive diminution in the application of agro-chemicals in farming practices without affecting yield or quality of the crops can only be possible with the advancement of new generation technologies. During the last couple of decades, the biotechnological advancements specifically in agriculture have unlocked new avenues for the augmentation of the agricultural productivity in a sustainable mode and have made possible exploitation of soil microorganisms for improving the crop health (Khan *et al.*, 2009). These in turn, have presented an economically feasible and ecologically sound alternative to minimize the common agricultural practices which result into decreased soil fertility and also affect the long term stability of soil ecosystem (Oves *et al.*, 2009).

The complexity of the soil ecosystem is established by the numerous and diverse interactions among its physical, chemical and biological components (Buscot, 2005). The variable genetic and functional activities of the heterogeneous microbial populations have a vital effect on soil functions, as such microbes are considered powerful forces for specific enzyme mediated fundamental metabolic processes (Ahemad *et al.*, 2009). Plant growth promoting rhizobacteria (PGPR) in the soil habitat play key roles in ecosystem functioning through controlling nutrient cycling reactions essential for maintaining soil fertility and also contributing to the genesis and maintenance of soil structure (Kirk *et al.*, 2004). Moreover, these microorganisms enhance the biogeochemical cycling of nutrients, production of phytohormones (IAA, GA) and control of plant pathogens and consequently help plants to grow better both under normal and stressed soil environment (Wani *et al.*, 2008).

Salt stressed soils are known to suppress the growth of plants. Saline stress decreases photosynthetic capacity and plants suffer from membrane destabilization and general nutrient imbalance (Parida and Das, 2005). Salinity also has a negative impact on the sustainability of beneficial microorganisms associated with the plant rhizosphere. In this context, the plant-beneficial microbe interaction is of much importance. The beneficial attributes of these organisms such as antagonism against bacterial and fungal pathogens of agricultural crops, nutrient recycling in the rhizosphere microcosm and root colonization are some of the challenges under stress. Salinity stress was shown to reduce the attachment of *Azospirillum brasilense* Cd to maize and wheat roots (Jofre *et al.*, 1998). Increase in salinity has been known to inhibit nitrogen fixation at the level of *nifH* expression and nitrogenase activity in *Azospirillum* sp. (Tripathi *et al.*, 2002).

Salinity presents a multifold challenge to all organisms in terms of perturbed osmotic balance, ionic disequilibria and generation of toxic metabolites. All organisms exhibit characteristic responses towards such abiotic stress and possess means to combat these. Depending upon the genetic make-up of the organism and the environment it normally dwells in, these responses vary greatly although some reactions seem to be universal for steady survival in salt environment. The ability to adapt to altered osmolarity of the surrounding growth medium is of fundamental importance for uni- and multi-cellular organisms. A dynamic and important property of cells is their ability to rapidly adapt to changes in external media, for example, increasing NaCl. As a general rule of thumb, bacteria and eukaryotes usually accumulate neutral compatible solutes whereas archaea prefer negatively charged solutes. Organic osmolytes fall into three general chemical categories: (i) zwitterionic solutes (ii) non-charged solutes and (iii) anionic solutes. Intertwined with these organic solutes are K and Na, which also contribute to osmotic balance in cells (Roberts, 2005). Abiotic stress indeed is a complex process, which signals cells to adapt themselves at the molecular, biochemical and physiological levels. Plants follow a number of salt-tolerant mechanisms such as synthesis of osmolytes and polyamines, reducing reactive oxygen species, the antioxidant defense mechanism, ion transport and compartmentalization (Agarwal and Jha, 2010).

Bacteria adapt to the fluctuations in soil salinity and osmolarity by either modulating the composition of the cell envelope (Lopez *et al.*, 2000) or accumulating osmolytes such as potassium ions, glycine betaine, proline, proline betaine, trehalose, ectoines and others (Galinski, 1995). These compatible solutes confer protection against the deleterious effects of low water activity, maintain the appropriate cell volume and protect intracellular macromolecules (Csonka and Epstein, 1996). Under osmotic stress, the accumulation of these osmoprotectants is achieved by either endogenous *de novo* synthesis or uptake mediated by specific transporters (Kempf & Bremer, 1998). Recent studies indicate that osmolytes can also function as free-radical scavengers or chemical chaperones by directly stabilizing membranes and proteins (Diamant *et al.*, 2001).

PGPR are a group of microorganisms that colonize the root of plants and enhance plant growth either directly or indirectly (Lugtenberg and Kamilova, 2009). Directly, PGPR promote plant growth by a higher acquisition of nutrients, increased availability of phytohormones and a greater uptake of phosphorus and nitrogen (Richardson *et al.*, 2009). Indirectly, PGPR affect the status of plants by eliciting an Induced Systemic Resistance (ISR) or Systemic Acquired Resistance (SAR), not allowing the soil-borne phytopathogens to inhibit plant growth (Bloemberg and Lugtenberg, 2001). PGPR have improved seed germination, plant weight, harvest yield and disease resistance (Kloepper *et al.*, 1999). Zhang *et al.* (2008) reported that PGPR regulate photosynthesis by decreasing glucose sensing and abscisic acid level in *Arabidopsis* plants. Yang *et al.* (2010) coined the term "Induced Systemic Tolerance" for PGPR-elicited tolerance in plants against abiotic stress.

There have been reports however that, when plant roots were treated with beneficial rhizobacteria, the extent of growth suppression (due to salt stress) was decreased and the bacterially treated plants accumulated more fresh and dry weights than untreated plants (Mayak *et al.*, 2004), indicating the beneficial role of rhizobacteria in alleviating the debilitating effects of salt stress. Nadeem *et al.* (2007) observed that inoculation with selected rhizobacteria increased the total chlorophyll contents (a, b and carotenoids) of maize and opined that it may be the result of an increased photosynthetic leaf area of the plant by rhizobacteria inoculation, compared with the un-inoculated control where the leaf area was reduced owing to stress.

Various reports have been published that elucidate the effect of PGPR in relieving abiotic stress in different crop plants (Zahir *et al.*, 2009 and Sandhya *et al.*, 2010). Majority of root-associated bacteria that display beneficial effects on plant growth have been shown to produce IAA and inoculation of various plant species with such bacteria has resulted in increased root growth (Long *et al.*, 2008). Promotion of root growth results in a larger root surface and can, therefore, have positive effects on water acquisition and nutrient uptake (Paul *et al.*, 2005) that is expected to alleviate the stress effects in the plant. Rhizobacteria mediated root proliferation has been well proved and it also works in stressed soils. PGPR promote plant growth by changing the selectivity of Na⁺, K⁺ and Ca²⁺ and maintain a higher K/Na ratio in inoculated plants under salt stress (Hamdia *et al.*, 2004).

PGPR-inoculated plants show a reduction in membrane potential, enhanced lateral root development due to higher nitric acid and IAA production, accelerated osmotic adjustment and production of ACC deaminase, which further reduces the ethylene-induced stress in plants (Dimkpa *et al.*, 2009). Rhizobacteria act as a sink for ACC (Saleem *et al.*, 2007), hydrolyzing it to ammonia and α -ketobutyrate and thereby lowering the level of ethylene in stressed plants. This has been shown to nullify the detrimental effect of ethylene. The enzyme ACC deaminase has been found in a wide range of rhizobacteria, such as the genera *Achromobacter*, *Acidovorax*, *Alcaligenes*, *Bacillus*, *Enterobacter*, *Klebsiella*, *Methylobacterium*, *Pseudomonas*, *Rhizobium* and *Variovorax* (Siddique *et al.*, 2011). Wheat plants inoculated with two salt-tolerant PGPR strains, *Bacillus subtilis* SU47 and *Arthrobacter* sp. SU18, either individually or in consortia, gave significant reduction in the activities of three antioxidant enzymes under different salinity regimes (2–6 dS/m) indicating that consortia of PGPR could alleviate the adverse effects of soil salinity on wheat growth (Upadhyay *et al.*, 2011).

Based on this background information, the present investigation was initiated to understand the role of salt-tolerant agriculturally important microorganisms (AIMs), their stress alleviating mechanisms in crop plants and their impact on growth and nutrient uptake of wheat with the following objectives.

1. To characterize and identify the salt tolerant agriculturally important groups of microorganisms.
2. To analyze agriculturally important functions among the different groups.
3. To understand the biochemical basis for salt tolerance.
4. To test the selected potential isolates for growth promotion of wheat plants under salt stress condition.
5. To test the performance of efficient isolates and their consortia in wheat under field condition.
6. To identify the presence/ absence of genes encoding tolerance/ resistance to high salt concentration.

2. REVIEW OF LITERATURE

The objective of the present investigation was to study the role of salt-tolerant agriculturally important microorganisms (AIMs) in developing sustainable systems for crop production under abiotic stress conditions. In this study, available research information on salt-stress has been gathered as soil salinity, which limits crop yield and restricts use of land, is a major constraint in food production. Research findings obtained by various investigators on salt-tolerant AIMs and their role in management of abiotic stress are reviewed and presented below.

2.1 Abiotic stress and its management

Numerous stresses caused by complex environmental conditions, *e.g.* bright light, UV, too high and low temperatures, freezing, drought, salinity, heavy metals and hypoxia, lead to substantial crop losses worldwide (Mahajan and Tuteja, 2005 and Mittler, 2006). These abiotic stresses might increase in the near future even because of global climate change (<http://www.ipcc.ch>). Among the abiotic factors that are shaping plant evolution, water availability is the most important (Kijne, 2006). Water stress in its broadest sense encompasses both drought and salt stress. Soil salinities are affecting extensive areas of land in both developed and developing countries. The agricultural intensification, together with unfavourable natural conditions, have accelerated soil salinity in many parts of the world. According to the FAO Land and Plant Nutrition Management Service, over six per cent of the world's land is *salt-affected* (Table 1a).

The term *salt-affected* refers to soil that are saline or sodic (Szabolcs, 1989). Saline soil has an excess of soluble salt in the soil solution, the liquid located between aggregates of soil. A sodic soil has too much sodium associated with the negatively charged clay particles. Salinity occurs through natural or human-induced processes that result in the accumulation of dissolved salts in the soil water to an extent that inhibits plant growth. Natural salinity results from the accumulation of salts over long period of time and is caused by two natural processes. The first is the weathering process that breaks down rock and release soluble salts of various type, mainly chlorides of sodium, calcium and magnesium and to a lesser extent, sulphates and carbonates. Sodium chloride is the most soluble salt. The second is the deposition of oceanic salt carried in wind and rain. Human-induced salinity results from human activity that changes the hydrologic balance of the soil between water applied (irrigation or rainfall) and water used by crops (transpiration). The most common causes are (i) land clearing and the replacement of perennial vegetation with annual crops and (ii) irrigation schemes using salt-rich irrigation water or having insufficient drainage (Munns and Tester, 2008).

Salinity and water deficit disrupt photosynthesis and increase photorespiration, altering the normal homeostasis of cells and cause an increased production of reactive oxygen species (ROS) such as the super oxide radical, hydrogen peroxide and hydroxyl radical (Miller *et al.*, 2010). Under optimal growth conditions, ROS are mainly produced at low level in organelles such as chloroplasts, mitochondria and peroxisomes (Apel and Hirt, 2004). The enhanced production of ROS during stress can pose a threat to cells but it is thought that ROS also acts as signal for the activation of stress-response and defence pathways (Pitzschke *et al.*, 2006).

The direct effects of salt on plant growth also involve nutrient imbalance caused by the loss of control on nutrient uptake and/or transport to the shoot leading to ion deficiencies (Munns, 2002). The main reason for these nutrient deficiencies can be related to the abundant presence of ions, like Na^+ and Cl^- , in the soil solution. Abundance of these soluble ions can decrease the activity of other essential elements in the soil and can lead to reduction in accessibility and uptake of some elements by the plants. Several studies show that plants exposed to environmental stresses require additional supplies of mineral nutrients to minimize the adverse effects of stress (Endris and Mohammed, 2007 and Heidari and Jamshid, 2010). In particular, it is known that salt stress causes reduction in P accumulation in plants, which develop P-deficiency symptoms. Several authors reported that, addition of soluble P to saline growth medium resulted in increased crop growth and yield (Grattan and Grieve, 1999 and Nadeem *et al.*, 2007).

Table 1a. Regional distribution of salt-affected soils, in million hectares (www.fao.org)

Regions	Total area (Mha)	Saline soils		Sodic soil	
		Mha	%	Mha	%
Africa	1899	39	2.0	34	1.8
Asia, the Pacific and Australia	3107	195	6.3	249	8.0
Europe	2011	7	0.3	73	3.6
Latin America	2039	61	3.0	51	2.5
Near East	1802	92	5.1	14	0.8
North America	1924	5	0.2	15	0.8
Total	12781	397	3.1	434	3.4

Agricultural scientists from different disciplines have made several approaches towards the development of sustainable agriculture in which yield losses are reduced and plant growth is improved. Such approaches include reclamation of salt-affected soils, agronomic manipulations, crop improvement, application of AIMS and development of transgenic crops and microbes.

2.1.1 Soil reclamation

Various technologies developed by soil scientists and agricultural engineers are in practice for the management of salt affected soils. The principal objective of the recovery of soils affected by salts is to reduce the concentration of soluble salts and of exchangeable sodium in the soil profile, to a level that does not prejudice the development of crops. A decrease in the degree of salinity involves the process of dissolution and consequent removal by percolation water, whereas a decrease in the exchangeable sodium content involves its displacement from the exchange complex by calcium before the leaching process. Since it is of low cost and relatively abundant in many parts of the world, gypsum is the corrective most used to recover sodic and saline-sodic soils (Gharaibeh *et al.*, 2009).

2.1.2 Agronomic manipulation

The increase in the frequency and severity of abiotic stresses is one of the main consequences of climate change. In particular, extreme weather events will result in more frequent drought and salinity. To mitigate the effects of these stresses, appropriate crop management techniques will be needed to ensure sufficient production of food from crop plants by increasing the productivity per unit of land area. Several low-technology management systems, such as crop rotation, intercropping, skip rows (decreasing planting density by omitting rows), mulching (with natural or synthetic mulches), protected cropping (enclosing the aerial environment of the crop under glass, plastic or netting) and biofertilization (single or mixed inoculation treatments) can be used to improve crop productivity (Davies *et al.*, 2010).

2.1.3 Crop improvement

To deal with saline soil and minimize crop loss, scientists have searched for salt-tolerant cultivars and have attempted to develop salt-tolerant crops through breeding (Araus *et al.*, 2008; Dwivedi *et al.*, 2010 and Sreenivasulu *et al.*, 2007). However, gaps in understanding the complex physiological, biochemical, developmental and genetic mechanisms that underlie environmental stress tolerance and the subsequent difficulty in combining favourable alleles to create improved high yielding genotypes, are the major constraints to improve crop yield under abiotic stress. Furthermore, it appears certain that domestication has narrowed the genetic diversity within crops for stress tolerance and thus limited options in traditional crop breeding.

Scientists are also using transgenic approaches to obtain genetically modified salt-tolerant plants (Ashraf and Akram, 2009 and Mittler and Blumwald, 2010). These approaches are time consuming and costly due to the impressive charges required to validate the consumption or cultivation of genetically modified plants.

Indeed, the development of transgenic plants with increased stress tolerance is primarily based on the performance of transgenic lines produced and tested under controlled conditions as greenhouse and can be found only few reports where the performance of transgenic cultivars was tested under field conditions. Several factors limit the success of producing salt-tolerant cultivars through genetic engineering. 1) In most cases only a single gene has been transformed, although salt stress resistance is polygenic. If, for example, osmoprotectant-producing, transcription factor expressing, ion homeostasis-maintaining and antioxidant enzymatic activities are all incorporated into a single species, there is a strong possibility that all these activities could work in concert to overcome concurrently present abiotic stresses. Transforming recipient plants with many genes or crossing plants containing different stress tolerance genes is very time consuming. 2) Transformation of agronomically important crops and identification of uncovered tolerance determinants or stress inducible promoters that direct the expression at proper time and place must be further explored to maximize salt tolerance.

2.1.4 Microbial management

For a microbial inoculant to be effective in soil, near optimum conditions for its survival and multiplication are required. However, in stressed ecosystem, very rarely such favourable conditions exist. Generally, a progressive decrease in the numbers of introduced organisms occurs following weeks after inoculation even under normal soils. Nevertheless, several approaches have been tried to overcome the stress effects on the survival and functioning of AIMS in soils. These include selection of salt-tolerant strains, adaptive host-micro symbiont combinations, strain improvement, rhizosphere engineering, better formulations, improved inoculation methods and management practices.

2.2 AIMS in the stress ecosystem

Microorganisms are ubiquitous in nature and are known to colonize different ecological niches and encounter several extreme environments on this planet. Such environments include extremities of temperature, acidity, salinity, pressure, desiccation and radiation. Microbial presence has been detected in hot vents where temperatures reach 113°C, polar regions which remain frozen for the better part of the year, at pH levels ranging from 0-9, salinities of 2-5 M sodium chloride (NaCl), deep sea environments where pressure reach 130 MPa, extreme desiccation and radiation prone environments (Rothschild and Mancinelli, 2001). Thus, it is the ubiquity and ability of microorganisms that set them apart from all other forms of life.

2.2.1 Characterization and identification

Methods used for discrimination of genera, species and isolates can be divided into phenotypic and genetic procedures. Phenotypic procedures take advantage of biochemical, physiological and biological phenomena, whereas genetic procedures aim to detect polymorphisms at the level of nucleic acids or to detect allelic variation at the level of enzymes (Louws *et al.*, 1999). Identification and additional typing can be done on the basis of their phenotypic characteristics such as cultural and biochemical characteristics like growth on selective or differential medium, general features such as odor and colony form and their action on various substrates and antibiotic susceptibility.

The shortcomings of phenotypically based typing methods have led to the development of typing methods based on the microbial genotype or DNA sequence, like (i) random whole-genome analysis; (ii) specific gene variation and (iii) mobile genetic elements.

The use of random amplified polymorphic DNA (RAPD), repetitive extragenic palindromic-PCR (rep-PCR), pulse field gel-electrophoresis (PFGE) and amplified fragment length polymorphism (AFLP), denaturing or temperature gradient gel electrophoresis (DGGE, TGGE) or single strand confirmation polymorphism (SCCP), DNA dot blot technique, fluorescent *in-situ* hybridization (FISH) etc minimize the problems with typeability and reproducibility and, in some cases, enable the establishment of large databases of characterized organisms (Olive and Bean, 1999). These techniques help to analyze the whole genome and provide a skeleton of polymorphic sites with exact genomic positions as whole-genome sequence data become available.

Different genes provide different levels of evolutionary information for determining isolate relatedness depending on whether they are highly variable (prone to recombination events and horizontal transfer), housekeeping genes with only a small number of single nucleotide differences between isolates. Comparative analyses of these different gene classes can provide enhanced information about isolate relatedness.

Mobile genetic elements such as insertion sequences, transposons, plasmids and bacteriophages integrate into the bacterial genome at specific (e.g. tRNA genes) or non-specific sites to alter band patterns produced by PFGE, RAPD, rep-PCR or AFLP.

Combination of all data, including those gathered by phototypic methods and those by the use of high-resolution molecular techniques, can lead to unequivocal identification and, in some cases, can even discriminate among isolates of a given species. Determination of unique characteristics of a microorganism enables the establishment of phylogenetic relationships (Belkum, 1994).

The use of ribosomal RNA (rRNA) as molecular marker to identify microorganisms is now routinely used in microbial ecology. By the pioneering work of Woese (Woese, 1987), it is now clear that all organisms on earth can be classified by comparative sequence analysis of their small subunit ribosomal RNA. PCR-amplified 16S rRNA sequencing is the most successful molecular technique used by various investigators across the world for the identification of salt-tolerant rhizobacteria isolated from various stress ecosystems (Stivaletta *et al.*, 2012) whereas internal transcribed spacer (ITS) regions is increasingly used to identify the fungi (Khan *et al.*, 2012).

2.2.2 Functional characterization

Plant growth-promoting rhizobacteria (PGPR) are free-living bacteria of beneficial agricultural importance. The PGPR present encourage beneficial effects on plant health and growth, suppress disease-causing microbes and accelerate nutrient availability and assimilation (Hayat *et al.*, 2010). Thus, in the quest to improve soil fertility and crop yield and to reduce the negative impacts of chemical fertilizers on the environment, there is a need to exploit PGPR for continued beneficial agricultural purposes. PGPR exist in the rhizosphere and this is defined as the region around the root. PGPR compensate for the reduction in plant growth caused by weed infestation (Babalola *et al.*, 2007), drought stress (Zahir *et al.*, 2008), heavy metals (Kumar *et al.*, 2008), salt stress (Egamberdieva, 2008 and Kaymak *et al.*, 2009) and some other unfavorable environmental conditions. One of the mechanisms by which bacteria are adsorbed onto soil particles is by simple ion exchange and a soil is said to be naturally fertile when the soil organisms are releasing inorganic nutrients from the organic reserves at a rate sufficient to sustain rapid plant growth. These bacteria belong to the genera *Acetobacter*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Derxia*, *Enterobacter*, *Gluconacetobacter*, *Herbaspirillum*, *Klebsiella*, *Ochrobactrum*, *Pantoea*, *Pseudomonas*, *Rhodococcus*, *Serratia*, *Stenotrophomonas*, *Zoogloea* etc and have been subject of extensive research throughout the years. Thus it is important to understand the microbial response to environmental stress, such as high concentrations of salts, heavy metals, temperature etc in the soil.

Stress can be detrimental for sensitive microorganisms and decrease the activity of surviving cells, due to the metabolic load imposed by the need for stress tolerance mechanisms. Soil salinity is a stress factor relating to microbial selection process and can reduce bacterial diversity and control microbial abundance, composition and functions (Ibekwe *et al.*, 2010). The salt requirement and tolerance properties of the bacteria are highly variable and may vary according to the growth temperature and the nature of the nutrients available (Quillaguaman *et al.*, 2004).

Several classifications have been proposed; one that is very well accepted considers the optimum growth of the microorganisms at different salt concentrations. Kushner (1978) defined several categories of microorganisms on the basis of their optimal growth: (1) non-halophiles are those that grow best in media containing less than 0.2 M NaCl (1% salt); (2) slight halophiles grow best in media with 0.2 to 0.5 M NaCl (1-3% salt); (3) moderate halophiles grow best with 0.5 to 2.5 M NaCl (3-15% salt) and (4) extreme halophiles show optimal growth in media containing 2.5 to 5.2 M NaCl (15- 32% salt) (Ventosa, 2006).

Lbekwe *et al.* (2010) reported that salinity and pH caused severe decrease in the rhizosphere bacterial population. The salinity negatively affects biological activity by high osmotic strength (low water potential) which can be attributed to the toxic effect on microbial growth, except tolerant halophilic bacteria (Yildirim *et al.*, 2008).

2.2.2.1 Dinitrogen fixation

Free living prokaryotes with the ability to fix atmospheric di-nitrogen are ubiquitous in the soil. The capacity for dinitrogen (N_2) fixation is widespread among bacteria. The estimated contribution of free-living N_2 fixing prokaryotes to the N_2 input of soil ranges from 0 to 60 kg ha^{-1} year $^{-1}$ (Mirzakhani *et al.*, 2009). Efficient strains that are area specific with selective traits to perform well under adverse conditions like soil acidity, salinity and moisture stress need to be developed and tested (Wani, 1992).

Saline habitats are nitrogen poor and have their own microbial population adapted to their environment (Zahran, 1997). Therefore N-input is very important in this environment. One of the sources of N-input in saline habitats is N_2 fixation (Whiting and Morris, 1986). Biological nitrogen fixation (BNF) offers nonpolluting source of nitrogen and could improve crop production and decrease the use of synthetic fertilizers (Cocking, 2003). In view of the impeding cost of fertilizers, the biological N_2 -fixation in terrestrial ecosystems has received vast attention in recent time.

The salinity of the soil plays a prominent role in the microbial selection process as environmental stress has been shown to reduce bacterial diversity (Borneman *et al.*, 1996). Earlier reports also indicate that bacteria isolated from a saline environment are more likely to survive inhibitory salt concentrations than their counterparts from non-saline habitats (Douka *et al.*, 1978 and Hua *et al.*, 1982).

The field of salt-tolerant rhizobacteria was recently reviewed by Egamberdiyeva and Islam (2008). The information on halotolerant diazotrophic PGPR is still limited; some examples of salt-tolerant PGPR include *Azospirillum halopraeferens* (Reinhold *et al.*, 1987), *Swaminathania salitolerans* (Loganathan and Nair, 2004) and the *Azospirillum brasilense* strain NH (Nabti *et al.*, 2007).

Saline stress inhibits nitrogen fixation quite significantly, inhibiting both the synthesis and activity of nitrogenase (Montserrat *et al.*, 2005). According to Moradi *et al.* (2011) increase in soil salinity is inversely proportional to the population of diazotrophs as well as their function. With increase in medium salt concentration from 0.44 to 1.02 M NaCl (equal with EC 35 and 70 dS/m of soil), the free-living diazotrophic populations decreased significantly. The authors concluded that, soil salinity is a stress factor relating to microbial selection process and can reduce bacterial diversity and control microbial abundance, composition and functions. Use of soil halotolerant or halophilic bacterial strains which can fix atmospheric nitrogen will be environmental friendly approach for nutrient management and ecosystem function for saline soils.

Fluctuations in salinity over the season affect diazotrophic populations as well as the amount of N_2 fixation in rice field soils of the coastal region of West Bengal, India. Recovery of microorganisms and N_2 fixation gradually decreased with extraneous addition of NaCl in the culture media (Barua *et al.*, 2011). Indigenous dinitrogen fixing bacterial strain *Azotobacter chroococcum* N-21 isolated from the hyper saline soils of Kolhapur district of Maharashtra, India was found to tolerate 16 per cent NaCl and reported to fix maximum elemental nitrogen at 4 per cent NaCl (Dhanraj *et al.*, 2012).

Novelty in terms of identity or the report has been proposed by several authors for their strains isolated from various stress ecosystems. Loganathan and Nair (2004) proposed a novel salt-tolerant genus and species, *Swaminathania salitolerans* gen. nov., sp. nov., which was isolated from the roots of salt-tolerant, mangrove-associated wild rice (*Porteresia coarctata* Tateoka) and reported to have dual properties of nitrogen fixation and P solubilization at 3 per cent NaCl. Similarly, a nitrogen fixing and P solubilizing bacterial strain *Rhodococcus fascians* was first time reported its association with the halophyte *Salicornia* in Bandar Abbas Iran desert. The isolate had an affinity to grow at 0 to 0.75 M of NaCl and tolerate 30 to 55°C (Rueda-Puente *et al.*, 2011). New halotolerant diazotrophic bacteria were isolated from roots of halophyte *Salicornia brachiata*.

The isolates were identified as *Brachybacterium saurashtrense* sp. nov., *Zhihengliuella* sp., *Brevibacterium casei*, *Haererehalobacter* sp., *Halomonas* sp., *Vibrio* sp., *Cronobacter sakazakii*, *Pseudomonas* spp., *Rhizobium radiobacter* and *Mesorhizobium* sp. These bacteria tolerated 3-4% NaCl concentrations in NFb semisolid medium and 15-20% NaCl concentrations in complex NB medium (Jha *et al.*, 2012).

It was long believed that there were no nitrogen-fixers among strains of the genus *Pseudomonas sensu stricto* (Young, 1992). Indeed, consistent with this assumption, most of the strains described as putative nitrogen-fixing *Pseudomonas* were later reassigned to genera in α - and β - proteobacteria (Chan *et al.*, 1994). It now seems that several strains unambiguously classified as true *Pseudomonas* spp. can be added to the list of nitrogen fixers, on the basis of physiological properties, nitrogenase assays, phylogenetic studies and detection of *nifH* DNA by hybridization or PCR amplification (Vermeiren *et al.*, 1999). Two nitrogen-fixing isolates belonging to the species *Pseudomonas stutzeri* CMT9A and *P. stutzeri* A15 were isolated from the roots of sorghum (Krotzky and Werner, 1987) and rice in China (You and Zhou 1989). Strain A15 was initially identified as an *Alcaligenes faecalis* and was later reassigned to *P. stutzeri* (Vermeiren *et al.*, 1999). *P. stutzeri* strains form a heterogeneous group comprising seven genomic subgroups, called genomovars, identified by DNA-DNA hybridization (Sikorski *et al.*, 1999).

2.2.2.2 Solubilization of phosphorous

Soil phosphorus dynamics is characterized by physicochemical and biological processes (Khan *et al.*, 2009). Soil microorganisms are considered to play an important eco-physiological role in soil P dynamics and subsequent availability of phosphate to plants. Among the whole microbial population in soil, phosphate solubilizing bacterial (PSB) isolates constitutes 1-50%, while phosphate solubilizing fungi (PSF) are only 0.1-0.5% in phosphorus solubilization potential (Chen *et al.*, 2006; Khan *et al.*, 2009). Microorganisms are central to the soil P cycle and play a significant role in mediating the transfer of P between different inorganic and organic soil P fractions, subsequently releasing available P for plant acquisition (McLaughlin *et al.*, 1988 and Oberson *et al.*, 2001). There are two aspects in microbial P solubilization: firstly, inorganic phosphate (Pi) released by solubilization processes (Rodriguez and Fraga, 1999) and secondly, Pi released from accumulated P in biomass of microorganisms (Oehl *et al.*, 2001).

Soil complexity determines multiple beneficial or stress effects depending on the ecological characteristics of a given area. Particularly in degraded ecosystems, soils with low level of soluble phosphate affect the activity of P solubilizing microorganisms by high salinity and drought, including additional stress factors such as pH, temperature, *etc.* Therefore, it is of great importance for the establishment, development and management of the plant cover to isolate soil microorganisms tolerant to more than one stress factors. For this reason, a number of studies appeared in the scientific literature that describes P solubilizing microorganisms isolated from soils characterized by various stress factors. Since the beginning of last century, many PSB have been isolated including, for example, those in *Bacillus*, *Pseudomonas*, *Erwinia*, *Agrobacterium*, *Serratia*, *Flavobacterium*, *Enterobacter*, *Micrococcus*, *Azotobacter*, *Bradyrhizobium*, *Salmonella*, *Alcaligenes*, *Chromobacterium*, *Arthrobacter*, *Streptomyces*, *Thiobacillus* and *Escherichia* (Zhao and Lin, 2001). The microorganisms functioning similarly also include some fungi in genus *Penicillium*, *Aspergillus*, *Rhizopus*, *Fusarium* and *Sclerotium* (Zhao and Lin, 2001). Unfortunately, most PSB isolated previously performed relatively low in salinity tolerance, being less appropriate for saline-alkali soil-based agriculture.

The establishment and performance of phosphate solubilizing microorganisms are severely affected by environmental factors, especially under stressful conditions (Mehta and Nautiyal, 2001). The halophilic bacterium, surviving in the saline environments, is an ideal organism for screening the salt-tolerant PSB to improve phosphorus release of salt-affected soils. There are strong evidences that some PSB naturally occur in the rhizosphere of halobiotic plant and take part in phosphorus geophysics cycles in the saline soils (Sharan *et al.*, 2008 and Chaiarn and Lumyong, 2009).

Many bacterial strains with P solubilizing abilities have been examined in previous studies, but only a few of them have been found to function well at high NaCl concentration.

A novel stress tolerant, phosphate-solubilizing bacterial strain *Xanthomonas campestris* isolated from saline-alkali soils has been reported to solubilize phosphorus in the presence of 8% NaCl concentration. It appeared to have potential to be used in saline soils as earlier reports could only identify bacteria capable of solubilizing P up to 5% NaCl (Sharan *et al.*, 2008). Moderately halophilic bacteria reported to grow well at 20% medium NaCl concentration was isolated from a solar saltern in Spain. It was identified as *Halomonas indalini* sp. nov. (Cabrera *et al.*, 2007). These studies paved an effective avenue of isolating halophilic and phosphorus-solubilizing bacteria from marine environment in order to promote saline-alkali soil-based agriculture.

In a large study, Johri *et al.* (1999) isolated 857 bacteria capable of solubilizing phosphate. Eighteen of them confirmed their activity in solid media with high salt presence and in liquid media supplemented with different salts (NaCl, CaCl₂ and KCl). It is noteworthy that the level of P solubilization of some bacterial strains was induced by salt addition to the medium but also enhanced by temperature increase to 37 °C.

Bacteria isolated from the rhizosphere of chickpea and tolerant to 0-20 % (w/v) NaCl were found to exhibit P solubilizing activity *in vivo* at temperatures as high as 37- 45 °C (Nautiyal *et al.*, 2000) and even 50 °C (Banerjee *et al.*, 2010). All tested strains were able to grow on different nitrogen and carbon sources and tolerated high pH values (8-12). Addition of Ca²⁺ and EDTA (up to 10 mg/ml) were found to enhance phosphate solubilization in four bacteria isolated from the rhizosphere of chickpea. Various biochemical additional analyses showed simultaneous production of plant growth metabolites and biocontrol activity thus confirming the potential of P solubilizing microorganisms with multiple beneficial functions. In some cases, indigenous bacteria tolerant to drought and salinity exhibited biochemical characteristics such as enhanced cell wall degrading enzyme activity combined with two or three plant growth promoting traits (Chaiharn and Lumyong, 2009).

Recently, the strain YCWA18 which can survive at high concentrations of NaCl (up to 20%) and pH range of 4.0-10.0 was isolated from the sediment of Daqiao saltern in China. The strain was reported to solubilize P in the presence of 6% NaCl concentration. The bacterium was identified as *Kushneria* according to its 16S rRNA gene sequence (Zhu *et al.*, 2011).

Several workers have reported the P solubilizing ability of filamentous fungi and their capacity to tolerate several abiotic stress factors like drought and salinity. Fungi have been detected in rhizosphere and in samples of every plant species examined to date (Blackwell, 2000). Their effect determines the relationships with plants and varies from pathogenic to mutually beneficial (Berbee, 2001). Plant beneficial endophytic fungi are gaining more attention due to the inability of arbuscular mycorrhizal fungi to be produced easily without plants. The most potent P solubilizers such as *Penicillium* and *Aspergillus* are cosmopolitan filamentous fungi which are usually described as epiphytic, but may also occur endophytically (Schulthess and Faeth, 1998). Particularly some *Aspergillus* species are now recognized as plant endophytes with strong capability of solubilizing inorganic and organic phosphates in arid soil (Barrow and Osuna, 2002).

Aspergillus ussuriensis isolated from the saltbush *Artiplex canescens* grown on arid soil was reported to solubilize insoluble inorganic phosphates typical for desert soil. This dark septate fungus which cannot be removed by heat and sterilization is suggested to have a significant ecological role in arid south-western rangelands in the USA. Its presence in seeds and plant tissues in dominant desert plants, low specificity, ability to form large mycelial network and dissolution of Ca-P complexes demonstrate well drought adapted functions that can be further studied and exploited. Similarly, tolerance to various abiotic stress factors such as drought, high temperature, high pH and high salinity, acidity, aluminium and iron was recently described for *Aspergillus niger*, *Aspergillus japonicus*, *Penicillium simplicissimum* and *Eupenicillium parvum* (Srividya *et al.*, 2009; Xiao *et al.*, 2011 and Yvas *et al.*, 2007). Normally, different strains demonstrate differential tolerance to one or the other stress factor (Xiao *et al.*, 2011).

The endemic microbial pool of a given soil may contain highly efficient genotypes and the indigenous strains are likely to perform better than the exotic strains (Yvas *et al.*, 2007).

For example, the results of the effects of temperature, pH and salt concentrations on growth of the fungus *Eupenicillium parvum*, as well as ability to tolerate desiccation, aluminum and iron showed that the maximum fungal biomass was obtained at 36 °C and pH 4.5. The indigenous fungus isolated from a tea soil was able to grow in up to 40% PEG 6000, 10% NaCl, 100 mM aluminum and 100 mM iron for up to 9 days of incubation in potato dextrose broth although a decline was registered in solubilization under these stress conditions. The latter is not always valid as Yadav *et al.* (2011) found that addition of salt (CaCl₂) to the fermentation system and increased pH stimulated P-solubilization by *Penicillium citrinum*.

Srinivasan *et al.* (2012) isolated salt-tolerant 23 P solubilizing bacteria (PSB) and 35 fungi (PSF) from salt affected soils and examined for P solubilization under different salt concentrations. They have reported increase in percent Pi release with increase in NaCl concentration up to 0.8 M for bacterial solubilization and declined thereafter but P-solubilizing ability of PSF decreased with increase in medium salt concentration.

2.2.2.3 Biocontrol activity

Soil salinization is reducing the area under cultivation by 1-2% every year, hitting hard the arid and semi-arid regions (FAO, 2002). As a result of soil salinization, plants are under saline or water imbalance stress and become more vulnerable to diseases, often caused by pathogenic fungi (Paul and Nair, 2008).

Under such circumstances one of the possible measures to improve crop health is to use salt-tolerant bacterial inoculants which can control diseases and/or which promote plant growth (Lugtenberg and Kamilova, 2004 and Mayak *et al.*, 2004) and it allows a considerable decrease in the use of agrochemicals. Development of such a stress-tolerant microbial strain associated with roots of agronomic crops can lead to improved fertility of salt-affected soils (Mayak *et al.*, 2004; Egamberdieva and Kucharova, 2009). There is also evidence that the use of beneficial microbes can enhance plant's resistance to adverse environmental stresses, *e.g.*, drought, salts, nutrient deficiency and heavy metal contaminations (Glick *et al.*, 2007). Presently, some bacterial inoculants consisting of *Pseudomonas*, *Bacillus*, *Streptomyces* and *Agrobacterium* strains have been commercialized, but there still is a need to improve the efficacy of these bacterial inoculants for stressed environmental conditions (Chebotar *et al.*, 2000; Copping, 2004 and Lugtenberg and Kamilova, 2004).

The *Pseudomonas* species, especially fluorescent pseudomonads, are particularly suitable to be used as biocontrol agents in crop protection because they can produce large amounts of secondary metabolites to protect plants from phytopathogens and stimulate plant growth in stressed saline soils (Rangarajan *et al.*, 2003; Berg *et al.*, 2005; Saravanakumar and Samiyappan, 2007 and Mishra *et al.*, 2010). The root colonization potential of the salt tolerant *Pseudomonas* strains was not hampered with higher salinity in soil (McInnes *et al.*, 1994 and Paul and Nair, 2008), thus confer on such isolates a potential competitive advantage to survive in arid and saline regions of the world. Biocontrol potential of salt-tolerant, root-colonizing bacterial strain *Pseudomonas chlororaphis* TSAU13 was evaluated under green house condition and registered to control foot and root rot of cucumber and tomato caused by *Fusarium solani* under conditions of soil salinity (Egamberdieva, 2012).

Seven bacterial strains isolated from rhizosphere of common bean growing at Uttarakhand Himalaya showed potential plant growth promoting (PGP) and antagonistic activities. Based on 16S rRNA gene sequence, the isolate BPR7 was identified as *Bacillus* sp. BPR7 which strongly inhibited the growth of several phytopathogens such as *Macrophomina phaseolina*, *Fusarium oxysporum*, *F. solani*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani* and *Colletotricum* sp. *in vitro*. Cell-free culture filtrate of strain BPR7 also caused colony growth inhibition of all test pathogens. PGP and antifungal activities of *Bacillus* sp. BPR7 suggest that it may be exploited as a potential bioinoculant agent for *Phaseolus vulgaris* (Pankaj Kumar *et al.*, 2012).

2.2.2.4 Phytohormone production

PGPR can alter root architecture and promote plant development with the production of different phytohormones like indole 3-acetic acid (IAA), gibberellic acid (GA) and cytokinins (Kloepper *et al.*, 2007). While IAA is involved in root initiation, cell division and cell enlargement, GA is involved in extension of stem tissue and both the hormones are produced by many PGPR (Salisbury, 1994).

Barea *et al.* (1976) observed 50 bacterial P solubilizing isolates positive for IAA, GA and cytokinin production of which 17 *Pseudomonas* isolates produced auxin and GA.

Reports describing factors that alter the level of phytohormone biosynthesis and/or the expression of biosynthetic genes in bacteria are numerous. However, it is not yet possible to integrate these factors into a comprehensive regulatory scheme of phytohormone biosynthesis in bacteria. A first class of factors influencing IAA biosynthesis in diverse bacteria is related to environmental stress, including acidic pH, osmotic and matrix stress and carbon limitation (Spaepen *et al.*, 2007).

Several authors have addressed the importance of phytohormones and their role in plant growth promotion under different extreme stress conditions like salinity, alkalinity, sodicity, drought and flooding (Spaepen *et al.*, 2007; Sachdev *et al.*, 2009 and Sadeghi *et al.*, 2012). In the studies reported so far, most of the authors have tested the IAA producing ability of the microbial isolates under normal conditions and very few under stress conditions and explored their possible application in plant growth promotion. Green house and field performance of the potential IAA positive isolates was extremely good under various stress conditions.

Simultaneously, several reports on effect of other stress factors like pH, carbon source etc on IAA and GA biosynthesis in various soil microorganisms are available but literature pertaining to IAA and GA biosynthesis under salt stress is limited. Despite the diversity in stress factors modulating IAA expression in different bacteria, a common feature can be identified (Spaepen *et al.*, 2007 and Kloepper *et al.*, 2007). The expression of genes involved in IAA biosynthesis is fine-tuned to encounter environmental stresses associated with the soil and plant environment.

Sachdev *et al.* (2009) isolated nine *Klebsiella* strains, out of which 6 strains demonstrated IAA production *in vitro* at varied NaCl concentrations (0-5% w/v). One strain K8 showed maximum IAA production of 21.9 µg per ml at 0.5 per cent NaCl and minimum of 11.2 µg per ml in absence of NaCl in the medium. On the contrary, Upadhyay *et al.* (2009) reported that, addition of NaCl results in gradual decline in both IAA and GA production. Ten salt-tolerant rhizobacterial isolates tolerating eight per cent NaCl were screened for IAA and GA production at different NaCl concentrations (2, 4, 6 and 8%). Addition of eight per cent NaCl to the growth medium showed reduction in IAA and GA production by 17 to 66 and 33 to 52 per cent respectively.

Sadeghi *et al.* (2012) studied the effect of graded concentration of NaCl (0, 100, 200, 300, mM NaCl) on the IAA biosynthesis in *Streptomyces* and reported gradual increase in IAA production with increase in salt concentration. The amount of IAA production was increased from 2.4 to 4.7 µg per ml with addition 300 mM NaCl.

2.3 Osmoadaptation in AIMS

Saline stress can gain importance, especially in agricultural soils where the high salinity may be a result of faulty irrigation practices and the excessive application of chemical fertilizers. Research has been carried out on naturally occurring saline soils where in the detrimental influence of salinity on the microbial soil communities and their activities is noted by several workers (Batra and Manna, 1997; Zahran, 1997; Rietz and Haynes, 2003 and Sardinha *et al.*, 2003). The effect is always more pronounced in the rhizosphere according to the increase in water absorption by the plants due to transpiration. The simple explanation for this is that life in high salt concentrations has a high bio-energetic taxation, since the microorganisms need to maintain osmotic equilibrium between the cytoplasm and the surrounding medium, excluding sodium ions from inside the cell. As a result, energy sufficient for osmoadaptation is required (Jiang *et al.*, 2007).

Exposure of microorganisms to high osmolality environments triggers rapid fluxes of cell water along the osmotic gradient out of the cell, thus causing a reduction in turgor and dehydration of the cytoplasm. Microorganisms actively respond to variations in the osmolality of their habitat (Miller and Wood, 1996). To counteract the outflow of water, microorganisms increase their intracellular solute pool by accumulation of very high intracellular concentrations of ions or by amassing large amounts of organic osmolytes.

This stress reaction, generally referred to as osmoregulation or osmoadaptation, is aimed at maintaining turgor and volume within boundaries acceptable for normal cellular physiology (Record *et al.*, 1998). Bacterial adaptations to increase in salinities in the environment not just involve turgor adjustment but are much broader on a cellular basis. It involves structural changes in the cell envelope, in the cytoplasmic membrane and a major reprogramming of the cell metabolism that is only poorly understood.

2.3.1 Bacterial accumulation of intracellular concentrations of ions

Under salinity stress, growth depression results from water deficiency and ion imbalance (Wyn Jones, 1981). In response to hyper osmotic stress, bacteria adjust their cell turgor by controlling fluxes of ions across cellular membranes. The cytoplasm is exposed to high ionic strength to achieve osmotic equilibrium by maintaining a cytoplasmic salt concentration similar to that of the surrounding media. The most rapid response to osmotic up-shock, both in gram-positive and gram-negative bacteria, is a stimulation of potassium (K) uptake (Whatmore *et al.*, 1990). Potassium acts as second messenger for stress signaling and as regulatory element for transcription control as well as counter ion for glutamate and other compounds during osmotic stress response. Among inorganic ions, increased potassium influx is believed to be a hallmark of bacterial response to hyper osmotic stress, K⁺ being the most abundant inorganic ion in the cell cytosol (Balaji *et al.*, 2000). The impact of potassium for bacterial life is indicated by the diversity of transport systems present for this particular ion. According to Shabala (2009) bacterial exposure to hyper osmotic stress, though results in an increased cellular accumulation of K⁺ it is the severity and a nature of hyper osmotic stress that determines the direction of net K⁺ efflux across the membrane and, ultimately, the intracellular K⁺ content and suggested that the process is, at least partially, explained by the voltage gating of K⁺ transporters. Even though much information about voltage gating of bacterial K⁺-permeable channels is not available yet, it has been reported in *Escherichia coli* that the K⁺ efflux is mediated by glutathione activated kefB/kefC proteins (Miller *et al.*, 2000). The occurrence of active potassium transport systems correlates potassium dependent bioenergetic homeostasis and survival of bacterial cells under stress conditions. In many enteric bacteria primary active P-type ATPases of the Kdp-type are present in addition to secondary carriers of the Trk-type. Ktr-type systems are related to Trk transporters and are present in many gram-positive bacteria (Ochrombel *et al.*, 2011). In many gram-positive and gram-negative bacteria, another uptake system that is present is the secondary Kup-type transporter. A comparison of potassium transport systems in certain bacterial strains revealed that in contrast to *Corynebacterium glutamicum* (harboring KtrAB), *Mycobacterium tuberculosis* harbored genes encoding the high affinity potassium ATPase Kdp while *Corynebacterium diphtheriae* carried a KtrAB transport system in addition to a CglK-type channel (Ochrombel *et al.*, 2011).

At least two different phases of turgor readjustment have been reported *viz.*, rapid and slower adaptive phase. In the rapid adaptive phase, K⁺ is taken up from the medium to quickly counter balance the external osmolarity. The corresponding anion (glutamate) that is synthesized *de novo* forming potassium glutamate. In the slower adaptive phase, K⁺ is expelled while compatible solutes are synthesized. At increased salinity, systems involved in sodium homeostasis also have been reported to be activated. Shabala (2009) reported an increase in internal Na⁺ concentration from several mM at low NaCl concentrations to over 1000 mM at 10% NaCl.

2.3.2 Intracellular biosynthesis of osmotically active compounds

An adaptation to counter balance the osmotic difference is accumulation of low molecular weight hydrophilic molecules, which do not interfere with cell metabolism. Furthermore, bacteria take up suitable compounds from their surroundings (da Costa *et al.*, 1998). These molecules do not inhibit overall cellular functions in a cell and this led to labeling them as 'compatible solutes' (Brown, 1976). The compatible solutes also called osmolytes include sugars, amino acids and their derivatives (Lamosa *et al.*, 1998). Polyols and derivatives, sugars and derivatives, amino acids and derivatives, betaines and ectoines and occasionally peptides are among the various types of organic molecules used for osmotic balance (Galinski, 1995). Compatible solutes could be synthesized *de novo* or if present in the medium, can be taken up by the organisms.

According to Lamosa *et al.* (1998) the latter strategy is preferred when appropriate substances are present in the environment or in the growth medium. In addition to their well studied function as osmoprotectants, compatible solutes also have protein stabilizing properties that support the correct folding of polypeptides under denaturing conditions both *in vitro* and *in vivo* (Street *et al.*, 2006). The stabilizing effect of compatible solutes on macromolecules and biosynthetic processes probably contribute to their physiological functions as protectants against stress.

Among the compatible solutes, glycine betaine, carnitine and proline are the major ones in bacterial osmoadaptation (Beumer *et al.*, 1994). Glycine betaine is the preferred compatible solute for majority of bacteria. Carnitine as an osmoprotectant was first reported by Kets *et al.* (1994) and has been shown that for the majority of bacteria carnitine is transported from the external environment rather than being synthesized endogenously. Proline has been shown to accumulate to high intracellular concentrations in a variety of bacteria, following exposure to osmotic stress (Measures, 1975). Many species of gram-positive bacteria accumulate proline by increased synthesis (Sandhu *et al.*, 1981), while gram-negative bacteria accumulate by enhanced transport (Le Rudulier and Bouillard, 1983). In a study conducted by Paul and Nair (2008) with the rhizobacteria, *Pseudomonas fluorescence* MSP-393, accumulation of Aspartic acid and Glutamic acid was found to be nearly 5 fold in cells when exposed to increasing concentration of salt while concentration of serine increased more than 7 fold. Among the compatible amino acids synthesized by MSP-393, concentration of Glutamic acid was the highest (up to 25,000 nM). Glutamate has been shown to be a common compatible solute during low level osmoadaptation in many bacteria and Archaea (da Costa *et al.*, 1998; Galinski 1995 and Csonka, 1989) while Aspartate is generally found at very low intracellular solute pool (Tempest *et al.*, 1970). The intracellular solute pool of Threonine, Glycine and Alanine were also found to increase with increasing salinity, in MSP-393.

Apart from these there are certain disaccharides acting as osmoprotectants in bacteria which include sucrose, maltose, cellobiose, gentibiose, turanose and palatinose (Gouffi and Blanco, 2000). These disaccharides do not get accumulated as cytosolic osmolytes (or immediate osmolyte precursors) in salt-stressed bacterial cell but get catabolised during early exponential growth, contributing indirectly to enhance the levels of two endogenously synthesized osmolytes, glutamate (two-fold increase) and N-acetyl-glutamyl-glutamine amide (six-fold), facilitating growth at elevated osmolarities (Sleator and Hill, 2001). There have been many reports of rhizobacteria making use of the plant exuded osmoprotectants and that the rhizobacterial osmoregulatory traits, to an extent depend on the type of compatible solutes excreted by the roots of the host plant (Sandhu *et al.*, 1981). The nitrogen fixing *Azospirillum* that colonize the intercellular spaces within plant roots gets immediate access to the osmoprotectants accumulated in the root and the energy demands for accumulation of these osmoprotectants are suggested to be effectively met through the catabolism of nutrients available in the root exudates (Tripathi and Mishra, 1998).

It is well-documented that model bacteria, such as *Escherichia coli* and *Bacillus subtilis*, can accumulate many more types of compatible solutes than they can synthesize *de novo*. Many uptake systems are well characterized, including high affinity uptake systems that belong to the group of primary active ABC-type transporters and symporters using H⁺ or Na⁺ gradients at the cytoplasmic membrane. Their expression and transport activity are stimulated by salt. Different classes of mechano-sensitive channel proteins exist in various bacteria. Three types of channels, named according to their electrical properties mechano-sensitive channels of large (MscL), small (MscS) and mini (MscM) conductance, has been first defined in *Escherichia coli* (Berrier *et al.*, 1996). In the gram-positive soil bacterium *Corynebacterium glutamicum*, the mscCG gene product (which is similar to the *E. coli* MscS protein) was related to the glutamate efflux activity (Nakamura *et al.*, 2007). Disruption of MscCG resulted in decreased glutamate efflux and mutations in the mscCG gene were shown to be correlated with constitutive glutamate secretion, but an unequivocal proof for MscCG being directly responsible for glutamate efflux has not yet been provided. Börngen *et al.* (2010) provided evidence that mechano-sensitive channels are key players in the response to hyper osmotic stress in bacteria. As a consequence, the physiological significance of mechano-sensitive efflux channels seems to be two fold, both as emergency efflux valves (hypo osmotic conditions) and as regulatory elements in fine tuning solute accumulation (hyper osmotic challenge).

In studies with *Lactococcus lactis* glycine betaine transport has been characterized at the genetic level to reveal the opuA (osmoprotectant uptake) operon (Bouvier *et al.*, 2000) which has since been renamed busA (betaine uptake system). The busA glycine betaine transport operon encodes an ABC-type transporter composed of busAA, the gene for the ATP-binding protein and busABC, a gene fusion of the permease protein and the substrate binding protein (Bouvier *et al.*, 2000).

2.3.3 Restructuring of the cell envelope

Bacterial cells that use the compatible solute for salt adaptation have the advantage that no great adjustments in the enzymatic machinery are necessary as the cytoplasmic conditions are kept as they are by the accumulation of compatible solutes. However, the cell surface is challenged by drastic changes and has to adapt to higher salt concentrations. Severe salt stress has been shown to have pleiotropic effects on the physiology of the soil bacterium, *B. subtilis* as the composition of the cytoplasmic membrane is altered (Lopez *et al.*, 2006), cell wall properties are adjusted and swarming capability of the cells is largely impaired (Steil *et al.*, 2003). Salinity stress also has been reported to alter the cell envelope composition of the rhizobacteria resulting in changes in proteins, periplasmic glucans and capsular, exo- and lipo-polysaccharides (Jofre *et al.*, 1998 and Altabe *et al.*, 1994) and is suggested that composition of the cell envelopes play an important role in osmoadaptation (López *et al.*, 2000).

Certain bacteria like *Pseudomonas* survive under stress conditions due to the production of exopolysaccharides (EPS), which protect microorganisms from hydric stress and fluctuations in water potential by enhancing water retention and regulating the diffusion of carbon sources in microbial environment (Sandhya *et al.*, 2009).

Azospirillum brasilense has been shown to produce large amounts of EPS which significantly improve their survival under stress (Chowdhury *et al.*, 2007). Changes in fatty acid composition of the lipid membrane also occur in response to salt stress. Klein *et al.* (1999) reported changes in the fatty acid composition of the bacterial membrane under osmotic stress, by varying the length of acyl chains, number of double bonds or branching of acyl chains by methyl groups.

Perhaps the most obvious adaptation strategy involves an increase in the proportion of anionic over zwitterionic phospholipids. This would add additional surface charge to the membrane that helps to maintain hydration of the interface and has a pronounced effect on lipid phase behavior (Sutton *et al.*, 1991). Salt stress induced higher levels of cyclo-propane fatty acid ($\Delta C19:0$) and lower levels of oleic acid (C18:1) in the lipid membrane of *Lactobacillus lactis* are known (Guillot *et al.*, 2000).

Cyclo-propane can be considered as an unsaturated fatty acid and as such, there is no net change in the ratio of unsaturated fatty acids to saturated fatty acids. It has been proposed that this change in the composition of the lipid membrane may regulate the activity of the glycine betaine transporter; however the mechanism is yet to be established (Guillot *et al.*, 2000). A cross linking of the peptidoglycan has also been shown to respond to variations in the salinity stress; NaCl stressed cells had shorter peptidoglycan inter-peptide bridges than the unstressed cells (Piuri *et al.*, 2005).

Osmotolerance in rhizobacteria have also been reported to be species specific and strain specific and also depends on the extent of salinity of the habitat from where the strain is isolated (Hartmann, 1989). Mechanical properties of bacteria, including cell wall elasticity change significantly as a result of forces acting on bacterial structure (Thwaites and Mendelson, 1991). Francius *et al.* (2011) showed that the presence of loose, flexible surface appendage around the bacteria under low electrolyte concentration condition acts as a protective barrier, thereby attenuating the impact of changes in extracellular ionic strength and lowering the osmotic pressure constraint.

2.4 Alleviation of abiotic stress in plants by salt-tolerant AIMS

Salinity, drought, temperature and other abiotic stresses lead to metabolic toxicity, membrane disorganization and generation of reactive oxygen species (ROS), inhibition of photosynthesis, reduced nutrient acquisition and altered levels of hormones.

Table 1b. Bacterially mediated plant tolerance to abiotic stress

Stress type	Bacterial inoculant	Plant species	Reference
Salt	<i>Azospirillum brasilense</i>	Chickpea (<i>Cicer arietinum</i>) Faba bean (<i>Vicia faba</i>)	Hamaoui <i>et al.</i> (2001)
Salt	<i>Achromobacter piechaudii</i>	Tomato (<i>Lycopersicon esculentum</i>)	Mayak <i>et al.</i> (2004)
Salt	<i>B. amylolequifaciens</i> , <i>B. insolitus</i> <i>Microbacterium</i> sp., <i>Pseudomonas syringae</i>	Wheat (<i>Triticum aestivum</i>)	Ashraf <i>et al.</i> (2004)
Salt	<i>A. piechaudii</i>	Tomato (<i>Lycopersicon esculentum</i>)	Mayak <i>et al.</i> (2004)
Salt	<i>Aeromonas hydrophila/caviae</i> <i>Bacillus insolitus</i> , <i>Bacillus</i> sp.	Wheat (<i>Triticum aestivum</i>)	Ashraf <i>et al.</i> (2004)
Salt	<i>Azospirillum</i>	Maize (<i>Zea mays</i>)	Hamdia <i>et al.</i> (2004)
Salt	<i>Piriformaspora indica</i>	Barley (<i>Hordeum vulgare</i>)	Waller <i>et al.</i> (2005)
Salt	<i>Scytonema</i>	Rice (<i>Oryza sativa</i>)	Rodriguez <i>et al.</i> (2006)
Salt	<i>Azospirillum</i>	Lettuce (<i>Lactuca sativa</i>)	Barassi <i>et al.</i> (2006)
Salt	<i>Pseudomonas fluorescens</i>	Groundnut (<i>Arachis hypogea</i>)	Saravanakumar and Samiyappan (2007)
Salt	<i>Pseudomonas syringae</i> , <i>P. fluorescens</i> <i>Enterobacter aerogenes</i>	Maize (<i>Zea mays</i>)	Nadeem <i>et al.</i> (2007)
Salt	<i>Azospirillum</i> sp.	Pea (<i>Pisum sativum</i>)	Dardanelli <i>et al.</i> (2008)
Salt	<i>Bacillus subtilis</i>	<i>Arabidopsis thaliana</i>	Zhang <i>et al.</i> (2008)
Salt	<i>A. brasilense</i>	Barley (<i>Hordeum vulgare</i>)	Omar <i>et al.</i> (2009)
Salt	<i>Pseudomonas mendocina</i>	Lettuce (<i>Lactuca sativa</i>)	Kohler <i>et al.</i> (2009)
Salt	<i>Pseudomonas pseudoalcaligenes</i> <i>Bacillus pumilus</i>	Rice (<i>Oryza sativa</i>)	Jha <i>et al.</i> (2010)
Salt	<i>Bacillus megaterium</i>	Maize (<i>Zea mays</i>)	Marulanda <i>et al.</i> (2010)
Salt	<i>B. pumilus</i> , <i>P. mendocina</i> , <i>Arthrobacter</i> sp., <i>Halomonas</i> sp., and <i>Nitrincola lacsaponensis</i>	Wheat (<i>Triticum aestivum</i>)	Tiwari <i>et al.</i> (2011)
Salt	<i>Arthrobacter globiformis</i>	Tomato (<i>L. esculentum</i>)	Goel <i>et al.</i> (2011)

Table 1 contd.....

Stress type	Bacterial inoculant	Plant species	Reference
Salt	<i>Brachybacterium saurashtrense</i> , <i>Brevibacterium casei</i> and <i>Haererothalobacter</i> sp.	Groundnut (<i>Arachis hypogaea</i>)	Shukla <i>et al.</i> (2012)
Salt	<i>Pseudomonas chlororaphis</i>	Tomato (<i>L. esculentum</i>)	Egamberdieva (2012)
Salt	<i>Bacillus aryabhatai</i> , <i>Brevibacterium epidermidis</i> and <i>Micrococcus yunnanensis</i>	Pepper (<i>Capsicum annuum</i>)	Siddikee <i>et al.</i> (2012)
Salt	<i>Raoultella planticola</i> and <i>Klebsiella oxytoca</i>	Cotton (<i>Gossypium hirsutum</i>)	Wu <i>et al.</i> (2012)
Drought	<i>Pantoea agglomerans</i>	Wheat (<i>Triticum aestivum</i>)	Amellal <i>et al.</i> (1998)
Drought	<i>Paenibacillus polymyxa</i>	<i>Arabidopsis thaliana</i>	Timmusk and Wagner (1999)
Drought	<i>A. brasilense</i>	Bean (<i>Phaseolus vulgaris</i>)	German <i>et al.</i> (2000)
Drought	<i>Rhizobium</i> sp.	Sunflower (<i>Helianthus annus</i>)	Alami <i>et al.</i> (2000)
Drought	<i>A. brasilense</i>	Maize (<i>Zea mays</i>)	Casanovas <i>et al.</i> (2002)
Drought	<i>Azospirillum</i> sp.	Wheat (<i>T. aestivum</i>)	Creus <i>et al.</i> (2004)
Drought	<i>Achromobacter piechaudii</i>	Tomato (<i>L. esculentum</i>)	Mayak <i>et al.</i> (2004)
Drought	<i>Azospirillum</i>	Wheat (<i>T. aestivum</i>)	Creus <i>et al.</i> (2004)
Drought	<i>Bacillus</i>	Lettuce (<i>Lactuca sativa</i>)	Arkhipova <i>et al.</i> (2007)
Drought	<i>Ensifer meliloti</i>	Bean (<i>Phaseolus vulgaris</i>)	Mnasri <i>et al.</i> (2007)
Drought	<i>Bradyrhizobium elkanii</i>	Flat crown (<i>Albizia adianthifolia</i>)	Swaine <i>et al.</i> (2007)
Drought	<i>P. mendocina</i>	Lettuce (<i>Lactuca sativa</i>)	Kohler <i>et al.</i> (2008)
Drought	<i>Rhizobium tropici</i> <i>Paenibacillus polymyxa</i>	Bean (<i>Phaseolus vulgaris</i>)	Figueiredo <i>et al.</i> (2008)
Drought	<i>Pseudomonas</i> sp.	Pea (<i>Pisum sativum</i>)	Arshad <i>et al.</i> (2008)
Drought	<i>P. mendocina</i> <i>Glomus intraradices</i>	Lettuce (<i>Lactuca sativa</i>)	Kohler <i>et al.</i> (2008)
Drought	<i>Pseudomonas</i> spp.	Asparagus (<i>Asparagus officinalis</i>)	Liddycoat <i>et al.</i> (2009)
Drought	<i>Pseudomonas putida</i> P45	Sunflower (<i>Helianthus annus</i>)	Sandhya <i>et al.</i> (2009)
Drought	<i>Pseudomonas</i> spp.	Maize (<i>Zea mays</i>)	Sandhya <i>et al.</i> (2010)
Drought	<i>Azospirillum</i> sp.	Wheat (<i>T. aestivum</i>)	Arzanesh <i>et al.</i> (2010)
Drought	<i>Bacillus amyloliquefaciens</i> and <i>A. brasilense</i>	Wheat (<i>T. aestivum</i>)	Kasim <i>et al.</i> (2012)

Accumulation of osmoprotectants, production of superoxide radical scavenging mechanisms, exclusion or compartmentation of ions by efficient transporter and symporter systems, production of specific enzymes involved in the regulation of plant hormones are some of the mechanisms that plants have evolved for adaptation to abiotic stresses (Des Marais and Juenger, 2010; Parida and Das, 2005 and Santner *et al.*, 2009).

Besides developing mechanisms for stress tolerance, microorganisms can also impart some degree of tolerance to plants towards abiotic stresses like salinity, drought, chilling injury, metal toxicity and high temperature. In the last decade, bacteria belonging to different genera including *Rhizobium*, *Bacillus*, *Pseudomonas*, *Pantoea*, *Paenibacillus*, *Burkholderia*, *Achromobacter*, *Azospirillum*, *Microbacterium*, *Methylobacterium*, *Variovorax*, *Enterobacter* *etc.* have been reported to provide tolerance to host plants under salt and drought stress environments (Table 1b). Use of these microorganisms *per se* can alleviate stresses in agriculture thus opening a new and emerging application of microorganisms. A variety of mechanisms have been proposed for the microbial elicited stress tolerance in plants (Fig. 1).

Plants in their natural environment are colonized both by endocellular and intracellular microorganisms (Gray and Smith, 2005). Rhizosphere microorganisms, particularly beneficial bacteria and fungi, can improve plant performance under stress environments and, consequently, enhance yield both directly and indirectly (Dimkpa *et al.*, 2009). Some plant growth-promoting rhizobacteria (PGPR) may exert a direct stimulation on plant growth and development by providing plants with fixed nitrogen, phytohormones, iron that has been sequestered by bacterial siderophores and soluble phosphate (Hayat *et al.*, 2010 and Rodriguez and Fraga, 1999). Others do this indirectly by protecting the plant against soil-borne diseases, most of which are caused by pathogenic fungi (Lugtenberg and Kamolova, 2004).

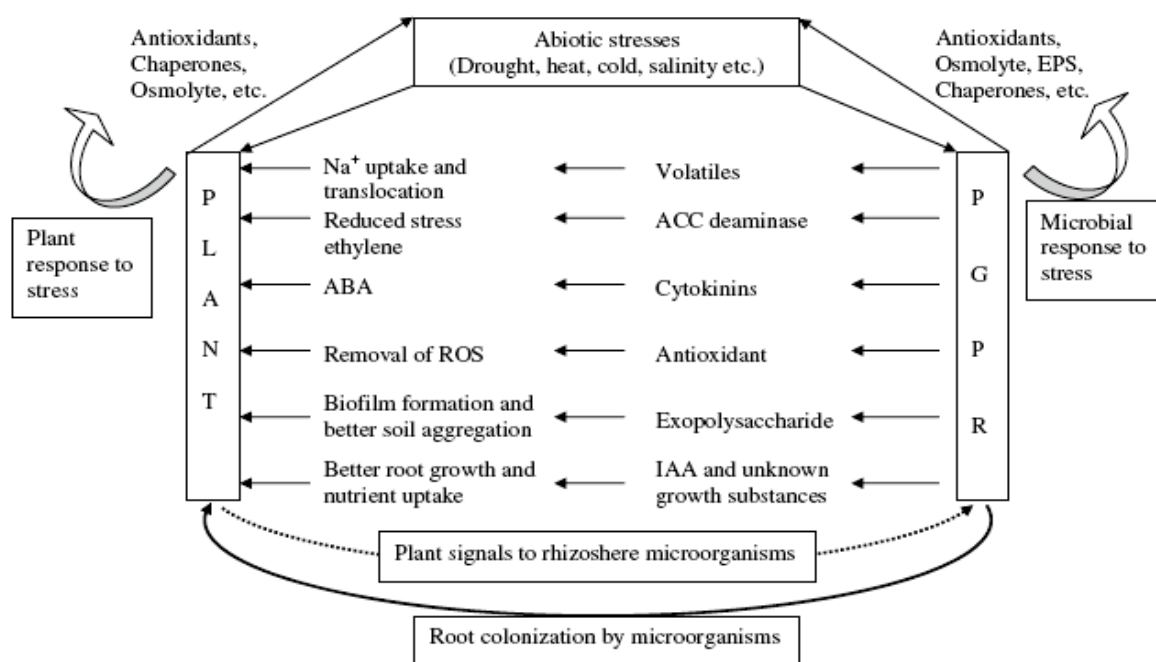


Fig 1. Conceptual diagram, on the plant-microbe interactions under abiotic stress (Grover *et al.*, 2011)

2.4.1 Elicitation of antioxidative enzymes activity

In plants ROS such as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen are continuously produced as byproducts of various metabolic pathways localized in different cellular compartments (Apel and Hirt, 2004). A common feature of these species is their capacity to cause oxidative damage to proteins, DNA and lipids. Since internal O₂ concentrations are high during photosynthesis, chloroplasts are especially prone to generate activated oxygen species (Gill and Tuteja, 2010). Under physiological steady state conditions, these molecules are scavenged by different antioxidative defence components that are often confined to particular compartments (Apel and Hirt, 2004). Under normal growth conditions, the production of ROS in cells is low, whereas, during stress their rate of production is enhanced. ROS accumulation during stress results from the imbalance between production and scavenging of ROS. Major ROS scavenging mechanisms of plants include superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) enzymes. Antioxidants such as ascorbic acid and glutathione, which are found in high concentration in chloroplasts and other cellular compartments, are also crucial for plant defence against oxidative stress (Miller *et al.*, 2010). For the detoxification of excess ROS in plant, the overall balance between different antioxidants is crucial for determining the steady state level of superoxide radicals and hydrogen peroxide and has to be tightly controlled (Mittler, 2002).

Induction of antioxidant enzymes (catalase and total peroxidase) is involved in the alleviation of salinity stress in lettuce plants inoculated with PGPR strains (Kohler *et al.*, 2010). Under non-saline conditions, inoculation with *Pseudomonas mendocina* and fertilization led to similar increases in plant growth (about 30% greater than the control plants). Salinity decreased the dry weight of the shoots and roots for all lettuce plants. However, the plants inoculated with *P. mendocina* had significantly greater shoot biomass than the control plants at both medium and high salinity levels.

Recent study reports the potential of PGPR strains in alleviating drought stress effects in maize. Maize plants inoculated with drought tolerant plant growth promoting *Pseudomonas entomophila*, *P. stutzeri*, *P. putida*, *P. syringae* and *P. montelli* were subjected to drought stress and the effects of inoculation on growth, osmoregulation and antioxidant status was investigated. Stress-tolerant PGPRs were found to decrease the antioxidative enzymes activity in the inoculated plants significantly when compared to uninoculated plants (Sandhya *et al.*, 2010). It was also reported that, increase in soil salinity leads to a significant increase of catalase and peroxidase activities in salt-stressed leaves of barley. While, inoculation of barley with *Azospirillum brasilense* was found to decrease the magnitude of increase in catalase and peroxidase activity as compared to uninoculated plants and significantly ameliorate the deleterious effects of salinity thus improves the crop productivity (Omar *et al.*, 2009).

Plant growth promotional activity of two PGPR strains viz., *Bacillus subtilis* SU47 and *Arthrobacter* sp. SU18, tolerating 8% NaCl were evaluated in wheat under different salinity regimes (2–6 dS/m). The activity of antioxidant enzymes in wheat leaves decreased under salinity stress after PGPR co-inoculation, suggesting the role of salt-tolerant PGPR strains to ameliorate the salt stress in wheat plants. Both strains were found to decrease the activity of three antioxidant enzymes in wheat most notably that of catalase activity at a salinity of 6 dS/m, when compared to uninoculated control. It was concluded that, co-inoculation with *B. subtilis* and *Arthrobacter* sp. could alleviate the adverse effects of soil salinity on wheat growth (Upadhyay *et al.*, 2011). On the contrary, *Medicago* plants inoculated with IAA-overproducing PGPR strains showed high antioxidant enzymes activity which contributed to enhance plant protection against salt stress (Bianco and Defez, 2009).

These results, which apparently seem to be in contradiction with the assumption that stress resistance in plants is related to more effective antioxidant systems, are an implication of the same positive effect and indicate that inoculated plants felt less stress as compared to uninoculated plants.

2.4.2 Phytohormones synthesis and modulation

Plants are sessile organisms with a high level of physiological plasticity, enabling survival under a wide variety of environmental stresses.

This is due to the continuously active shoot and root meristems and their capability to generate new organs after embryogenesis (Wolter and Jurgens, 2009). They have developed an extensive array of defensive responses that includes changes in the root morphology. The root architecture of the plants, which is determined by the pattern of root branching (lateral root formation) and by the rate and direction of growth of individual roots (Malamy, 2005), constitutes an important model to study how developmental plasticity is translated into growth responses under several environmental stresses.

Morphogenesis is tightly linked to hormonal homeostasis, with several hormones controlling cell elongation, cell division and re-orientation of growth. The physiologically most active auxin in plants is indole-3-acetic acid (IAA) and the fact that no fully auxin-deficient mutant plants have been identified so far reflects the importance of auxin in plant development. There is a high capacity for auxin biosynthesis not only in young aerial tissues, but also in roots, particularly in the meristematic primary root tip (Teale *et al.*, 2006).

Auxin and its fine concentration gradients have powerful effects on plant development and in particular on lateral root formation and branching, two key components of the response phenotype induced in plants under stress conditions (Potters *et al.*, 2009). Alteration in the pattern of lateral root formation and emergence in response to P availability is mediated by changes in auxin sensitivity in *Arabidopsis thaliana* roots. These changes alter the expression of auxin-responsive genes and stimulate pericycle cells to proliferate (Perez-Torres *et al.*, 2008).

Exogenous auxin application results in formation of branched root and, similarly, mutants that accumulate high levels of auxin, or mutants with an altered auxin distribution, produce excess of lateral roots.

A broad range of abiotic stresses induce lateral root formation, therefore auxin may be an intermediate between the action of a stressor and the realization of response phenotype. Several mechanisms have been proposed to explain stress-induced changes in auxin metabolism and/or receptiveness; however, evidences for stress-induced changes in auxin transport and catabolism are predominantly found in literature.

For example, water and osmotic stresses impact on auxin transport by altering the expression of PIN genes and/or by inhibition of polar auxin transport (Potters *et al.*, 2009). Moreover, auxin conjugates and the respective hydrolases were shown to be involved in the reaction of plant to stress (Muller, 2011). Interestingly, overexpression of an auxin-amidohydrolase in *Arabidopsis* is associated with a reduced inhibition of root elongation and increased resistance to salt stress. This effect was probably due to the increase in the content of free auxin sufficient to provide a protective effect against salt stress (Junghans *et al.*, 2006).

As more plant tissues are analyzed for the presence of bacteria, an increased number of IAA-producing PGPR strains are detected inside the plant tissue (Spaepen *et al.*, 2008). Various plant species inoculated with such bacteria showed increased root growth and/or enhanced formation of lateral roots and roots hairs (Dimkpa *et al.*, 2009).

For example, the stimulatory effect of *Azospirillum* strains on the development of roots is well documented. Morphological plant root changes have been observed repeatedly upon *Azospirillum* inoculation and have been attributed to the production of plant-growth promoting substances: auxins, cytokinins and gibberellins, with auxin production being quantitatively the most important (Spaepen *et al.*, 2008). Specific evidences for the involvement of auxins produced by *Azospirillum* in roots proliferation were obtained in many cases. Addition of filter-sterilized culture supernatants of *A. brasiliense* to rice roots grown in hydroponic tanks increased root elongation, root surface area, root dry matter and development of lateral roots and root hairs, compared with untreated roots (El-Khawas and Adachi, 1999). Similarly, a cell-free supernatant of *A. brasiliense* Cd applied to soybean plants induced many roots and increased root length (Molla *et al.*, 2001). Exogenous application of IAA to bean roots resembled responses of these plants to inoculation with *Azospirillum* (Remans *et al.*, 2008). More direct evidence for the importance of IAA was provided when several IAA-attenuate mutants were compared with their parental wild types for their effect on plant growth. A mutant of *A. brasiliense* with low production of phytohormones, but high N₂-fixing activity, did not enhance root growth over uninoculated controls (Kundu *et al.*, 1997).

Bianco and Defez (2009) analyzed the growth of *Medicago truncatula* plants nodulated by *Sinorhizobium meliloti* strains under severe salt stress conditions. *Medicago* plants nodulated by the IAA overproducing strain RD64 (*Mt*-RD64) showed a phytohormone re-modulation, with a higher IAA content in nodules and roots and a reduced accumulation of IAA in the shoot as compared to the plants nodulated by the wild-type strain 1021 (*Mt*-1021). Transcriptional analysis of the main ethylene signalling genes showed that, *Mt*-1021 inoculated plants showed induction of ethylene pathway when 150 mM NaCl was imposed whereas it was not induced in plants inoculated with IAA overproducing strain *Mt*-RD64, indicating the role of IAA in protecting the *Medicago* plants against abiotic stress. Production of indole acetic acid, gibberellins and some unknown determinants by PGPR, results in increased root length, root surface area and number of root tips, leading to enhanced uptake of nutrients thereby improving plant health under stress conditions (Egamberdieva and Kucharova, 2009).

2.4.3 Suppression of ethylene production

Ethylene is well known to function in various physiological events such as triple responses of seedlings, senescence including leaf abscission and fruit ripening (Abeles *et al.*, 1992). Ethylene is also well known to work as a stress hormone. It has been shown that plants produce ethylene at two different phases in response to stressful stimuli. In the first phase, the small amount of ethylene produced promotes the activity of stress-related genes. In the second phase (1-3 days after stimulus application) the larger amount of ethylene produced lead to inhibition of growth and harmful effects on plants including senescence, chlorosis and abscission (Glick *et al.*, 2007).

The enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (E.C. 4.1.99.4) cleaves ACC, the immediate precursor of ethylene in plants, to form ammonia and α -ketobutyrate. This multimeric enzyme is a common component of many soil microorganisms, both bacteria and fungi. It has also been suggested, based largely on sequence similarities, that some plants may contain ACC deaminase genes. However, it has not yet been unequivocally demonstrated that these putative ACC deaminase genes encode an enzyme with ACC deaminase activity.

Until recently, bacterial strains that contain ACC deaminase have been identified in a wide range of genera such as *Achromobacter*, *Agrobacterium*, *Alcaligenes*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Ralstonia* and *Rhizobium* (Blaha *et al.*, 2006) and regulation of ACC is a principal mechanism by which bacteria exert beneficial effects on abiotically stressed plants (Saleem *et al.*, 2007). Bacteria possessing this enzyme can use the immediate ethylene precursor ACC as a source of nitrogen. Bacterial hydrolysis of ACC leads to a decrease in plant ethylene level, which in turn, results in increased root growth (Belimov *et al.*, 2009 and Long *et al.*, 2008). Considering the relationship between IAA and ethylene precursor ACC (Dimkpa *et al.*, 2009), the positive effects of IAA on root growth can be either direct or indirect through the reduction of ethylene levels (Lugtenberg and Kamilova, 2009).

Degradation of the ethylene precursor ACC into 2-oxobutanoate and ammonia by bacterial ACC-deaminase lowers the ethylene concentration in plant roots, relieves the ethylene repression of auxin response factor synthesis and indirectly increases plant growth (Kang *et al.*, 2010). It has been proposed that ACC might be exuded from plant roots and that soil bacteria containing ACC-deaminase could convert this for their growth. As a result, the hydrolyzed ACC products would enhance bacterial growth. Taken together, the ACC-deaminase function seems to be mutually beneficial between plants and PGPR, because ethylene in plants can be reduced by continuous ACC secretion and degradation by bacteria and bacteria can use metabolized ACC (Glick *et al.*, 2007).

ACC deaminase-containing PGPR strains have found practical application in protecting different plant species against growth inhibition caused by various environmental stresses. Mayak *et al.* (2004) reported that *Achromobacter piechaudii* having ACC deaminase activity significantly increased the fresh and dry weights of tomato seedlings grown in the presence of NaCl (up to 172 mM).

Pseudomonas fluorescens strain TDK1 containing ACC deaminase activity enhanced the saline resistance in groundnut plants and increased yield as compared to plants inoculated with *Pseudomonas* strains lacking ACC deaminase activity (Saravanakumar and Samiyappan, 2007). *Pseudomonas putida* UW4, which produces IAA and ACC deaminase, protected canola seedlings from growth inhibition by high levels of salt. Siddikee *et al.* (2010) have also confirmed that inoculation with 14 halotolerant bacterial strains ameliorate salt stress in canola plants through the reduction of ethylene production via ACC deaminase activity. Inoculation of maize plants with *Pseudomonas fluorescens* containing ACC deaminase boosted root elongation and fresh weight significantly under saline conditions (Kausar and Shahzad, 2006).

Inoculation with *Pseudomonas* spp. containing ACC deaminase partially eliminates the effects of drought stress on growth, yield and ripening of pea (*Pisum sativum* L.) (Arshad *et al.*, 2008). Nadeem *et al.* (2010) reported that rhizobacteria capable of producing ACC deaminase mitigate salt stress in wheat. Inoculation with ACC deaminase containing bacteria induce longer roots which might be helpful in the uptake of relatively more water from deep soil under drought stress conditions, thus increasing water use efficiency of the plants under drought conditions (Zahir *et al.*, 2008).

Two potential plant growth-promoting rhizobacterial strains Rs-2 and Rs-5 with ACC deaminase activity were isolated from salinized soils using ACC as the sole nitrogen source. Rhizobacterial strains were identified as *Raoultella planticola* Rs-2 and *Klebsiella oxytoca* Rs-5 and reported to exhibit multiple functions viz., nitrogen fixation, P and K solubilization.

Inoculation of these strains to cotton increased the germination rate and the activity of protective enzymes under salinity stress conditions and reported to have tremendous capability in alleviating salinity stress by raising IAA contents and regulating ethylene and ABA changes in cotton plants (Wu *et al.*, 2011 and 2012).

Siddikee *et al.* (2012) reported the role of ACC deaminase producing halotolerant bacteria in regulation of ethylene biosynthesis under salt stress in red pepper (*Capsicum annuum* L.). Inoculation of halotolerant bacterial strains viz., *Bacillus aryabhattai* RS341, *Brevibacterium epidermidis* RS15 and *Micrococcus yunnanensis* RS222, showed reduction in ethylene production by 47-64% in salt-stressed (150 mM NaCl) red pepper seedlings compared to uninoculated controls.

2.4.4 Accumulation of protective compounds

Several studies correlated accumulation of nitrogen-containing compounds (NCC) with drought and salt tolerance in plants (Parida and Das, 2005). The most frequently accumulating NCC includes amino acids, amides, imino acids, proteins, quaternary ammonium compounds and polyamines.

Proline is often synthesized by plants in response to various abiotic, as well as biotic stresses, mediating osmotic adjustment, free radical scavenging and subcellular structure stabilization (Hare and Cress, 1997). Very high accumulation of cellular proline (up to 80% of the amino acids pool under stress and 5% under normal conditions) due to increased synthesis and decreased degradation under a variety of stress conditions such as salt and drought has been documented in many plant species (Szabados and Savoure, 2009). Several comprehensive studies using transgenic plants or mutants demonstrate that proline metabolism has a complex effect on development and stress responses. Proline has been proposed to act as a compatible osmolyte and to be a way to store carbon and nitrogen. Salinity and drought are known to induce oxidative stress. Studies have also indicated that proline may have an antioxidant activity acting as a ROS scavenger. Proline may also function as molecular chaperones that are able to stabilize the structures of proteins and enhance the activity of different enzymes and its accumulation play a role in maintenance of cytosolic pH and regulation of intracellular redox potential (Hare and Cress, 1997 and Verbruggen and Hermans, 2008).

Under abiotic stress conditions, increased proline biosynthesis was observed for various plant species inoculated with different PGPR (Jha *et al.*, 2010; Kohler *et al.*, 2009; Sandhya *et al.*, 2010 and Vardharajula *et al.*, 2011).

The synthesis of proline as well as other compatible solutes require an energy cost (41 moles of ATP) and occur at the expense of plant growth, but may allow the plant to survive and recover from the presence of high external salt concentration (Munns and Tester, 2008). Bianco and Defez (2009) found a significant correlation between reduced symptoms of senescence, such as chlorosis, necrosis and drying and 2-fold increased proline content in the shoot of *Mt*-RD64 as compared to *Mt*-1021 plants, after exposure to 150 mM NaCl.

Chen *et al.* (2007) correlated proline accumulation with drought and salt tolerance in plants. Introduction of *proBA* genes derived from *Bacillus subtilis* into *Arabidopsis thaliana* resulted in production of higher levels of free proline resulting in increased tolerance to osmotic stress in the transgenic plants. Increased production of proline along with decreased electrolyte leakage, maintenance of relative water content of leaves and selective uptake of K⁺ ions resulted in salt tolerance in *Zea mays* co-inoculated with *Rhizobium* and *Pseudomonas* (Bano and Fatima, 2009). Proline protects membranes and proteins against the adverse effects of high concentration of inorganic ions and temperature extremes. It also functions as a protein-compatible hydrotope and as a hydroxyl radical scavenger (Smirnoff and Cumbes, 1989). Accumulation of proline buffers cellular redox potential under environmental stresses (Wahid and Close, 2007). Proline synthesis has been shown to be increased in abiotically stressed plants in the presence of beneficial bacteria such as *Burkholderia* (Barka *et al.*, 2006), as well as *Arthrobacter* and *Bacillus*; however, in pepper, proline was also accumulated in the absence of abiotic stress in *Arthrobacter*- and *Bacillus* treated plants, suggesting that these bacteria could cause some biotic stress on the pepper (Sziderics *et al.*, 2007).

Trehalose metabolism in rhizobia is a key for signaling plant growth, yield and adaptation to abiotic stress and its manipulation had a major agronomical impact on leguminous plants (Suarez *et al.*, 2008). Figueiredo *et al.* (2008) reported increased plant growth, N content and nodulation of *Phaseolus vulgaris* L. under drought stress due to coinoculation of *Rhizobium tropici* and *Paenibacillus polymyxa*. Inoculated plants recorded higher trehalose-6-phosphate synthase activity. Microarray analysis of 7,200 expressed sequence tags from nodules of plants inoculated with strain overexpressing trehalose-6-phosphate synthase gene revealed upregulation of genes involved in stress tolerance, suggesting a signaling mechanism for trehalose.

The rhizobacterial-produced volatile organic compounds (VOCs) constitute an important mechanism for the elicitation of plant growth by rhizobacteria. Ryu *et al.* (2004) recorded some PGPR strains namely *Bacillus subtilis* GB03, *B. amyloliquefaciens* IN937a and *Enterobacter cloacae* JM22 that released a blend of volatile components, particularly, 2, 3-butanediol and acetoin, which promoted growth of *Arabidopsis thaliana*. It has now been established that the VOCs produced by the rhizobacterial strains can act as signaling molecules to mediate plant-microbe interactions as volatiles produced by PGPR colonizing roots are generated at sufficient concentrations to trigger the plant responses (Ryu *et al.*, 2004). Farmer (2001) identified low-molecular weight plant volatiles such as terpenes, jasmonates and green leaf components as potent signal molecules for living organisms in different trophic levels. However, to acquire a clear appreciation on the mechanisms of VOCs in signaling plants to register plant defence, more investigations on the volatile components in plant-rhizobacteria are necessary. Some of the VOCs emitted by *Bacillus* (Ryu, 2004) are bacterial determinants involved in induced systemic tolerance (IST). The volatiles emitted by PGPR down regulate hkt1 (high affinity K⁺ transporter 1) expression in roots but up-regulates it in shoots, orchestrating lower Na⁺ levels and recirculation of Na⁺ in the whole plant under stress conditions (Zhang *et al.*, 2008). Root colonization of *Arabidopsis thaliana* by *Pseudomonas chlororaphis* O6 induced tolerance in the plants against biotic and abiotic stresses due to the production of a volatile metabolite, 2R, 3R-butanediol. Studies with *Arabidopsis* mutant lines indicated that induced drought tolerance requires salicylic acid (SA), ethylene and jasmonic acid-signalling pathways (Cho *et al.*, 2008).

It has been reported that sodium uptake by plants is not altered upon inoculation with rhizobacteria. Yet, the suppression of photosynthesis observed in tomato plants exposed to salt stress was less severe in plants inoculated with *Achromobacter*.

While the exact mechanisms remain unclear, it has been suggested that apart from bacterial ACC deaminase activity, elevated phosphorous and potassium uptake might play a role in this process (Mayak *et al.*, 2004). Nadeem *et al.* (2007) found that inoculation of salt-stressed maize with ACC deaminase containing *Pseudomonas syringae*, *Enterobacter aerogenes* and *P. fluorescens* resulted in higher K^+/Na^+ ratios in combination with high relative water, chlorophyll and low proline contents. In this case, salt tolerance appears to be dependent on different mechanisms. High K^+/Na^+ ratios were also found in salt-stressed maize in which selectivity for Na^+ , K^+ and Ca^{2+} was altered upon inoculation with *Azospirillum* (Hamdia *et al.*, 2004). Furthermore, a decrease of Na^+ uptake was observed in wheat seedlings inoculated with strains of exo-polysaccharide producing bacteria (Ashraf *et al.*, 2004). The authors suggested that a higher proportion of the root zone of inoculated seedlings was covered in soil sheaths, which caused a reduced apoplastic flow of sodium ions into the stele. Similarly, inoculation of pepper with *Bacillus* sp. TW4 led to relief from osmotic stress, which is often manifested as salinity (and/or drought) stress. In these plants, genes linked with ethylene metabolism under abiotic stress such as *caACCO* (encoding ACC oxidase) and *caLTPI* (an abiotic stress-inducible gene encoding a lipid transfer protein) were down-regulated because *Bacillus* sp. TW4 showed ACC deaminase activity, the authors speculated that the enzyme may be involved in the lower expression of these genes (Sziderics *et al.*, 2007).

2.4.5 Improvement in growth and yield of different crops

Survival and productivity of crop plants exposed to environmental stresses are dependent on their ability to develop adaptive mechanisms to avoid or tolerate stress (Munns and Tester, 2008). Accumulating evidence suggests that mineral nutritional status of plants greatly affects their ability to adapt to adverse environmental conditions and in particular to abiotic stress factors. Impairment of the mineral nutrition status of plants exacerbates the adverse effects of abiotic stresses and the exogenous addition of high levels of macronutrients can alleviate the adverse effects of stress on plant growth (Baligar *et al.*, 2001; Endris and Mohammed, 2007 and Heidari and Jamshid, 2010).

Interestingly, the beneficial effects of inoculation with plant growth-promoting root zone bacteria have been reported to be most significant under unfavourable conditions such as salinity, flooding, drought, metal toxicity or nutrient deficiency. This is consistent with results from a computer simulation showing that the survival rate of introduced rhizobacteria in any given microbial community is an important factor determining the degree of plant stimulation. Because competition for limited resources is crucial and bacteria are also susceptible to environmental stressors, the most prominent beneficial effects of inoculation with a potential PGPR is to be expected in poor soils, when the development of the indigenous microbial community is inhibited (Egamberdiyeva, 2007).

The availability of nutrients for plants is regulated by the rhizospheric microbial activity. Thus any factor affecting this community and its functions influences the availability of nutrients and growth of the plants. One of the microbial function that plays a significant role in plant growth is the internal recycling of nitrogen (N) by way of immobilization and re-mineralization. In the majority of studies, the immobilization of NH_4^+-N is reported to be quicker than that of NO_3^--N , whilst the re-mineralization of the N immobilized in NH_4^+ is slower than that immobilized in NO_3^- (Herrmann *et al.*, 2005). However, little has been reported about immobilization/ re-mineralization in the two forms of N under conditions of salinity. Since nitrification is more or less inhibited in the presence of salts (Sethi *et al.*, 1993) resulting in an accumulation of NH_4^+-N , the cycling of the two forms of N will have a significant impact on the dynamics and availability of N for the plants. The presence of NaCl retards the N immobilization process. Both re-mineralization and nitrification were significantly retarded in the presence of NaCl, maximum inhibition occurring with 4000 mg NaCl kg^{-1} soil. The inhibitory effect of NaCl on N re-mineralization was relatively higher in soils treated with NH_4^+-N . The results of this study suggest greater sensitivity to NaCl by microorganisms that have assimilated NO_3^- . However, N re-mineralization in the population that had assimilated NO_3^- was less affected by salinity when compared to the population that had assimilated NH_4^+ (Azam and Ifzal, 2006).

After nitrogen, phosphorous is the second major nutrient for plant growth as it is an integral part of different biochemical constituents like nucleic acids, nucleotides, phospholipids and phosphoproteins. In most cases salinity decreased P accumulation in plant, resulting in development of P-deficiency symptoms (Parida and Das, 2005; Rogers *et al.*, 2003). The reduction in P availability in saline soils was suggested to be a result of ionic strength effects that reduce the activity of phosphate and the tight control of P concentrations by sorption processes and by low solubility of Ca-P minerals.

Phosphorus exists in two forms in soil, as organic and inorganic phosphate and like other nutrient elements such as potassium, iron, zinc and copper and it has limited mobility in the soil (Hayat *et al.*, 2010). The conversion of insoluble phosphate compounds (both organic and inorganic) in a form accessible to the plants is an important trait of PGPR strains. PGPR strains belonging to various genera have the ability to solubilize insoluble inorganic phosphate compounds such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite and rock phosphate (Richardson *et al.*, 2009 and Khan *et al.*, 2009).

It is generally accepted that the major mechanism of mineral phosphate solubilization is the action of organic acids synthesized by soil microorganisms (Rodriguez and Fraga, 1999). Production of organic acids results in acidification of the microbial cell and its surroundings. Consequently, P may be released from a mineral phosphate by proton substitution for Ca^{2+} . The production of organic acids has been well documented for different PGPR genera such as *Pseudomonas*, *Erwinia*, *Rhizobium* and *Bacillus* (Rodriguez and Fraga, 1999). Under P-limiting conditions, the IAA-overproducing RD64 strain showed high P-mobilizing activity that is connected to the synthesis of high levels of acid phosphatase enzymes and the secretion into the growth medium of malic, succinic and fumaric acids in large quantities. As compared to *Mt*-1021 inoculated plants, *Mt*-RD64 inoculated plants released large amount of another P-solubilizing organic acid, 2-hydroxyglutaric acid and showed significant increase in both shoot and root fresh weights, when grown under P-deficient conditions (Bianco and Defez, 2010).

Plant growth promoting bacteria have been found to improve growth of tomato, pepper, canola, bean and lettuce under saline conditions (Barassi *et al.*, 2006 and Glick *et al.*, 1997). Studies have shown that inoculation with endophytic bacteria can mitigate the effects of salt stress in different plant species. *Azospirillum*-inoculated seeds of lettuce (*Lactuca sativa* L., cv.Mantecosa), for instance, showed better germination rates and vegetative growth than non-inoculated control plants when exposed to NaCl (Barassi *et al.*, 2006).

Timmusk and Wagner (1999) were among the first to show that inoculation of *Paenibacillus polymyxa* confers drought tolerance in *Arabidopsis thaliana* through the induction of drought responsive gene, *erd15* (early response to dehydration 15). Inoculation of *Azospirillum brasilense* Sp245 in wheat (*Triticum aestivum*) under drought stress resulted in a better water status and an additional “elastic adjustment” resulting in better grain yield and mineral quality (Mg, K and Ca) at harvest.

Bacteria of the genus *Azospirillum* are free-living, surface colonizing and, sometimes, endophytic diazotroph and plant growth promoting rhizobacteria. *Azospirillum* strains had no preference for crop plants or weeds or for annual or perennial plants and can be successfully applied to plants that have no previous history of *Azospirillum* in their roots. Although reports about isolating *Azospirillum* from graminaceous plants are common, other reports showed that the bacterium is a natural inhabitant of many non-graminaceous plants. It appears that *Azospirillum* is a general root colonizer and is not a plant-specific bacterium (Bashan and Holguin, 1997). *Azospirillum* strains are capable of increasing yield of important crops growing in various soils and climatic regions. It has been reported that root elongation rate as well as uptake of mineral N, P and K and microelements are consequently improved after *Azospirillum* inoculation (Bashan *et al.*, 2004), even under stressful environmental conditions (Askary *et al.*, 2009).

Co-inoculation is based on mixed inoculants, combination of microorganisms that interact synergistically, or when microorganisms such as *Azospirillum* are functioning as “helper” bacteria to enhance the performance of other beneficial microorganisms.

In the rhizosphere the synergism between various bacterial genera such as *Bacillus*, *Pseudomonas* and *Rhizobium* has been demonstrated to promote plant growth and development. Compared to single inoculation, co-inoculation improved the absorption of nitrogen, phosphorus and mineral nutrients by plants (Figueiredo *et al.*, 2010 and Yadegari *et al.*, 2010).

A significant increase in root and shoot biomass was observed in chickpea plants when co-inoculated with *Mesorhizobium* and *Pseudomonas* (Sindhu *et al.*, 2002). Increased nodule weight, root and shoot biomass and total nitrogen of chickpea plants were also reported due to co-inoculation of *Rhizobium*, *Pseudomonas* and *Bacillus* (Parmar and Dadarwal, 1999). Co-inoculation with *Bradyrhizobium japonicum* and *Pseudomonas fluorescens* increased colonization by *B. japonicum* on soybean roots, nodule number and acetylene reduction assay (Tchebotar *et al.*, 1998). Combined inoculation of *Rhizobium* with *Pseudomonas striata* or *Bacillus megaterium* led to increased dry matter, grain yield and phosphorus uptake significantly over the uninoculated control in legumes (Elkoca *et al.*, 2008).

Verma *et al.* (2010) have studied the impact of application of *Rhizobium* spp. and plant growth promoting rhizobacteria on nodulation, plant biomass and yield of chickpea plants. In field studies, the grain and straw yields were significantly increased in co-inoculation of *Rhizobium* with *P. fluorescens* followed by *B. megaterium* and *Azotobacter chroococcum* over uninoculated control.

Co-inoculation of *Pseudomonas* spp. with *Rhizobium* is reported to improve growth and symbiotic performance of fodder galega (Egamberdieva *et al.*, 2010). The greenhouse experiment showed that co-inoculation of fodder galega with *Rhizobium galegae* and *P. trivialis* or with *R. galegae* and *P. extremorientalis* improved plant growth, nodulation and N content compared to plants inoculated with *R. galegae* alone in potting soil containing low levels of nitrogen. Co-inoculation of plants with *P. trivialis* and *R. galegae* showed the highest stimulatory effect.

The mechanisms behind these effects are only partially understood. One of the mechanisms used by these PGPR strains is the production of phytohormones such as auxins, gibberellins and cytokinins, which steadily contribute to the plant auxin “pool” in a way that the effect of PGPR inoculation can be mimicked by exogenous auxin application.

The endogenous IAA level in plant regulates growth of the shoots and roots and in the case of legumes, nodule formation (Teale *et al.*, 2006). It has been observed that low concentrations of exogenously given pure IAA stimulated shoot and root growth of wheat in non-saline and saline conditions and similar effects were induced by IAA-producing PGPR strains (Egamberdieva, 2009).

Dual inoculation of legumes with *Rhizobium* and *Azospirillum* significantly increases several plant-growth variables when compared with single inoculations (Hamaoui *et al.*, 2001 and Remans *et al.*, 2007). *Azospirillum* is considered as a *Rhizobium* helper by stimulating nodulation, nodule function and possibly plant metabolism (Molla *et al.*, 2001 and Verma *et al.*, 2010). Phytohormones produced by *Azospirillum* promote epidermal cell differentiation in root hairs that increased the number of potential sites for rhizobial infection leading to the formation of more nodules. Morphological and physiological changes in root system are also stimulated (Bashan and Levanony, 1990 and Volpin and Kapulnik, 1994). An increase in the number of lateral roots and root hairs cause addition of root surface available for nutrients and water uptake. Higher water and nutrient uptake by inoculated roots cause an improved water status of plant which in turn could be the main factor for enhanced plant growth (Dalla Santa *et al.*, 2004 and Mostajeran *et al.*, 2002).

Positive effects of co-inoculation were also observed on symbiotic performance of common bean, which is usually considered a poor nitrogen-fixing legume. Poor nodulation and variable response to inoculation is mainly attributed to intrinsic characteristics of the host plant, particularly the great sensitivity to nodulation-limiting factors, such as high rate of N fertilizer used in intensive agriculture, high temperature and soil dryness (Bais *et al.*, 2006 and Egamberdiyeva, 2007). Indeed, Yadegari and Rahmani (2010) showed that co-inoculation of three *Phaseolus vulgaris* cultivars with two *Rhizobium* strains, *Pseudomonas fluorescens* and *A. lipoferum* resulted in increased seed yield, number of pods per plant, weight of seeds, seeds protein yield and number of seeds per pod.

Inoculation of common bean or alfalfa (*Medicago sativa*) with *Azospirillum brasilense* in the absence of *Rhizobium* resulted in a more persistent exudation of flavonoids by legumes roots. *Azospirillum-Rhizobium* co-inoculation positively affected the expression of *nod* genes and production of nodulation factor patterns in *Rhizobium tropici* and *Rhizobium etli* in the presence or absence of NaCl at 50 mM. A significant increase of total and upper nodule numbers was observed at different concentrations of *Rhizobium* inoculum (Dardanelli *et al.*, 2008).

Some of the root-colonizing bacteria which have been shown to possess protection against different types of biotic stresses can also enhance a plant's tolerance to abiotic stresses. For example, *P. fluorescens* TDK1, *Pseudomonas putida* UW4, *Bacillus* sp. and *Arthrobacter* sp. have been shown to enhance resistance against various soil-borne pathogens and also mitigate salt, as well as drought stress in different plants (Mayak *et al.*, 2004 and Saravanakumar and Samiyappan 2007). *Bacillus subtilis* is widely known for its biocontrol properties and has recently been shown to enhance tolerance to iron toxicity (Asch and Padham 2005; Terre *et al.*, 2007). Similarly, siderophore production by beneficial bacteria, which protects plants against pathogenic bacteria through better competition for iron, has also been shown to protect plants from metal-induced oxidative stress (Dimkpa *et al.*, 2009). Ideally, however, future commercially available biocontrol agents should simultaneously provide cross-protection against various stress factors, making agricultural systems environmentally and economically more sustainable by reducing the need for pesticides, irrigation and other ecologically problematic and costly crop management strategies.

2.4.6 Enhancement of soil enzyme activity and microbial biomass

Soil enzymes are a group of enzymes that are continuously playing an important role in maintaining soil ecology, physical and chemical properties, fertility and soil health. The enzymatic activity in the soil is mainly of microbial origin, being derived from intracellular, cell-associated or free enzymes. A unique balance of chemical, physical and biological (including microbial especially enzyme activities) components of soil may contribute in maintaining the soil health. Soil enzymes are important in catalyzing several vital reactions necessary for the life processes of microorganisms in soils and the stabilization of soil structure, the decomposition of organic wastes, organic matter formation and nutrient cycling, hence playing an important role in agriculture (Dick, 1997).

Amongst the diverse soil enzymes, dehydrogenase, β -glucosidase, urease and the phosphatases are important in the transformation of different nutrients for plants. The activity of dehydrogenase reflects the total oxidative capacity of the microbial biomass (Nannipieri *et al.*, 1990) and is involved in the central aspect of metabolism. β -glucosidase is an important enzyme in the land carbon cycle, in the production of glucose, which constitutes an important energy source for the microbial mass (Tabatabai, 1994). Thus, the determination of β -glucosidase activity, amongst other hydrolytic enzyme activities, has been suggested as a good indicator of soil quality (Dick *et al.*, 1996).

The phosphatases play an important role in the transformation of organic phosphorus into inorganic forms more appropriate for plants. Phosphorus (P) is one of the essential nutrients for the plant growth and the greater part of soil phosphorus occurs in the organic form. Urease predominates amongst the enzymes involved in the N cycle of the soil (Tabatabai and Bremner, 1972 and Cookson, 1999). It catalyzes the hydrolysis of urea into ammonia or the ammonium ion, depending on the pH of the soil and carbon dioxide. Urease and catalase are the enzymes responsible for the decomposition of vegetable residues. The activity of these enzymes transforms the residue into humus, which is then completely decomposed into the free nutrients (Ahmad and Khan, 1988). On the other hand, amylase hydrolyzes the polysaccharides, converting them into simpler constituents. The activity of this enzyme is associated with high productivity of the crops (Ahmad and Khan, 1988).

Under laboratory conditions, salinity influences soil enzyme activity negatively, although the degree of inhibition varies according to the enzyme analyzed and the nature and amount of soil added (Frankenberger and Bingham, 1982). Dehydrogenase activity was severely inhibited whereas the hydrolases showed a milder degree of inhibition.

The reduction of enzyme activity in saline soils could be due to the osmotic dehydration of the microbial cells that liberate intracellular enzymes, which become vulnerable to the attack by soil proteases, with a consequent decrease in enzyme activity. The salting-out effect modifies the ionic conformation of the protein-enzyme active site and specific ionic toxicity causes a nutritional imbalance for microbial growth and subsequent enzyme synthesis (Frankenberger and Bingham, 1982).

According to Rietz and Haynes (2003) the increase in salinity due to an influx of salty water under controlled conditions, decreased the carbon content of the soil microbial biomass and enzymes. Other researchers, for example Omar *et al.* (1994) and Jialiang (2008) also indicated the effects of soil salinity on the carbon of microbial biomass and on enzyme activity. Increase in soil salinity inhibited the enzyme activities of benzoyl argininamide alkaline phosphatase and β -glucosidase and also microbial respiration (Garcia and Hernandez, 1996). Invertase and urease activities were also severely reduced by an increasing concentration of sodium chloride (NaCl) during incubation. In addition, the effect was inhibitory to nitrate reductase in the majority of the treatments (Omar *et al.*, 1994). On comparing the enzyme activities of saline soil with those of normal soil, Ahmed and Khan (1988) also observed a decline in amylase, catalase, phosphatase and urease activities with increasing salinity.

Controlled conditions (laboratory) do not usually reflect the natural situation prevailing in coastal region soils, where the salinity varies temporally. Tripathi *et al.* (2007) studied the influence of the salinity of arable soils in Indian coastal regions on the microbial biomass and the activities of dehydrogenase, β -glucosidase, urease and acid and alkaline phosphatases, in three different seasons of the year. The microbial and biochemical parameters were adversely affected by the salinity and the most extreme situation occurred in the summer. Of the enzymes studied, the activity of dehydrogenase was the most affected.

Another particular ecosystem is the mangrove swamps, areas restricted to zones between coastal seas and islands in tropical regions, associated with estuaries, bays and lagoons in places protected from the impact of waves, where the salinity is between 5 and 30‰, but can reach 90‰ (Una, 2011). This is a highly degraded natural environment for a variety of reasons, amongst which the discharge of domestic and industrial effluents. Variations in the salinity of environment can affect the retention of the pollutants and the microbiological responses as a function of the discharge of effluent. On investigating such areas, Tam (1998) observed that the addition of effluent to mangrove swamps, independent of their salinity, stimulated microbial growth and increased the activities of dehydrogenase and alkaline phosphatase. According to the author these effects were due to supplementation with additional carbon sources and other nutrients, provided by the effluent.

Considering the forecast for an increase in saline and sodic areas, an understanding of the effects of salinity and sodicity on the soil carbon (C) stock and flow is fundamental for environmental management. Wong *et al.* (2008) evaluated the effects of salinity and sodicity on the microbial biomass and on soil respiration, under controlled conditions, submitting perturbed soil samples to leaching after receiving different salts concentrations. The highest soil respiration rates were observed in soils with low salinity and the lowest in soils with medium salinity, whilst the microbial biomass was greater in the treatments with high salinity and lower in those with low salinity. The results were attributed to a greater availability of substrate in high salt concentrations, or by an increase in the dispersion of the aggregates of soil or from the dissolution or hydrolysis of the organic material in the soil, which can compensate, at least in part, the stress to which the microbial population is submitted in high salt concentrations. The apparent disparity between the evolution of respiration and that of the biomass could be due to a change induced in the microbial population from one dominated by more active microorganisms to one dominated by less active microorganisms.

The microbial biomass is an important labile fraction of the soil organic matter, functioning both as an agent of transformation and recycling of the organic matter and soil nutrients, as also of a source of nutrients for the plants. Microbial biomass decreased with increasing level of salinity, underlying the detrimental effect of salinity on soil microorganisms (Shah and Shah, 2011). High salinity also affects amino acid capture and protein synthesis, respiration (Gennari *et al.*, 2007) and causes increases and decreases in C and N mineralization (Wichern *et al.*, 2006). Since the soil organic matter and consequently.

The biomass and microbial activity, are generally more relevant in the first few centimeters at the surface of the soil, salinization close to the surface can significantly affect a series of microbiologically mediated processes. This is a considerable problem, since the microbial processes of the soil control its ecological functions and fertility.

2.5 Characterization of genes encoding for salt tolerance

Accumulation of osmolyte compounds has long been proposed as an adaptative mechanism for salt and drought tolerance. It has received increasing interest during the last 20 years. In response to drought and/ or high salinity stresses, which disturb the intracellular water balance, many plants and bacteria synthesize and accumulate several kinds of compatible osmolytes, such as proline (Chen *et al.*, 2007), glycinebetaine (Gu *et al.*, 2008), ectoine (Reshetnikov *et al.*, 2011) etc that function as osmotica.

Among the compatible solutes, proline appears to be the most widely distributed osmolyte accumulated under stress conditions not only in plants but also in eubacteria. Genes encoding proline biosynthesis and involved in salt tolerance have been isolated and characterized in several halophilic and halotolerant bacteria. The pathway of proline biosynthesis comprises three enzymatic steps. The corresponding genes of *proB*, *proA* and *proC*, encode γ -glutamyl kinase (γ -GK), γ -glutamyl-phosphate reductase (γ -GPR) and pyrroline- 5-carboxylate (P5C) reductase respectively (Chen *et al.*, 2007).

Glycine betaine is known to function as a preferential protective solute which can be accumulated either through biosynthesis or by being taken up directly from surroundings. A common biosynthetic pathway for glycine betaine is from precursor choline, utilizing a two-step oxidation process with betaine aldehyde as intermediate. The genetic and biochemical mechanisms of this pathway have been elucidated for nonhalophilic and some halotolerant microorganisms such as Gram-negative *Escherichia coli* (Lamark *et al.*, 1991) and *Sinorhizobium meliloti* (Osteras *et al.*, 1998) and Gram-positive *Arthrobacter pascens* (Rozwadowski *et al.*, 1991), *Bacillus subtilis* (Boch *et al.*, 1996) and *Staphylococcus xylosus* (Rosenstein *et al.*, 1999).

The molecular details of the choline to glycine betaine synthesis pathway encoded by the *betTIBA* gene cluster have been fully characterized in *Escherichia coli* (Lamark *et al.*, 1991). The genes encoding the glycine-betaine biosynthesis were also analyzed in other bacteria and shared many characteristics with their homologs found in *E. coli*. Import of the precursor choline is mediated by the high-affinity BetT transporter. The *betA*- and *betB*-encoded proteins are responsible for the enzymatic conversion of choline and glycine betaine aldehyde to glycine betaine. BetI is a choline-sensing repressor (Lamark *et al.*, 1991) that can bind, *in vitro*, the operator sequence (Rokenes *et al.*, 1996). The *betT* and *betIBA* genes are divergently oriented and the overlapping *betT* and *betI* promoters are negatively regulated by BetI.

Trehalose, a non-reducing disaccharide is another major compatible solute, which maintains fluidity of membranes and protects the biological structure of organisms under stress. Four pathways for trehalose biosynthesis in bacteria have been described to date (Avonce *et al.*, 2006; Elbein *et al.*, 2003 and Schiraldi *et al.*, 2002). The common OtsAB pathway involves the condensation of glucose-6-phosphate with UDP-glucose by trehalose-6-phosphate synthase (OtsA) to form trehalose-6-phosphate, with subsequent dephosphorylation by trehalose-6-phosphate phosphatase (OtsB), yielding free trehalose. The TreYZ pathway involves the conversion of maltodextrins, such as glycogen, into trehalose. The terminal α -1, 4-glycosylic bond at the reducing end of the glucan polymer is converted into α -1, 1-glycosidic bond via transglycosylation by maltooligosyltrehalose synthase (TreY). Free trehalose is subsequently released from the end of the polymer via hydrolysis by maltooligosyltrehalose trehalohydrolase (TreZ). The TreS pathway involves a reversible transglycosylation reaction in which trehalose synthase (TreS) converts the α -1, 4 bond of maltose to the α -1,1 bond of trehalose. The recently discovered fourth pathway involves trehalose glycosyltransferring synthase (TreT), which catalyzes the reversible formation of trehalose from ADP-glucose and glucose. TreT has been characterized in hyperthermophilic bacteria (Qu *et al.*, 2004 and Ryu *et al.*, 2005).

The genes coding for ectoine biosynthesis have been characterized in several bacterial species and are organized in the gene cluster *ectABC*. The gene cluster *ectABC* coding for diaminobutyric acid (DABA) acetyltransferase (EctA), DABA aminotransferase (EctB) and ectoine synthase (EctC) was isolated and characterized by several researchers (Canovas *et al.*, 1998; Ono *et al.*, 1999 Kuhlman and Bremer, 2002) and reported to have role in salt tolerance of bacteria.

3. MATERIAL AND METHODS

The present investigation on functional analysis and molecular characterization of salt-tolerant microorganisms of agricultural importance was carried out at the Microbial Genetics Laboratory, Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad. The objective of the present study was to characterize and identify the salt-tolerant agriculturally important groups of microorganisms (nitrogen fixers, phosphate solubilizers and fluorescent pseudomonads) isolated from the stress ecosystems of Karnataka state and analyze their functional properties under salt stress, biochemical basis of their and genes encoding tolerance/ resistance. Attempts were also made to assess the ability of selected efficient strains to enhance growth, nutrient uptake and yield of wheat under saline conditions. The materials used and the methods followed in the present investigations are outlined below.

3.1 Characterization of the salt-tolerant isolates

3.1.1 Salt-tolerant isolates

One hundred ninety five salt-tolerant bacteria (36 associative nitrogen fixers, 43 free-living nitrogen fixers, 55 phosphate solubilizers and 61 fluorescent pseudomonads) and three salt-tolerant phosphate solubilizing fungi isolated from different soil and root samples collected from saline, arid and waterlogged areas of Karnataka and maintained in the culture bank of the Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad, were used in the present study (Table 2).

3.1.2 Morphological characterization

All the isolates were examined for the colony morphology, pigmentation, cell shape, gram reaction and ability to form spores, as per the standard procedures given by Anon (1957) and Barthalomew and Mittewar (1950).

3.1.3 Biochemical characterization

The isolates were subjected to biochemical characterization by employing the standard procedures given by Cappuccino and Sherman (1992). Associative N₂ fixers were subjected for biochemical tests like oxidase, urease and cellulose hydrolysis whereas free-living N₂ fixers were examined for oxidase, catalase, acid and indole production. Similarly, the P-solubilizing bacterial isolates (PSB) were subjected to oxidase, catalase, urease, indole production, acid and gas production, hydrogen sulfide production, Methyl Red (MR), Voges-Proskauer (VP), gelatin liquefaction, citrate utilization, denitrification and hydrolysis of lipid, starch and casein. Fluorescent pseudomonads (FP) isolates were examined for oxidase, denitrification, gelatin liquefaction and hydrolysis of starch and agrinine.

3.1.4 Physiological characterization

3.1.4.1 Effect of temperature

For ascertaining the ability of the fluorescent pseudomonads (FP) isolates to grow at 4° C and 41° C temperature and free-living N₂ fixers at 60° C, 10 µl of 24 h old cultures of the test organisms were spotted on duplicate set of TSA plates and each set was incubated for 48 h at 4° C and 41° C and 60° C temperature respectively. The observations on growth were recorded at the end of incubation period.

3.1.4.2 Utilization of different carbon sources

Minimal agar medium (M9) (Appendix I) was amended with different carbon (C) sources viz., glucose, sucrose, meso-inositol, trehalose, rhamnose, mannitol, geraniol, L-valine, β-alanine, xylose, ribose, lactose and maltose @ 10 g/l and autoclaved. Sterilized petri plates were poured with 15-20 ml medium with different carbon sources and allowed to solidify. After solidification, 10 µl of 24 h old cultures of the associative N₂ fixers were spotted on plates with glucose, sucrose and meso-inositol as C source while free-living N₂ fixers on plates with rhamnose, mannitol and meso-inositol as C sources. Similarly, the FP isolates were spotted on plates with glucose, sucrose, meso-inositol, trehalose, geraniol, L-valine, β-alanine, xylose, ribose, rhamnose, lactose, mannitol and maltose as C source.

Table 2. Details of samples collected from stress-ecosystems of Karnataka for isolation of AIMS

Sl. No.	Isolate code	Location	Type of sample	Soil pH	Soil EC (dS/m)	Plant species associated
1.	ASANFII S22E	Gokak, Belagavi (Dist.)	Root	7.6	15.1	Grass
2.	ASANFII S26E	Gokak, Belagavi (Dist.)	Root	8.2	0.43	Wheat
3.	ASANFIV S40E	Hooli, Belagavi (Dist.)	Root	7.6	0.24	Wheat
4.	ASANFIV S61E	Chinchli, Belagavi (Dist.)	Root	8.3	0.64	Weed
5.	ASANFIV S63E	Biradi, Bagalkot (Dist.)	Root	8.2	4.52	Sweet potato
6.	ASANFIV S78E	Rabkavi, Bagalkot (Dist.)	Root	8.7	13.50	<i>Cynodon dactylon</i>
7.	ASANFIV S83E	Batakurki, Bagalkot (Dist.)	Root	8.0	7.83	Sugarcane
8.	ASANFIV Dr8E	Mullur ghat, Belagavi (Dist.)	Root	7.3	0.22	<i>Commelina bengalensis</i>
9.	ASANFVDr18bE	Veniveerapur, Bellary (Dist.)	Root	8.8	0.21	Linseed
10.	ASANFV WL12E	Ashapur, Bellary (Dist.)	Root	8.7	0.76	Paddy
11.	ASANFIX S121E	Atharga, Bijapur (Dist.)	Root	8.9	0.29	Lucerne
12.	ASAZOIX S125E	Hirebevir, Bijapur	Root	8.2	1.23	<i>Cyperus rotundus</i>
13.	ASANFIX S132E	Chikpadasalagi, Bagalkot	Root	7.9	0.48	<i>Prosopis julifera</i>
14.	ASANFX S135E	Chikahalalli, Bijapur	Root	8.1	3.86	Sorghum
15.	ASANFX S137E	Mulibagilu, Kolar	Root	7.6	20.80	Ragi
16.	ASANFX S138E	Hoskote, Bangalore	Root	8.1	0.19	Grass
17.	ASANFX S141E	Ukkada, Mandya	Root	7.9	0.39	Grass
18.	ASANFX S144E	Mandya	Root	7.9	0.19	Aap
19.	ASANFX S145E	Sosale, Chamarajnagar	Root	7.5	0.91	Sugarcane
20.	ASANFX S148E	Yadamole, Chamarajnagar	Root	8.1	3.07	Sugarcane
21.	ASANFX S149E	Chikkandawadi, Mysore	Root	8.0	4.00	Paddy
22.	ASANFX S150E	Chikkandawadi, Mysore	Root	8.2	20.4	Grass
23.	ASANFX S154E	Chitradurga	Root	7.3	6.43	<i>Cassia tora</i>
24.	ASANFX Dr29aE	Gamanahalli, Chitradurga	Root	8.8	0.84	Ragi
25.	ASANFX Dr31E	Bellur cross, Raichur	Root	8.1	0.13	<i>Lantana camara</i>
26.	ASANFX WL13E	Pyadee, Mandya	Root	8.1	0.39	<i>Cyperus rotundus</i>
27.	ASANFX WL15E	Doddamadenahalli, Mandya	Root	7.9	4.83	Aap
28.	ASANFXI S158E	Korahalli, Gulbarga	Root	7.7	23.16	Lettuce
29.	ASANFXI S160E	Inchageri, Gulbarga	Root	8.1	20.10	<i>Commelina bengalensis</i>
30.	ASANFXI S167E	Hotapate, Gulbarga	Root	8.2	0.48	Cotton
31.	ASANFXI S170E	Diggi, Gulbarga	Root	9.2	12.86	Weed
32.	ASANFXI S173E	Gogi, Gulbarga	Root	8.0	0.56	Paddy
33.	ASANFXI S182E	Hebbal, Gulbarga	Root	7.1	1.89	Grass
34.	ASANFXI S187E	ARS, Malanur, Gulbarga	Root	8.9	0.47	Parthenium
35.	ASANFXI WL17E	Korahalli, Gulbarga	Root	8.8	0.90	Lettuce
36.	ASANFXI WL19E	Afzalpur, Gulbarga	Root	7.9	10.86	<i>Cyperus rotundus</i>
37.	ASFNFI S17(2)S	Menadal, Koppal	Soil	8.5	0.42	Weed
38.	ASFNFI S19(2)S	Menadal, Koppal	Soil	8.5	0.31	<i>Aloe vera</i>
39.	ASFNFI Dr4(1)	Kustagi, Koppal	Soil	8.7	0.32	<i>Agave</i> sp.
40.	ASFNFI Dr2(5)	Vajrabhandi cross, Koppal	Soil	9.4	9.98	<i>Cassia</i> sp.
41.	ASFNFI Dr3(3)	Kustagi, Koppal	Soil	8.8	0.39	Anesondi
42.	ASFNFII S22S	Gokak, Belagavi	Soil	7.6	15.10	Grass
43.	ASFNFII S22R	Gokak, Belagavi	Root	7.6	15.10	Grass
44.	ASFNFIII S32S	Talawai, Dharwad	Soil	9.1	0.55	Chickpea
45.	ASFNFIII S31R	Talawai, Dharwad	Root	9.3	0.47	Weed
46.	ASFNFIII S33E	Talawai, Dharwad	Root	8.7	0.43	Sugarcane
47.	ASFNFIV S36(1R)	Hirekumbi, Belagavi	Root	7.9	0.91	Parthenium
48.	ASFNFIV S37R	Hirekumbi, Belagavi	Root	7.9	0.91	Wheat
49.	ASFNFIV S40(1)R	Hooli, Belagavi	Root	7.6	0.24	Wheat
50.	ASFNFIV S40(2)R	Hooli, Belagavi	Root	7.6	0.24	Wheat

Contd...

(Table 2 contd...)

Sl. No.	Isolate code	Location	Type of sample	Soil pH	Soil EC (dS/m)	Plant species associated
51.	ASFNFIV S43(2)R	Haralakatti, Belagavi	Root	8.8	54.4	Weed
52.	ASFNFIV S44R	Haralakatti, Belagavi	Root	8.8	54.4	Weed
53.	ASFNFIV S45(1)R	Kagadal, Belagavi	Root	7.9	11.07	Sunflower
54.	ASFNFIV S57(2)R	Hidkal, Belagavi	Root	6.7	8.2	Coconut
55.	ASFNFIV S60(1)S	Biradi, Belagavi	Soil	7.2	6.61	Negli mullu
56.	ASFNFIV S61(1)S	Chinchuli, Belagavi	Soil	8.3	0.64	Weed
57.	ASFNFIV S62E	Biradi, Belagavi	Root	8.3	8.84	Sugarcane
58.	ASFNFIV S63(1)R	Biradi, Belagavi	Root	8.2	4.52	Sweet potato
59.	ASFNFIV S66R	Siraguppa, Bellary	Root	7.7	4.23	Sorghum
60.	ASFNFIV S75(1)R	Yabaratti, Bellary	Root	7.7	2.06	Sugarcane
61.	ASFNFIV S75(2)R	Yabaratti, Bellary	Root	7.7	2.06	Sugarcane
62.	ASFNFIV S75E	Yabaratti, Bellary	Root	7.7	2.06	<i>Cynodon dactylon</i>
63.	ASFNFIV S80(1)R	Yadalli, Bellary	Root	7.5	0.43	<i>Cynodon dactylon</i>
64.	ASFNFIV Dr8R	Mullur ghat, Belagavi	Root	7.3	0.22	<i>Commelina bengalensis</i>
65.	ASFNFIV WL3S	Bommanal, Belagavi	Soil	8.2	0.40	Aap
66.	ASFNFIV WL4(1)R	Ugar, Belagavi	Root	4.9	47.31	Aap
67.	ASFNFIV WL5(1)R	Ugar, Belagavi	Root	6.8	2.46	Aap
68.	ASFNFIV WL5(2)R	Ugar, Belagavi	Root	6.8	2.46	Aap
69.	ASFNFIV WL6R	Daroor, Bellary	Root	7.1	7.29	Wheat
70.	ASFNFIV WL7(2)R	Hanagadi, Bellary	Root	7.6	12.96	Aap
71.	ASFNFV S85R	Gajjarhal, Raichur	Root	7.7	0.33	Paddy
72.	ASFNFIX S127R	Hireibevanur, Bijapur	Root	9.1	0.47	<i>Achyranthus aspera</i>
73.	ASFNFIX Dr31R	Belur cross, Raichur	Root	8.1	0.13	<i>Lantana cameron</i>
74.	ASFNFIX S161E	Seerutagi, Gulbarga	Root	8.9	13.41	Grass
75.	ASFNFIX S174E	Gogi, Gulbarga	Root	8.1	0.69	Parthenium
76.	ASFNFIX WL16E	Shivanagi, Gulbarga	Root	7.7	13.86	Maize
77.	ASFNFIX S178E	Naganur, Koppal	Root	8.7	3.42	Weed
78.	ASFNFIX S190S	Mattur, Koppal	Soil	8.6	0.21	<i>Cassia</i> sp.
79.	ASFNFIX S190E	Mattur, Koppal	Root	8.6	0.21	<i>Cassia</i> sp.
80.	ASPSBIX S122R	Atharga to Thamba road, Bijapur	Root	9.0	0.49	Weed
81.	ASPSBIX S122(1)R	Atharga to Thamba road, Bijapur	Root	9.0	0.49	Weed
82.	ASPSBIX S124(1)R	Thamba to Indi road, Bijapur	Root	8.8	4.95	Weed
83.	ASPSBIX S124(2)S	Thamba to Indi road, Bijapur	Soil	8.8	4.95	Weed
84.	ASPSBIX S124E	Thamba to Indi road, Bijapur	Root	8.8	4.95	Weed
85.	ASPSBIX S125(6)S	Indi to Heribevanur road, Bijapur	Soil	8.2	1.23	<i>Commelina bengalensis</i>
86.	ASPSBIX S125R	Indi to Heribevanur road, Bijapur	Root	8.2	1.23	<i>Commelina bengalensis</i>
87.	ASPSBIX S125E	Indi to Heribevanur road, Bijapur	Root	8.2	1.23	<i>Commelina bengalensis</i>
88.	ASPSBIX S129R	Kelagi, Bijapur	Root	9.2	0.89	Karchikai
89.	ASPSBIX S129(1)R	Kelagi, Bijapur	Root	9.2	0.89	Karchikai
90.	ASPSBIX S132(2)S	Chikkapadasalagi, Bagalkot	Soil	7.9	0.48	<i>Prosopis julifera</i>
91.	ASPSBX S135S	Chikkahalalli, Bijapur	Soil	8.1	3.86	Sorghum
92.	ASPSBX S135R	Chikkahalalli, Bijapur	Root	8.1	3.86	Sorghum
93.	ASPSBX S137(1)S	Mulabagilu, Koppal	Soil	7.6	20.80	Ragi
94.	ASPSBX S138(1)S	Hoskote, Koppal	Soil	8.1	0.19	Grass
95.	ASPSBX S138(1)S	Hoskote, Koppal	Soil	8.1	0.19	Grass
96.	ASPSBX S138R	Hoskote, Koppal	Root	8.1	0.19	Grass
97.	ASPSBX S139(1)S	Narsipur, Mandya	Soil	7.7	0.29	<i>Achyranthus aspera</i>
98.	ASPSBX S139R	Narsipur, Mandya	Root	7.7	0.29	<i>Achyranthus aspera</i>
99.	ASPSBX S140S	Kambodi, Mandya	Soil	8.4	0.43	<i>Cynodon dactylon</i>
100.	ASPSBX S141(1)S	Ukkada, Mandya	Soil	7.9	0.39	Grass

Contd...

(Table 2 contd...)

Sl. No.	Isolate code	Location	Type of sample	Soil pH	Soil EC (dS/m)	Plant species associated
101.	ASPSBX S141R	Ukkada, Mandya	Root	7.9	0.39	Grass
102.	ASPSBX S141E	Ukkada, Mandya	Root	7.9	0.39	Grass
103.	ASPSBX S142(2)S	Mudavadi, Mandya	Soil	9.1	0.27	Wheat
104.	ASPSBX S144(1)S	Mandya	Soil	7.9	0.19	Aap
105.	ASPSBX S145(1)S	Sosale, Chamarajnagar	Soil	7.5	0.91	Sugarcane
106.	ASPSBX S147S	Mugur, Chamarajnagar	Soil	8.9	0.40	Paddy
107.	ASPSBX S150S	Chikkandawadi, Mysore	Soil	8.0	3.98	Paddy
108.	ASPSBX S150R	Chikkandawadi, Mysore	Root	8.0	3.98	Paddy
109.	ASPSBX Dr27S	Totagere, Raichur	Soil	8.5	0.29	<i>Lantana cameron</i>
110.	ASPSBX Dr27(1)S	Totagere, Raichur	Soil	8.5	0.29	<i>Lantana cameron</i>
111.	ASPSBX Dr29aS	Gamanahalli, Raichur	Soil	8.8	0.84	Wild horsegram
112.	ASPSBX Dr29aR	Gamanahalli, Raichur	Root	8.8	0.84	Wild horsegram
113.	ASPSBX Dr31(1)S	Belur cross, Raichur	Soil	8.1	0.13	<i>Lantana cameron</i>
114.	ASPSBX Dr32S	Jayachamrajapura, Raichur	Soil	8.8	0.76	Jatropha
115.	ASPSBX WL15S	Doddamadenahalli, Mandya	Soil	7.9	4.83	Aap
116.	ASPSBX WL15(3)S	Doddamadenahalli, Mandya	Soil	7.9	4.83	Aap
117.	ASPSBXI S156R	Sindagi, Bijapur	Root	9.1	0.39	Parthenium
118.	ASPSBXI S158S	Korahalli, Bijapur	Soil	7.7	23.16	Lettuce
119.	ASPSBXI S160S	Inchageri, Gulbarga	Soil	8.1	20.10	<i>Commelina bengalensis</i>
120.	ASPSBXI S160(1)E	Inchageri, Gulbarga	Root	8.1	20.10	<i>Commelina bengalensis</i>
121.	ASPSBXI S160(2)E	Inchageri, Gulbarga	Root	8.1	20.10	<i>Commelina bengalensis</i>
122.	ASPSBXI S169(1)R	Diggi, Gulbarga	Root	8.8	2.18	Weed
123.	ASPSBXI S172(2)S	Daranahalli, Gulbarga	Soil	8.0	0.32	Cotton
124.	ASPSBXI S175E	Gogi, Gulbarga	Root	9.5	11.83	<i>Cassia crutica</i>
125.	ASPSBXI S176S	Danadamallapur, Gulbarga	Soil	8.0	0.99	<i>Cyperus rotundus</i>
126.	ASPSBXI S177E	Aevur tanda, Koppal	Root	7.7	0.44	Cowpea
127.	ASPSBXI S181R	Mudnoor cross, Gulbarga	Root	7.9	0.65	<i>Prosopis julifera</i>
128.	ASPSBXI S187(2)S	ARS, Malnur, Gulbarga	Soil	8.9	0.47	Parthenium
129.	ASPSBXI WL18E	Korahalli, Gulbarga	Root	8.2	14.30	Lettuce
130.	ASPSBXI WL19E	Afzalpur, Gulbarga	Root	7.9	10.86	<i>Cyperus rotundus</i>
131.	ASPSBXI WL20S	Gogi, Gulbarga	Soil	7.1	5.30	Aap
132.	ASPSBXII S189E	Rajankoolur, Gulbarga	Root	7.9	0.14	Paddy
133.	ASPSBXII S190S	Mattur to Mudgal, Koppal	Soil	8.6	0.21	<i>Cassia</i> sp.
134.	ASPSBXII Dr33S	Rajankoolur, Gulbarga	Soil	7.9	0.24	Jatropha
135.	ASFPI S3(1)S	Jawalgera, Koppal	Soil	8.4	2.30	<i>Cynodon dactylon</i>
136.	ASFPI S3(2)S	Jawalgera, Koppal	Soil	8.4	2.30	<i>Cynodon dactylon</i>
137.	ASFPI S4(1)S	Jawalgera, Koppal	Soil	8.4	2.30	<i>Cynodon dactylon</i>
138.	ASFPI S6(1)S	Herur, Koppal	Soil	8.4	1.22	Paddy
139.	ASFPI S9(1)S	ARS, Gangavati, Koppal	Soil	8.2	3.50	Paddy
140.	ASFPI S14(3)S	ARS, Gangavati, Koppal	Soil	8.5	1.41	Mesta
141.	ASFPI S15(1)S	ARS, Gangavati, Koppal	Soil	8.2	4.14	Sunhemp
142.	ASFPI Dr6(8)S	Kustagi, Koppal	Soil	7.2	0.29	<i>Carissa caranda</i>
143.	ASFPIV S35R	Hirekumbi, Belagavi	Root	7.6	0.43	Safflower
144.	ASFPV S85R	Gajarhal, Raichur	Root	7.7	0.33	Paddy
145.	ASFPV S94(2)E	Gabbur, Raichur	Root	7.9	0.72	Sunflower
146.	ASFPV S97R	Kollur, Raichur	Root	8.6	0.21	Sunflower
147.	ASFPV S100E	Nandihal, Bellary	Root	8.2	1.58	Cotton
148.	ASFPV S112(a)R	Rampur, Bellary	Root	8.4	5.01	Sunflower
149.	ASFPV S158(2)R	Korahalli, Bijapur	Root	7.7	23.16	Lettuce
150.	ASFPV Dr10R	Tuntapur, Bellary	Root	8.3	0.12	Toddy palm

Contd...

(Table 2 contd...)

Sl. No.	Isolate code	Location	Type of sample	Soil pH	Soil EC (dS/m)	Plant species associated
151.	ASFPV Dr10E	Tuntapur, Bellary	Root	8.3	0.12	Toddy palm
152.	ASFPV Dr16E	Amarapura, Bellary	Root	8.2	0.57	Weed
153.	ASFPX S121(3)S	Atharga to Thamba road, Bijapur	Soil	8.9	0.29	Lucerne
154.	ASFPX S121R	Atharga to Thamba road, Bijapur	Root	8.9	0.29	Lucerne
155.	ASFPX S131S	Chikkalki Cross, Bijapur	Soil	8.3	0.49	Lucerne
156.	ASFPX S134(3)S	Basavanagar, Kolar	Soil	8.4	1.28	<i>Cynodon dactylon</i>
157.	ASFPX S149S	Chikkandawadi, Mysore	Soil	8.0	3.98	Paddy
158.	ASFPX S155S	Kumatagi, Chitradurga	Soil	8.5	2.86	Grass
159.	ASFPX S156R	Sindagi, Bijapur	Root	9.1	0.39	Parthenium
160.	ASFPX S156E	Sindagi, Bijapur	Root	9.1	0.39	Parthenium
161.	ASFPX S157(1)S	Rampur, Bellary	Soil	9.0	11.98	Sugarcane
162.	ASFPX S157(2)R	Rampur, Bellary	Root	9.0	11.98	Sugarcane
163.	ASFPX S158(1)R	Korahalli, Gulbarga	Root	7.7	23.16	Lettuce
164.	ASFPX S161(1)R	Seerutagi, Gulbarga	Root	9.0	13.43	Grass
165.	ASFPX S161(2)R	Seerutagi, Gulbarga	Root	9.0	13.43	Grass
166.	ASFPX S163S	Kodammanahalli, Raichur	Soil	7.1	0.49	Cotton
167.	ASFPX S164S	Hattigudur, Raichur	Soil	7.6	0.87	Redgram
168.	ASFPX S164(b)E	Hattigudur, Raichur	Root	7.6	0.87	Redgram
169.	ASFPX S167(2)S	Hotapate, Raichur	Soil	8.2	0.48	Cotton
170.	ASFPX S168R	Hutkal Tanda, Raichur	Root	8.4	1.43	Paddy
171.	ASFPX S169(2)S	Diggi, Gulbarga	Soil	8.8	2.18	Weed
172.	ASFPX S170R	Diggi, Gulbarga	Root	9.2	12.86	Weed
173.	ASFPX S172R	Daranahalli, Gulbarga	Root	8.0	0.32	Cotton
174.	ASFPX S173S	Gogi, Gulbarga	Soil	8.0	0.57	Paddy
175.	ASFPX S174S	Gogi, Gulbarga	Soil	8.1	0.69	Parthenium
176.	ASFPX S174(1)R	Gogi, Gulbarga	Root	8.1	0.69	Parthenium
177.	ASFPX S174(2)R	Gogi, Gulbarga	Root	8.1	0.69	Parthenium
178.	ASFPX S176S	Danadamallapur, Gulbarga	Soil	8.0	0.99	<i>Cyperus rotundus</i>
179.	ASFPX S178(1)S	Naganur, Koppal	Soil	8.7	3.42	Weed
180.	ASFPX S178(2)S	Naganur, Koppal	Soil	8.7	3.42	Weed
181.	ASFPX S178R	Naganur, Koppal	Root	8.7	3.42	Weed
182.	ASFPX S178E	Naganur, Koppal	Root	8.7	3.42	Weed
183.	ASFPX S180S	Kembhavi, Gulbarga	Soil	8.8	0.49	Weed
184.	ASFPX S180E	Kembhavi, Gulbarga	Root	8.8	0.49	Weed
185.	ASFPX S181R	Mudnoor cross, Gulbarga	Root	7.9	0.65	<i>Prosopis julifera</i>
186.	ASFPX S183R	Kachaknur, Gulbarga	Root	8.5	1.03	Redgram
187.	ASFPX S183E	Kachaknur, Gulbarga	Root	8.5	1.03	Redgram
188.	ASFPX S184R	Kachaknur, Gulbarga	Root	8.0	0.90	Sorghum
189.	ASFPX S186S	Kuppi cross, Gulbarga	Soil	8.6	2.13	Weed
190.	ASFPX S187S	Malnur ARS, Gulbarga	Soil	8.9	0.47	Parthenium
191.	ASFPX WL16R	Shivanagi, Gulbarga	Root	7.7	13.86	Maize
192.	ASFPX WL16(1)R	Shivanagi, Gulbarga	Root	7.7	13.86	Maize
193.	ASFPX WL17R	Korahalli, Gulbarga	Root	8.8	0.90	Lettuce
194.	ASFPXII S190R	Mattur to Mudgal, Koppal	Root	8.6	0.21	<i>Cassia</i> sp.
195.	ASFPXII Dr33R	Rajankoolur, Gulbarga	Root	7.9	0.24	Jatropha

The plates were incubated for 48 h at $28 \pm 2^\circ \text{C}$ and the ability of the isolates to grow on different carbon sources was noted.

3.1.4.3 Effect of hydrogen ion concentration (pH)

The above test was performed for characterization of ANF isolates only. For studying the effect of pH, the Okon's malate agar medium (Appendix I) was prepared and the pH was adjusted to 6.0 by addition of 0.1N HCl before adding agar-agar and autoclaving. Sterilized petri plates were poured with 15-20 ml medium and allowed to solidify. Then the plates were spotted with 10 μl of overnight grown cultures of the test organisms and incubated for 48 h at $28 \pm 2^\circ \text{C}$. The observations on the ability of the ANF isolates to grow at pH 6.0 were recorded.

3.1.5 Molecular characterization

3.1.5.1 Isolation of genomic DNA

The genomic DNA was isolated from the 91 salt-tolerant bacteria by following the protocol (Ausubel *et al.*, 1987) as described below.

Twenty five ml of overnight culture grown in Luria Bertani (LB) broth (Appendix I) at 30°C was centrifuged at 10,000 rpm for 10 min at 4°C and the supernatant was discarded. The pellet was resuspended in 2.5 ml each of 10 mM Tris-Cl and 100 mM sodium chloride solution and centrifuged at 10,000 rpm for 10 min at 4°C . The supernatant was discarded and the pellet was resuspended in 2.5 ml of $T_{50}E_{20}$ buffer and 500 μl of lysozyme (50 mg/ml of 10 mM Tris-Cl) was added. The solution was incubated on water bath for 20 min at 37°C . Similarly, 25 μl of RNase (10 mg/ml) was added and incubated for 10 min at room temperature. 2.5 ml of sarkosyl (2% in $T_{50}E_{20}$) was added and incubated on water bath for 45 min at 50°C followed by addition of 50 μl of proteinase-K (20 mg/ml) and incubation for 10 min at 50°C . After cooling, equal volume of phenol was added, mixed gently and centrifuged at 10,000 rpm for 10 minutes at 4°C . The aqueous phase was transferred to a new tube and extraction was repeated twice with equal volumes of phenol: chloroform (1:1) and chloroform: isoamylalcohol (24:1) separately, centrifuged and the aqueous phase was separated out. To the supernatant 1/10th volume of 3M sodium acetate was added and incubated on ice for 20 minutes. Equal volumes of cold isopropanol was then added and centrifuged. The supernatant was discarded and the pellet was washed with 70 per cent alcohol, dried and dissolved in 100 μl of $T_{10}E_1$.

3.1.5.1.1 Quantification of genomic DNA

Genomic DNA of each isolate was quantified by spotting 1 μl of sample in spectrophotometer (Nanodrop 1000). The concentration of the DNA calculated using an inbuilt software ND 1000 version 3.5.2 was recorded in ng per μl .

3.1.5.1.2 Agarose gel electrophoresis

The DNA samples were subjected to gel electrophoresis using one per cent agarose along with molecular weight marker λ DNA *EcoRI/HindIII* double digest. The gel was observed under UV transilluminator and documented (Syngene Bioimaging Private Ltd, Gurgaon, India).

3.1.5.2 Amplification of 16S rRNA gene

The 16S rRNA gene was amplified as described by Sye *et al.* (2001). Twenty μl of reaction mixture containing 100 ng template, 3 unit (U) of *Taq* polymerase, 1.0 mM each of dATP, dCTP, dGTP and dTTP, 10x assay buffer, 25 mM MgCl_2 (Bangalore genei, Bangalore, India) and 5 pM of 16S rRNA primer (16F 5'-ggagagtttagatcttggtcag-3' and 16R 5'-aaggaggggatccagccgca-3') were used for amplification (Weisburg *et al.*, 1991). The reactions were carried out in an polymerase chain reaction (PCR) machine (Mastercycler Gradient, Eppendorf) with an initial 5 min denaturation step, followed by 35 cycles of 95°C for 1 min, 50°C for 1 min and 72°C for 90 sec, with a final extension at 72°C for 45 min and thereafter cooled at 4°C .

3.1.5.3 Elution of the PCR amplicon from agarose gels

PCR amplicons were analyzed by loading 5 µl of sample to 1.5 per cent agarose gel and run for about 45 minutes at 50V in TAE 1X buffer (0.4 mM of Tris acetate and 1 mM of EDTA) and subsequently the gel image was acquired with gel doc (Syngene Bioimaging Private Ltd, Gurgaon, India). The single sharp amplicon of 1.45 kb size was separated out using sharp sterile scalpel by keeping the gel at low intensity UV transilluminator and collected in a sterile pre-weighed 2.0 ml microcentrifuge tube. The Qiagen's MinElute gel extraction kit was used to elute the DNA from the agarose block as described in user's manual.

3.1.5.4 Cloning of PCR product

The purified 1.45 kb PCR fragment was ligated to pTZ57R/T cloning vector (2868 bp) as described in InsT/Aclone™ PCR product cloning kit from MBI, Fermentas, USA.

3.1.5.5 Ligation

For ligation, an optimal 1:3 molar ratio of ends of vector: insert was calculated. The components of ligation mix (Appendix II) were mixed in 0.5 ml micro centrifuge tubes and incubated at 16° C for 16 h for ligation.

3.1.5.6 Preparation of competent cells

The competent cells of *E. coli* DH5α were prepared following the protocol described by Sambrook and Russell (2001) with minor modification as described below. An isolated colony from *E. coli* DH5α plate was inoculated into 5 ml Luria broth and incubated at 37° C overnight at 200 rpm. The next day, the culture was diluted to 1:100 using Luria broth and incubated for 2 to 3 h till it attained an OD of 0.3 to 0.4 at 600 nm. The culture was chilled on ice for 30 min and 25 ml of culture was dispensed into two 50 ml centrifuge tubes. The cells were pelleted at 6000 rpm for 5 min, the supernatant was discarded and pellet was suspended in 12.5 ml of ice-cold 0.1 M calcium chloride. The centrifuge tubes were again kept in ice for 45 min and later centrifuged at 4000 rpm for 10 min. The pellet was dispensed in 1 ml of 0.1 M calcium chloride and to this 88 µl of dimethyl sulfoxide (DMSO) was added. About 200 µl of cell suspension was distributed to each pre-chilled 1.5 ml micro centrifuge tubes and immediately used.

3.1.5.7 Transformation of *E. coli* DH5α

About 100 µl of freshly prepared competent cells were taken in a chilled centrifuge tube and 10 µl of ligation mixture was added into the tube and was mixed gently. The mixture was chilled on ice for 45 min and heat shock was given by shifting the chilled mixture into water bath at 42° C for 2 min and immediately, it was transferred onto ice to chill for 5 minutes. To this, 800 µl of Luria broth was added and incubated at 37° C at 200 rpm for 45 minutes, to allow bacteria to recover and express the antibiotic marker encoded by plasmid. The culture was centrifuged at 13,000 rpm for 1 min and about 700 µl of supernatant was discarded and the pellet was resuspended in the remaining supernatant and spread on the plates having Luria agar with Amp50, X-gal IPTG and incubated overnight at 37° C. The recombinant clones were identified by blue/white assay. After incubation, only white colonies, having recombinant vectors were picked up and streaked on plates having Luria agar with Amp100, X-gal, IPTG and incubated at 37° C overnight.

3.1.5.8 Confirmation of clones and sequencing

The recombinant clones were screened for the presence of cloned fragment through colony PCR using the M13 primers as well as restriction confirmation using *Xba*I and *Bam*HI.

A total of 10-16 recombinant clones (white colonies) from each of the isolates were subjected for PCR confirmation. Twenty µl PCR mixture containing 5 U *Taq* polymerase, 2 mM each of dATP, dCTP, dGTP and dTTP, 10x *Taq* buffer A, 25 mM MgCl₂ (Bangalore genei, Bangalore, India) and 10 pM of M13 primer (M13F 5'-gtaaacgacggccagt-3' and M13R 5'-caggaaacagctatgac-3') were used for amplification (Sambrook and Russell, 2001).

The reactions were carried out with an initial 5 min denaturation step, followed by 30 cycles at 94° C for 30 sec, 50° C for 1 min and 72° C for 1 min, with a final extension at 72° C for 45 min and thereafter cooled at 4° C. The size of the PCR product was checked in 1 per cent agarose gel and positive clones were selected.

Plasmid from the positive clones was isolated and subjected for restriction analysis using *Xba*I and *Bam*HI. Plasmid isolation was done using QIAprep spin MiniPrep kit as described in the User's manual. Twenty µl reaction mixture containing 15.5 µl nuclease free water, 2 µl of 10x buffer, 0.25 µl of *Xba*I and *Bam*HI (Hi Media Pvt. Ltd., Mumbai) and 2 µl of plasmid DNA was incubated for 1 h at 37° C and later for 20 min at 65° C. Restricted products along with the vector control were loaded on to 0.8 per cent agarose gel and run for about 45 minutes at 50V in TAE 1X buffer (0.4 mM Tris acetate and EDTA 1 mM) and subsequently the gel image was acquired with gel doc (Syngene Bioimaging Private Ltd, Gurgaon, India).

Recombinant plasmids containing the insert (1.45 Kb) were sent for sequencing to Applied Bio-Systems Private Ltd., Hyderabad. Sequencing was done using M13 primers by primer walking technique at AB Systems Private Ltd., Hyderabad.

3.2 Effect of salt concentration on agriculturally important functions of isolates

3.2.1 Nitrogen fixation

A total of 79 salt-tolerant isolates comprising 36 associative and 43 free-living N₂ fixers were subjected for quantitative estimation of the amount of nitrogen fixed in the broth culture by Microkjeldahl method of Bremner and Mulvaney (1982). Noriss N-free broth (Appendix I) supplemented with sodium chloride at 0.02, 5, 7.5, 10, 12.5 and 15 per cent (w/v) and Okon's semi-solid N-free malate medium supplemented with sodium chloride at 0.002, 5, 7.5 and 10 per cent (w/v) were prepared, autoclaved and used for the estimation of nitrogen fixation by free-living and associative N₂ fixers respectively. A loopful of 48 h old culture of each isolate was inoculated to 5 ml of respective medium and incubated for 48 h. One ml of this culture was inoculated to 50 ml of their respective medium in 250 ml flasks. The flasks were incubated at 28± 2° C for 10 days and 10 ml of this culture was used for estimation of nitrogen.

To the 10 ml of broth culture, 5 ml of concentrated H₂SO₄ and 200 mg catalyst mixture (potassium sulphate, copper sulphate and selenium in the ratio of 10:1:0.1) were added and allowed for digestion in block digester for 2 h to get clear digest. The clear digest was cooled and diluted with distilled water up to 10 ml. This was distilled in a distillation unit after addition of 20 ml of 40 per cent sodium hydroxide solution to make the digest alkaline, in a Parnas-Wayner type distillation unit. The evolved ammonia was absorbed in four percent boric acid with mixed indicator and finally titrated with 0.05 N H₂SO₄ for colour change from green to red. From the volume of acid consumed, total nitrogen content was calculated. Simultaneously, the protein content of each isolate was estimated as per Lowry's method and amount of N₂ fixed by each isolates was expressed as µg per mg protein.

3.2.1.1 Protein estimation

The protein content of each isolate was estimated by Lowry's method (Sadasivam and Manickam, 1991).

3.2.2 Phosphate solubilization

Qualitative assay for P-solubilization was done for 61 fluorescent pseudomonad isolates. The ability of FP isolates to solubilize insoluble inorganic phosphate was tested by spotting 10 µl overnight grown cultures on Pikovskaya's agar (Appendix I) supplemented with different salt concentration and incubating the plates at 28± 2° C for 2-3 days. The FP isolates which showed clear zone of solubilization of tricalcium phosphate (TCP) around the colony were noted as phosphate solubilizers and subjected for quantitative estimation.

A total of 81 salt-tolerant isolates (55 PSB, 23 FP and 3 P-solubilizing fungi) were subjected for quantitative estimation of amount of inorganic phosphate (Pi) released from TCP in Pikovskaya's broth (Appendix I) supplemented with different salt concentrations.

3.2.2.1 Quantitative estimation of Pi released from tricalcium phosphate (TCP)

The Erlenmeyer flasks containing 50 ml Pikovskaya's broth (Pikovskaya, 1948) supplemented with 0, 5, 7.5, 10, 12.5 and 15 per cent NaCl were inoculated with 500 µl of overnight grown culture of each isolate in two replicates and incubated for 7 days at $28 \pm 2^\circ \text{C}$. The amount of Pi released in the broth was estimated at 7 days of incubation in comparison with the uninoculated control. The TCP broth cultures were spun at 10,000 rpm for 10 minutes to separate the cells and insoluble phosphate. The available P content in the supernatant was estimated by phosphomolybdic blue colour method (Jackson, 1973).

3.2.3 Antagonistic activity

Sixty one salt tolerant FP isolates were subjected for their antagonistic activity *in-vitro* against five fungal plant pathogens, viz., *Alternaria carthami* Chowdhury, causing leaf spot/blight of safflower, *Fusarium oxysporum* f. sp. *carthami* Klisiewicz and Houston, causing wilt of safflower, *Sclerotium rolfsii* Sacc., causing wilt/root rot in a wide variety of crops, *Rhizoctonia bataticola* (Taub.) Butler, causing root rot in many crops and *Pyricularia oryzae* causing blast disease of rice.

The pure cultures of the all the plant pathogenic fungi maintained in the Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad were used for the present study.

The dual inoculation technique of Sakthivel and Gnanamanickam (1987) was followed to study the antagonistic activity of the fluorescent pseudomonads against above plant pathogens.

The fungal pathogens were grown on potato dextrose agar (PDA-Appendix I) plates till they covered complete agar surface. With the help of a sterile cork borer (10 mm diameter), discs of fungal growth from the plates were taken and placed at the center of the fresh PDA plates. Each test isolate was then streaked parallel on either sides of the fungal disc leaving 1.5 cm distance from the edge of the plate (Sakthivel and Gnanamanickam, 1987). The PDA plates inoculated with only fungal pathogens served as respective controls. The plates were incubated at 30°C for 96 h. The colony diameter of the fungus in control plate and the plates streaked with fluorescent pseudomonads was recorded. The zone of inhibition (ZOI) of each fungal pathogen by different isolates was calculated by using the following formula,

$$\text{ZOI} = \text{Colony diameter (control plate)} - \text{Colony diameter (in dual inoculated plates)}$$

3.2.4 Phytohormone production

3.2.4.1 Production of indole compounds

3.2.4.1.1 Extraction

All the 195 salt-tolerant isolates were tested for the production of indole 3-acetic acid (IAA) and gibberellic acid (GA). The flasks containing 50 ml Czapeck's solution (Appendix I) supplemented with NaCl at 0, 5, 7.5, 10, 12.5, 15 and 17.5 per cent were prepared and autoclaved. A 500 µl of 24 h old culture of associative N_2 fixers, free-living N_2 fixers, PSB and FP isolates were inoculated to 50 ml Czapeck's solution containing NaCl concentrations and ranging from 0-10, 0-15, 0-15 and 0-17.5 per cent respectively and incubated at 37°C for 7 days in dark. After incubation, the cultures were spun at 6000 rpm for 20 min. The supernatants were collected in Erlenmeyer flasks and used for estimation of IAA and GA.

3.2.4.1.2 Estimation of IAA

Twenty five ml supernatant of each isolate was dispensed in 100 ml Erlenmeyer flask and adjusted the pH to 2.8 using 1N HCl. Equal volume of diethyl ether was added to it and incubated in dark for 4 h. IAA was extracted at 4°C using separating funnel. The organic phase was discarded and the solvent phase was pooled and evaporated to dryness. This dried material was redissolved in 2 ml of methanol and the IAA present in the methanol extract was determined (Gorden and Paleg, 1957).

The methanol extract (0.5 ml) from each isolate was mixed with 1.5 ml distilled water and 4 ml Salper's reagent (1 ml of 0.5 M FeCl₃ in 50 ml of 35 per cent perchloric acid) and incubated for 1 h in dark. The pink colour developed was read at 535 nm in an UV-Vis spectrophotometer (Optima, Tokyo, Japan; Model-SP 3000 Plus). A standard curve was also prepared using graded concentrations of pure IAA (Sigma Aldrich Co., USA). Using standard curve, the quantity of IAA in the culture filtrate was determined. Simultaneously, the cultures were subjected for protein estimation as per the protocol mentioned in section 3.2.1.1 and quantity of IAA in the culture filtrate was expressed as µg per mg protein.

3.2.4.1.3 Estimation of GA

Twenty-five ml culture supernatant of each isolate was taken in a test tube. To this, 2 ml Zinc acetate was added followed by addition of 2 ml potassium ferrocyanide after 2 minutes. This was centrifuged at 1000 rpm for 15 minutes and the supernatant collected. To 5 ml of this supernatant, 5 ml of 30 per cent HCl was added and incubated at 20° C for 75 min. The blank sample was treated with 5 per cent HCl. The absorbance of the samples as well as blank was measured at 254 nm in an UV-Vis spectrophotometer (Pateg, 1965). The standard curve of GA was prepared by using graded concentrations of pure GA₃ (Sigma Aldrich Co., USA). The amount of protein in each isolate was quantified as per the protocol mentioned in section 3.2.1.1 and GA present in the extract was expressed as µg per mg protein.

3.2.4.2 Cytokinin production

3.2.4.2.1 Extraction of cytokinin

A total of 12 representative functionally important isolates comprising 3 each of associative N₂ fixers, free-living N₂ fixers, PSB and FP isolates tolerating higher salt concentration were also tested for the production of cytokinins. The respective culture broths supplemented with three concentrations of sodium chloride for each of the functional group were prepared and autoclaved. The sterilized culture tubes dispensed with Okon's, Norris, TCP and King's B broth (Appendix I) containing NaCl at 0, 5 and 10 per cent (w/v) were inoculated with 20 µl of overnight grown culture of associative N₂ fixers, free-living N₂ fixers, PSB and FP isolates respectively and incubated on a rotary shaker (150 rpm) at 30° C for 7 days. The broth cultures were centrifuged at 12,000 rpm for 10 min and supernatant were extracted twice with equal volume of n-butanol.

3.2.4.2.2 Quantification of cytokinins by HPLC method

The n-butanol fractions of the cell-free culture broths of each functional group of isolates were used for quantification of cytokinins by HPLC (Waters, 515 pump with 2487 dual λ absorbance detector). The n-butanol was evaporated, sediments were redissolved in 2 ml of HPLC grade methanol and filter sterilized using bacteriological filters (2 µm) and 20 µl of this extract was injected into the HPLC. Analysis was performed using a 5 (m particle size reverse phase column (C18 symmetry, 4.6 x 215 mm) with a solvent gradient of 30 per cent methanol in water at a flow rate of 1.5 ml per min and the operating pressure was 1600 psi (Tien *et al.*, 1979). The quantification of cytokinin compounds in the samples was done by comparison of retention time with peak height of pure zeatin (Sigma Aldrich Co., USA). The substances were quantified by integrating the areas under the peaks using UV detector at 254 nm.

3.3 Biochemical basis for salt tolerance

3.3.1 Exopolysaccharide production (EPS)

A total of 21 salt-tolerant bacterial isolates comprising 3 associative N₂ fixers and 6 each of free-living N₂ fixers, PSB and FP isolates were examined for EPS production. The flasks containing 25 ml Tryptone glucose yeast extract (TGY) broth (Appendix I) supplemented with 0, 5 and 10 per cent NaCl were prepared and autoclaved. One ml of overnight culture grown in Luria broth was inoculated to each flask and allowed to grow for 120 h at 28±2° C on a rotary shaker at 180 rpm. EPS was recovered from the culture as described by Ramirez *et al.* (1988). Culture was centrifuged at 10,500 rpm for 10 min at 4° C to remove the cells. The supernatant was then mixed with a 10 per cent (v/v) saturated KCl solution as an electrolyte, precipitated with an equal volume of 95 per cent ethanol and kept at 4° C overnight. The precipitate was then removed by centrifugation at 16,500 rpm for 15

min at 4° C, washed twice with 95 per cent ethanol and lyophilized. The dried pellet was weighed and content of EPS was expressed as µg per mg protein.

3.3.2 ACC deaminase activity

A total of 67 high salt-tolerant isolates comprising 18 associative N₂ fixers, 14 free living N₂ fixers, 23 PSB and 12 FP were assayed for ACC deaminase activity (Honma and Shimamura, 1978). M9 minimal medium broth without N source but supplemented with different salt concentration viz., 0.05, 5 and 10 per cent NaCl were prepared, autoclaved and used for the ACC deaminase assay. Initially, a loopful of culture of each test isolate was inoculated to 10 ml LB broth and incubated overnight on a rotary shaker at 200 rpm at 28±2° C. The cells were harvested by centrifugation at 8000 rpm for 10 min at 4° C and supernatant was discarded. The pellet was redissolved in 10 ml M9 minimal medium broth (without N source) containing different salt concentration and supplemented with 45 µl of 0.5 M ACC (filter sterilized). The cultures were incubated for 24 h in a shaker at 200 rpm at 28±2° C. The cells were harvested by centrifugation at 8000 rpm for 10 min at 4° C. The pellet was suspended in 5 ml of 0.1 M Tris-HCl, pH 7 and centrifuged at 16000 rpm for 5 min at 4° C. The pellet was suspended in 600 µl of 0.1 M Tris-HCl, pH 8.5 and 30 µl toluene. The toluenized cell suspension was assayed for ACCD activity as well as protein content (section 3.2.1.1).

The assay mixture comprising of 200 µl of the toluenized cells, 20 µl of 0.5 M ACC, 1 ml of 0.5 M HCl, 300 µl of 2, 4-dinitrophenyl hydrazine was mixed by vortexing and incubated for 30 min at 28±2° C. After incubation, 2 ml of 2 N NaOH was added and the absorbance was read at 540 nm in an UV-Vis spectrophotometer. The activity of ACC deaminase in each of the test isolate was calculated from the standard graph of α-ketobutyrate (100 mM) and expressed as nmol of α-ketobutyrate per mg protein per h.

3.3.3 Accumulation of osmolytes

A total of 67 high salt-tolerant isolates comprising 18 associative N₂ fixers, 14 free living N₂ fixers, 23 PSB and 12 FP were assessed for different osmolytes viz., proline, glycine-betaine, total sugar and potassium, accumulated in response to different salt concentrations in the medium.

Changes in the proline, glycine-betaine and total sugar content in response to changing salt concentration of the medium were monitored in all the 67 salt-tolerant isolates. Four sets of flasks containing 50 ml M9 minimal medium broth (Appendix I) supplemented with different salt concentrations viz., 0.05, 5, 7.5, 10, 12.5, 15 and 17.5 per cent NaCl were prepared and autoclaved. One ml of 24 h old culture of associative N₂ fixers, free-living N₂ fixers, PSB and FP isolates were inoculated to 50 ml M9 minimal medium broth containing NaCl concentrations ranging from 0.05-10, 0.05-15, 0.05-15 and 0.05-17.5 per cent respectively and incubated at 37° C for 10 days at 28± 2° C. After incubation, the amount of proline, glycine-betaine and total sugar content were estimated by following Ninhydrin method (Bates *et al.*, 1973), Periodide method (Storey and Jones, 1977) and Phenol-sulfuric acid method (Dubios *et al.*, 1956) respectively. The estimation protocol is given below.

3.3.3.1 Proline

Ten ml of the 10 days old culture was treated with 3 per cent sulphosalicylic acid and filtered using Whatman No.1 filter paper. The reaction mixture containing 5 ml of filtrate, 2 ml each of acid ninhydrin reagent (2.5 g of ninhydrin dissolved in 40 ml 6 M H₃PO₄ and 60 ml of glacial acetic acid) and glacial acetic acid were incubated on boiling water bath for 1 h. The contents were transferred to a separating funnel and treated with 6 ml of toluene, mixed thoroughly and allowed to stand for few minutes. The absorbance of the upper toluene layer containing the colour complex was read at 520 nm in an UV-Vis spectrophotometer. A standard curve was prepared using various concentrations of standard proline (100 ppm). The amount of proline in the test samples was calculated from the standard curve and expressed as µg per mg protein.

3.3.3.2 Glycine-betaine

Glycine-betaine content in each of the isolates was quantified as per the method described by Storey and Jones (1977). It involves pH dependent selective precipitation of betaine and choline compounds by the non-specific periodide method. Glycine-betaine content was calculated using the following formula.

Glycine-Betaine= Total quaternary ammonium compounds (QACs) (pH 2.0)–Choline (pH 8.0)

Five ml of culture was homogenized with equal volumes of methanol and the contents were evaporated to dryness, redissolved in water and stored at -20° C. Total QACs and choline were estimated as per the protocol mentioned below.

3.3.3.2.1 Estimation of total QACs

The acid potassium tri-iodide solution was prepared by dissolving 7.5 g I₂ and 10 g KI in 1 M HCl and filtered. Precisely, 0.2 ml of potassium tri-iodide reagent was added to a 25 ml culture broth and mixed properly and incubated on ice for 90 min with intermittent shaking followed by addition of 2 ml of ice cooled water and 20 ml of 1, 2-dichloroethane. Later, the above contents were transferred to separating funnel and refrigerated for 5 min at -20° C and the two layers mixed constantly for 5 min. The absorbance of the lower layer was measured at 365 nm in an UV-Vis spectrophotometer and the amount of glycine-betaine in each of the test isolate was calculated from the standard graph of glycine-betaine (50 ppm) and expressed as µg per mg protein.

3.3.3.2.2 Estimation of choline

Choline content was estimated as per the procedure mentioned in section 3.3.3.2.1 wherein, instead of acid potassium tri-iodide solution, alkaline solution with pH 8 was used. The potassium tri-iodide solution was prepared by dissolving 7.5 g I₂ and 10 g KI in a 0.4 M KH₂PO₄-NaOH buffer (pH 8.0) and filtered.

3.3.3.3 Sugar

Ten ml of the 10 days old culture was taken and the cells were removed by centrifugation at 6000 rpm at 4° C for 15 min. One ml of supernatant was taken in a test tube to which 1 ml of 5 per cent phenol and 5 ml of concentrated sulfuric acid were added and mixed thoroughly for 10 min. The tubes were incubated in a water bath at 25° C for 20 min. The yellow orange color developed was read at 490 nm against water blank in an UV-Vis spectrophotometer. Simultaneously, a standard curve was prepared by using various concentrations of glucose standard (100 ppm). The amount of total sugar was calculated from the standard curve and expressed as µg per mg protein.

3.3.3.4 Intracellular potassium (K) and sodium (Na) content

Changes in the intracellular K and Na content in response to changing salt concentration of the medium were monitored in all the 67 salt-tolerant isolates. Four sets of flasks containing 50 ml M9 minimal medium broth (Appendix I) supplemented with NaCl at 0.05, 5, 7.5, 10, 12.5, 15 and 17.5 per cent (w/v) were prepared and autoclaved. One ml of 24 h old culture of associative N₂ fixers, free-living N₂ fixers, PSB and FP isolates were inoculated to 50 ml M9 minimal medium broth containing NaCl concentrations ranging from 0.05-10, 0.05-15, 0.05-15 and 0.05-17.5 per cent respectively and incubated at 37° C for 10 days at 28±2° C. After incubation, the cells were prepared for estimation of intracellular K and Na contents as per the protocols given by Wase and Ratwatte (1985) and the elemental contents were analyzed using flame photometer (Jackson, 1973). The protocol used in the present study is given below.

Fifty ml of 10 days old culture was used for the estimation. The cells were separated using cellulose acetate membrane filter (0.45 µm pore size with 50 mm diameter). The cells on the filter paper were washed with 0.1 M MgCl₂ (pH 6.5) using suction pump and flask. The cells were carefully suspended in 5 ml of double distilled water and incubated in a water bath at 100° C for 5 min. The contents were cooled and stored at 4° C for further analysis.

The prepared cells suspended in water were subjected for elemental analysis using flame photometer. The cell suspension was used to estimate the K and Na contents and their ratio (Na to K) was worked out.

3.4 Growth promotional activity of potential salt-tolerant isolates

Growth promotional activity of 19 salt tolerant isolates comprising of 4 associative N₂ fixers, 5 each of free-living N₂ fixers and FP, 4 PSB and one P-solubilizing fungi (PSF) were tested in an environmental growth chamber at six soil electrical conductivity (saturation paste extract) (ECe) levels in wheat (cv. DWR 162).

3.4.1 Soil electrical conductivity (ECe) levels

Normal and saline soils having ECe of 0.68 and 98.3 dS m⁻¹ respectively were used to develop the required soil ECe level of 0.37, 2.32, 4.08, 5.38, 7.21 and 7.55 dS/m. The normal medium black soil (ECe 0.37 dS/m) was collected from 'E' blocks of Main Research Station, University of Agricultural Sciences, Dharwad and that of saline soil having soil ECe of 98 dS m⁻¹ from salt affected soils of Mudhol in B.agalkot district of Karnataka state. Both the soils were ground to a fine powder and passed through 2 mm sieve and mixed in a required ratio to get the desired ECe level. The mixed soil was checked for the soil ECe and filled into plastic cups at the rate of 300 g per cup.

3.4.2 Treatments

A two factorial experiment comprising salt-tolerant isolates as one factor and soil ECe levels as another factor. Experiment consisted of 120 treatment combinations involving 19 salt-tolerant isolates with un-inoculated control (UIC) and 6 soil ECe levels with 3 replications. The details of the treatments are given below.

Factor 1. Isolates		Factor 2. Soil ECe (dS m ⁻¹)
1. ASANFIV S61E	11. ASPSBIX S125R	1. 0.37
2. ASANFIV S40E	12. ASPSBIX S124E	2. 2.32
3. ASANFII S22E	13. ASPSBXI WL19E	3. 4.08
4. ASANFIV S78E	14. ASPSF WL9S	4. 5.38
5. ASFNFIV S63(1)R	15. ASFPV Dr12bR	5. 7.21
6. ASFNFIV S75(1)R	16. ASFPI S4(1)S	6. 7.55
7. ASFNFIV WL4(1)R	17. ASFPV Dr10R	
8. ASFNFIV S37R	18. ASFPI S6(1)S	
9. ASFNFXII S178E	19. ASFPV S100E	
10. ASPSBIX S122R	20. UIC	

3.4.3 Seed bacterization

Wheat (CV. DWR 162) seeds obtained from Seed unit, UAS, Dharwad were bacterized following the method of Weller and Cook (1983). All the bacteria were grown on Luria agar for 24 h. The bacterial growth was scraped and thoroughly mixed with 1 per cent sterile carboxy methyl cellulose (CMC) (Hi Media) suspension. Wheat seeds were surface sterilized with sodium hypochlorite (4%) for 25 minutes and then thoroughly rinsed twice with sterile water. The seeds were then placed in CMC based culture suspension and air dried overnight by placing in a laminar air flow chamber (LFC). Seed coated with only CMC suspension served as control.

3.4.4 Sowing and maintenance

The biocoated seeds were sown in cups at the rate of 10 seeds per plastic cup. After germination, thinning was done to retain five plants per cup and plants were allowed to grow up to 30 days. Three replications were maintained for each treatment and the cups were kept in a controlled environmental chamber (illuminated with 350 µmol m⁻² sec⁻¹ light intensity at 28/18° C day/night temperatures and 16/8 h light and dark cycle).

3.4.5 Observations

3.4.5.1 Seedling emergence

Emergence of wheat seeds in response to inoculation and increasing soil ECe level was recorded after 7 days after sowing and expressed as percentage.

3.4.5.2 Plant height

Height of all the five plants from each treatment was measured. The measurement was made from the base of the plant to the tip of longest leaf at 30 days after sowing (DAS). Mean of all the five plants was calculated and expressed in cm.

3.4.5.3 Total dry matter content

After 30 days of experimentation, all the plants from each treatment were removed and oven dried at 65° C to a constant weight to determine the total dry matter production. The data were expressed in mg per plant.

3.5 Field performance of potentially efficient salt-tolerant isolates and their consortia

3.5.1 Potential salt-tolerant isolates

Based on the salt tolerance ability, functional property and growth promotional activity, a total of five potential isolates (4 bacterial and 1 fungal) were selected for the field assessment. The isolates used in the experiment were *Enterobacter aerogenes* S63(1)R, *Azospirillum irakense* S173E, *Enterobacter cloacae* S125R, *Pseudomonas* sp. S4(1)S and *Aspergillus niger* WL9S. Besides, a consortia of all four bacterial isolates was also used in the field studies.

All the four bacterial isolates were first checked for their compatibility on LB agar. A loopful of each culture was cross streaked on LB agar (Appendix I) and incubated for 48 h at 28±2° C and checked for their growth.

3.5.2 Mass production of bioinoculants

3.5.2.1 Sterilization and packing of carrier

Hundred gram of dried lignite powder was neutralized with five per cent CaCO₃ and packed in polypropylene bags with clips and sterilized. After sterilization, clips were removed and bags were heat sealed aseptically.

3.5.2.2 Inoculum preparation and inoculation

One ml of overnight culture grown in Luria broth of each bacterial isolate was inoculated to 100 ml Luria broth separately. Similarly, 1 ml of 3 days old culture of *Aspergillus niger* WL9S was inoculated to potato dextrose broth (PDB). All the cultures were incubated on a rotary shaker with 150 rpm for 48 h at 30° C. The bacterial culture was inoculated @ 30 ml per 100 g pre-sterilized lignite powder based carrier aseptically with the help of a syringe and mixed thoroughly. Similarly, the multiplied fungal mycelia was homogenized aseptically in a sterile blender and mixed with sterilized lignite. The packed lignite based carrier was kept for curing of cells for a period of 7 days at 30° C and stored at 28±2° C.

3.5.3 Study area

A field experiment was conducted to assess the field performance of salt-tolerant agriculturally important microorganisms (AIMs) in wheat crop at varied soil ECe levels during Rabi 2010-11 at Agricultural Research Station (ARS), Gangavati (Koppal district of Karnataka state) under the jurisdiction of University of Agricultural Sciences (UAS), Raichur. The details of materials used and experimental techniques adopted are described below.

3.5.3.1 Experimental site

The experimental site is situated in the geographic location of 15° 25' 48" N longitude and 76° 31' 48" E latitude. The peculiarity of the experimental site was that it had a gradient of salinity ranging from 2.4 to 14.3 dS/m.

Table 3. Properties of the soils used in the present study

Properties	Soil ECe levels					Method followed
	S1	S2	S3	S4	S5	
pH (1:2.5)	8.3	8.9	8.9	9.0	9.3	Jackson (1973)
ECe (dS m ⁻¹)	2.4	4.9	8.7	11.2	14.3	
OC (g Kg ⁻¹)	14.1	10.1	9.6	9.3	8.4	
Microbial biomass carbon (MBC) (mg kg soil ⁻¹)	241	195	156	131	110	Vance <i>et al.</i> (1987)
Microbial biomass nitrogen (MBN) (mg kg soil ⁻¹)	63.6	27.2	20.1	12.3	9.6	
Dehydrogenase (µg TPF g soil ⁻¹ day ⁻¹)	36.5	29.2	21.7	13.1	9.1	Tabatabai (1982)
Urease (µg NH ₄ ⁺ -N g soil ⁻¹ day ⁻¹)	59.1	53.2	48.1	37.4	26.9	Tabatabai and Bremner (1972)
Phosphatase (µg pNP g soil ⁻¹ h ⁻¹)	167.2	142.4	121.7	112.3	98.5	Tabatabai and Bremner (1969)
Water soluble cations (1:2.5)						
Ca ²⁺ (me l ⁻¹)	3.6	5.4	9.2	21.6	26.9	Chapman and Pratt (1961)
Mg ²⁺ (me l ⁻¹)	2.1	11.7	15.9	30.3	38.5	
Na ⁺ (me l ⁻¹)	13.3	35.1	49.6	76.9	92.3	
K ⁺ (me l ⁻¹)	0.7	1.9	3.3	1.6	1.1	

Table 4. Mean monthly meteorological data of the experimental site at Agricultural Research Station, Gangavati, UAS, Raichur (Karnataka)

Months	Rainfall (mm)		Temperature (°C)			Relative humidity			
	Average (1979-2010)	2010-11	Average (1990-2010)	2010-11		8 a.m.		2 p.m.	
				Max.	Min.	Average (1979-2010)	2010-11	Average (1990-2010)	2010-11
April	13.0	8.0	37.5	38.0	22.0	78.2	84.1	51.2	67.7
May	33.8	101.3	37.8	42.0	23.0	79.6	81.6	51.5	62.1
June	66.6	51.3	34.6	34.0	22.0	80.3	85.8	59.3	69.7
July	62.7	110.3	31.9	31.0	21.0	82.8	84.1	64.4	79.6
August	94.3	259.8	29.2	30.9	21.1	84.2	82.3	70.1	77.8
September	128.9	67.5	30.0	30.6	20.6	84.3	83.8	67.1	81.3
October	122.7	38.8	29.0	30.5	14.8	85.5	82.6	71.6	80.7
November	30.5	85.5	29.3	29.5	19.1	79.1	85.1	68.3	80.9
December	11.3	-	28.8	29.6	19.3	77.2	77.0	70.6	79.7
January	0.7	-	29.5	29.0	20.0	77.9	77.5	60.2	76.7
February	0.7	-	31.5	30.5	17.0	74.5	75.2	59.7	67.8
March	1.0	-	33.8	34.1	17.9	75.0	69.3	55.7	75.7
Total	566.1	722.3	31.9	32.5	19.8	79.9	80.7	62.5	75.0

3.5.3.2 Soil and its characteristics

Soil type was saline vertisol. Soil depth in the study area ranged from 0.75 to 1.5 m. Soil physiochemical and biological properties were analyzed following standard procedures and are listed in Table 3.

3.5.3.3 Climatic conditions

The rainfall data and monthly mean maximum and minimum temperature data during 2010-11 is depicted in Table 4.

3.5.4 Experimental details

3.5.4.1 Treatments

The experiment was conducted with a total of 35 treatment combinations comprising 5 electrical conductivity levels and 7 bioinoculant treatments with 3 replications. The treatment details are mentioned below.

Factor A: Soil ECe level (E)	Factor B: Strains (S)
$S_1 = 2.4 \text{ dS m}^{-1}$	$T_1 = \textit{Enterobacter aerogenes}$ S63(1)R
$S_2 = 4.9 \text{ dS m}^{-1}$	$T_2 = \textit{Azospirillum irakense}$ S173E
$S_3 = 8.7 \text{ dS m}^{-1}$	$T_3 = \textit{Enterobacter cloacae}$ S125R
$S_4 = 11.2 \text{ dS m}^{-1}$	$T_4 = \textit{Pseudomonas}$ sp. S4(1)S
$S_5 = 14.3 \text{ dS m}^{-1}$	$T_5 = \textit{Aspergillus niger}$ WL9S.
	$T_6 = \text{Consortia } (T_1+T_2+T_3+T_4)$
	$T_7 = \text{Uninoculated control (UIC)}$

3.5.4.2 Design and layout

Experiments were laid out in a two factorial randomized complete block design (RCBD) with electrical conductivity as one factor and bioinoculants as another factor with 3 replications. The plan of layout of the experiments is depicted in Fig. 2.

3.5.4.3 Plot size

$$\begin{aligned}\text{Gross} & : 4.3 \text{ m} \times 4.0 \text{ m} = 17.2 \text{ m}^2 \\ \text{Net} & : 3.18 \text{ m} \times 3.1 \text{ m} = 9.86 \text{ m}^2\end{aligned}$$

3.5.4.4 Seed treatment

Wheat seeds (CV. DWR 162) coated with 15 per cent sugar solution (boiled and cooled at room temperature) were inoculated with lignite based bioinoculants (*Enterobacter aerogenes* S63(1)R, *Azospirillum irakense* S173E, *Enterobacter cloacae* S125R, *Pseudomonas* sp. S4(1)S and their consortia and *Aspergillus niger* WL9S) at the rate of 500 g per ha seeds separately. The treated seeds were dried in shade for few minutes and immediately used for sowing.

3.5.5 Cultural operations

3.5.5.1 Land preparation

The experimental area was brought to a fine tilth with a set of operations involving ploughing, clod crushing and harrowing. Initially, the experimental plot was ploughed to a sufficient depth with a mould board plough followed by clod crushing with a rotavator. Lastly, blade harrowing was done for two times. Bunds were raised for each plot and replication and the irrigation channels were prepared in the experimental area.

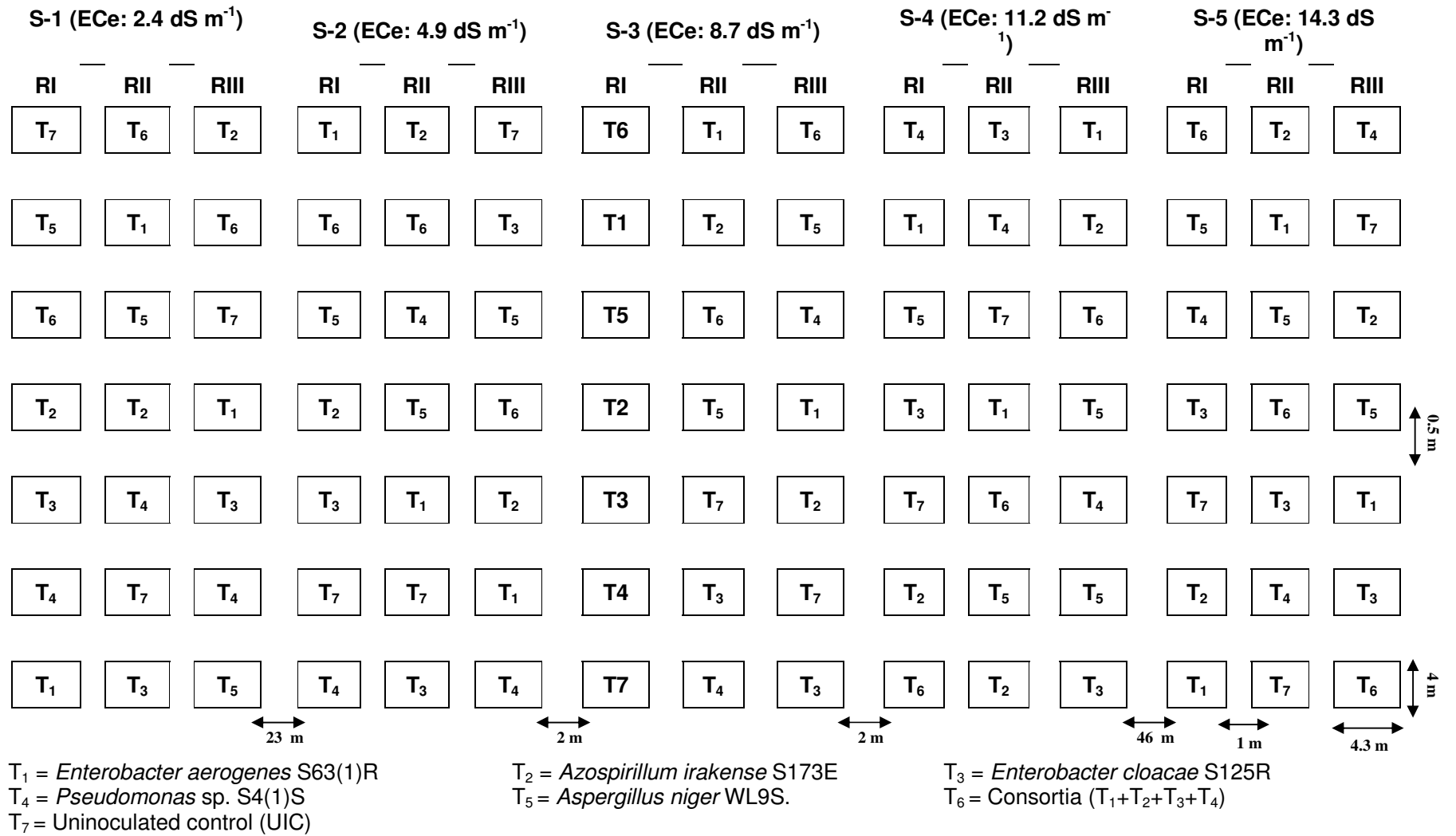


Fig. 2. Experimental layout at Agricultural Research Station, Gangavati

3.5.5.2 Fertilizer application

Based on the nutrient dose recommended for wheat (100: 75: 50 N: P₂O₅: K₂O kg/ha), the amount of fertilizer required per plot was calculated and applied to all the treatment combinations. Recommended dose of phosphorous and potassium were applied as a basal dose at sowing through single super phosphate and muriate of potash while nitrogen was applied in two equal splits viz., at basal and at 30 days after sowing (DAS) through urea.

3.5.5.3 Seeds and sowing

Wheat CV. DWR-162 seeds were procured from Karnataka State Seed Corporation (KSSC), Govt. of Karnataka, Dharwad. Seeds were weighed separately for each plot at the rate of 150 kg per ha. The seeds were hand dibbled to about four centimeters depth with a spacing of 22.5 cm x 7.5 cm and covered by soil.

3.5.5.4 Irrigation

A light irrigation was given before sowing for uniform and good germination. Then subsequent irrigations were given at an interval of 15-20 days to maintain adequate soil moisture in root zone. The broad basin method of irrigation was followed in the present study.

3.5.5.5 Weeding

To check weed growth, three manual weedings at 30, 45 and 60 DAS were carried out to keep the plots free from weeds and to provide better aeration.

3.5.5.6 Harvesting and threshing

The crop in the net plot area in each plot was harvested at 110 days after sowing. After complete drying, the weight of total dry matter from each net plot was recorded. After threshing, the produce was cleaned and weight of the grains per net plot was recorded.

3.5.6 Collection of experimental data

3.5.6.1 Growth parameters of wheat

3.5.6.1.1 Plant height

Five plants in the net plot area were selected randomly and tagged to measure the height at different stages of crop growth. The measurement was made from the base of the plant to the tip of longest leaf at 30 days after sowing (DAS) and from base of the plant to the base of panicle at 60, 90 DAS and at harvest.

3.5.6.1.2 Dry matter production

Destructive sampling was followed to determine the total dry matter content at 30, 60, 90 DAS and at harvest. Plant samples were collected from second row on either side of the plot to a row length of one meter and oven dried to a constant weight at 65° C. The total dry matter production was recorded and expressed as g per meter row length.

3.5.6.1.3 Chlorophyll content

Leaf chlorophyll content was estimated by measuring leaf greenness using a portable SPAD (Soil Plant Analysis Development)-502 chlorophyll meter (Minolta Camera Co. Ltd., Japan). SPAD readings were collected from first fully expanded flag leaf from the top of the plant at 30 and 60 DAS. Five plants were selected for recording the observations. In each plant five readings were recorded from leaf and they were averaged across each plot and expressed as SPAD reading per plant.

3.5.6.1.4 Total number of tillers

Total number of tillers in one square meter area within the net plot was recorded at 30 and 60 days of crop growth.

3.5.6.2 Yield and yield attributing characters

3.5.6.2.1 Number of productive tillers

Number of productive tillers per m² in each net plot area was recorded at 90 DAS and at harvest.

3.5.6.2.2 Spike length

The length of ten randomly selected spikes was measured and the average length was expressed in cm per spike.

3.5.6.2.3 Number of grains per spike

Twenty spikes selected at random from each plot at harvest were threshed to separate the grains. The total number of grains were counted and averaged to express number of grains per spike.

3.5.6.2.4 Thousand grain weight

Grain samples drawn from each net plot yield were used to record the test weight (1000 grains) and expressed as g.

3.5.6.2.5 Grain and straw yield

Net plot area of each treatment was harvested separately and the total biomass yield was recorded. After threshing, grains were separated, cleaned and weighed to record net plot yield. Straw yield per net plot was worked out by subtracting respective total grain weight from the total biomass yield. Later the grain and straw yields were computed on hectare basis from the respective net plot yields and expressed in q per ha.

3.5.6.2.6 Harvest index (HI)

From the yield of grains and biomass, the harvest index was calculated by using formula of Donald (1962) as follows.

$$\text{Harvest index (HI)} = \frac{\text{Grain yield (q/ha)}}{\text{Total biomass yield (q/ha)}}$$

3.5.7 Laboratory analysis

3.5.7.1 Bio-chemical parameters

3.5.7.1.1 Protein

Plant samples drawn at 7, 15 and 21 DAS from each experimental plot were assessed for protein content. Leaf proteins were extracted by grinding 1 g leaf sample in a 10 ml 100 mM potassium phosphate buffer containing 0.5 M EDTA. The homogenate was centrifuged at 10000 rpm for 15 min and the protein content of the supernatant was determined by Bradford method (Bradford, 1976).

3.5.7.1.2 Anti-oxidative enzymes (AOE)

Plant samples drawn at 7, 15 and 21 DAS were analyzed for changes in AOE activity. Leaf samples of wheat plants were collected, weighed (0.5 g), immediately frozen in liquid nitrogen and stored at -80°C until extraction. Frozen tissues were ground in liquid nitrogen with mortar and pestle and the powder was suspended in 0.5 ml of 0.1 M Tris (pH 8.0), 1 mM phenyl-methyl-sulfonyl fluoride (PMSF), 0.1 per cent (w/v) poly-vinyl-pyrrolidone and 1 per cent (v/v) β-mercaptoethanol. Extracts were centrifuged twice at 26000 rpm for 20 min at 4°C and the supernatant used for determination of enzyme activity. Enzyme activities were measured at 25°C using UV-Vis spectrophotometer. Protein content was determined by the method of Bradford (1976).

3.5.7.1.2.1 Catalase

Catalase activity (EC 1.11.1.6) was assayed in a reaction solution (3 ml) composed of 50 mM phosphate buffer (KH₂PO₄/K₂HPO₄), pH 7.0, to which 30 per cent (w/v) H₂O₂ was added until reaching an absorbance at 240 nm between 0.52 and 0.55. The reaction was started by adding the reaction solution to 10 µl of crude extract and the activity was assayed by monitoring the decrease in absorbance at 240 nm as a consequence of H₂O₂ consumption (Aebi, 1984) and the activities were expressed in U mg protein⁻¹.

3.5.7.1.2.2 Superoxide dismutase

Superoxide dismutase (EC 1.15.1.1) activity was assayed by measuring its ability to inhibit the photochemical reduction of nitro-blue tetrazolium (NBT) according to the method of Beauchamp and Fridovich (1971). About 3 ml of reaction mixture, containing 0.1 ml of 200 mM methionine, 0.01 ml of 2.25 mM nitro-blue tetrazolium (NBT), 0.1 ml of 3 mM EDTA, 1.5 ml of 100 mM potassium phosphate buffer, 1 ml distilled water and 0.05 ml of leaf extract, were taken in test tubes in duplicate. Two tubes without enzyme extract were taken as control. The reaction was started by adding 0.1 ml riboflavin (60 μ M) and placing the tubes below a light source of two 15 W florescent lamps for 15 min. Reaction was stopped by switching off the light and covering the tubes with black cloth. Tubes without enzyme developed maximal colour. A non-irradiated complete reaction mixture which did not develop colour served as blank. Absorbance was recorded at 560 nm and one unit of enzyme activity was taken as the quantity of enzyme which reduced the absorbance reading of samples to 50 per cent in comparison with tubes lacking enzymes. A_{560} was plotted as a function of fresh matter equivalent of enzyme extract used in the reaction mixture. From the resultant graph fresh matter equivalents of enzyme extract corresponding to 50 per cent inhibition of the reaction was read and considered as one enzyme unit. SOD activity was expressed in U mg protein⁻¹.

3.5.7.1.2.3 Peroxidase

Guaiacol peroxidase activity (EC 1.11.1.7) was assayed in a reaction solution (3 ml) containing 10 mM phosphate buffer (KH₂PO₄/K₂HPO₄), pH 7.0, 10 mM H₂O₂, 20 mM guaiacol and 40 μ l of crude extract (Maehly and Chance, 1954). The reaction was started by adding H₂O₂ and guaiacol and the activity determined by monitoring the increase in absorbance at 470 nm in an UV-Vis Spectrophotometer and the activity expressed in U/mg protein.

3.5.7.1.3 Osmoprotectants

Plant samples collected at 30 and 60 DAS were analyzed for following set of osmoprotectants.

3.5.7.1.3.1 Proline

The proline content in the leaf tissue of wheat was estimated as per the protocol suggested by Bates *et al.* (1973). The leaf sample of 0.5 g was homogenized in 10 ml of three per cent sulphosalicylic acid. Samples were mixed, centrifuged at 18000 rpm for 10 min and filtered using Whatman No.1 filter paper. The filtered homogenate was used for the estimation of proline.

Two ml of the filtrate was taken in a test tube to which 2 ml each of ninhydrin reagent (2.5 g of ninhydrin was dissolved in 40 ml of 6 M orthophosphoric acid and 60 ml of glacial acetic acid) and glacial acetic acid were added. The test tubes containing the mixture were placed in boiling water bath for one h, then transferred to ice bath for cooling and 4 ml of toluene was added. The contents of the test tube were transferred to a separating funnel and shaken thoroughly and allowed for few minutes for the two layer to separate. The lower layer was discarded and upper toluene layer was taken into a test tube. The colour was read at 520 nm in an UV-Vis spectrophotometer. A blank was maintained with all the reactants except the leaf extract. Proline concentrations were calculated using proline standard curve values (0–50 mg/ml) in identical manner using the following formula and the contents were expressed as μ g per g fresh weight. Later, they were expressed as μ mol per g fresh weight by dividing with molecular weight of proline.

$$\text{Proline content} \quad (\mu\text{g/g fr. wt.}) = \frac{36.23 \times \text{OD}_{520} \times \text{Volume of the aliquot made (ml)}}{\text{Volume of the aliquot (ml)} \times \text{Weight of the sample (mg)}}$$

3.5.7.1.3.2 Quaternary amino compounds (QACs)

The total QACs content in the leaf tissue of wheat was estimated as per the method of Storey and Jones (1977). Leaf sample of 50 mg was weighed into 1.5 ml centrifuge tubes and treated with 60 μ l drying reagent (prepared by dissolving 5 g of silver ortho-phosphate in 100 ml of anhydrous di-sodium hydrogen phosphate) and 1 ml extraction solvent (methanol: acetonitrile, 1: 9) and incubated for 20 min at room temperature. Samples were centrifuged at 18,000 rpm for 10 min and supernatant was taken for estimation.

The acid potassium tri-iodide solution was prepared by dissolving 7.5 g I₂ and 10 g KI in 1 M HCl and filtered. Precisely, 200 µl of potassium tri-iodide reagent and 20 µl of magnesium oxide (10%) were added to a 400 µl of sample, mixed properly and incubated on ice for 90 with intermittent shaking followed by addition of 2 ml of ice cooled water and 20 ml of 1, 2-dichloroethane. Later, the above contents were transferred to separating funnel and refrigerated for 5 min at -20° C and the two layers mixed constantly for 5 min. The absorbance of the lower layer was measured at 365 nm in an UV-Vis spectrophotometer and the amount of glycine-betaine in each of the test isolate was calculated from the standard graph of glycine-betaine (50 ppm) and expressed as µmol per g fresh weight.

3.5.7.1.3.3 Polyols

Polyols content in wheat plants was estimated as per the protocol described by Lewis and Smith (1967).

Contents of the leaf samples (1.0 g) were extracted with 10 ml of distilled water by maseration in a pestle and mortar. The extract was filtered and diluted to 25 ml with distilled water. The filtrate was deproteinized by adding 2 ml of 0.3 N barium hydroxide and 2 ml of 5 per cent zinc sulphate and allowed to precipitate for 1 h. The precipitate was filtered and an aliquot of filtrate was used for estimating sugar alcohol (polyol) content. An aliquot of 0.9 ml in duplicate was taken in each cuvette with 2 ml of sodium acetate buffer (pH 4.5). The absorbance was adjusted to zero after keeping reference and sample cuvettes. Simultaneously, 0.1 ml of 10 mM sodium periodate and water were added to sample and reference cuvettes respectively. Change in absorbance was recorded at 229 nm and the contents were calculated using the following formula and expressed as µg per g dry weight.

$$\text{Polyols } (\mu\text{g/g dry wt.}) = \frac{(\text{OD}_{\text{ref}} - \text{OD}_s) \times 941.66 \times \text{volume of extract} \times \text{fresh to dry weight ratio}}{\text{Fresh weight} \times \text{Vol of aliquot}} \times 1000$$

3.5.7.1.3.4 Sugar

One g of plant sample was taken in a 30 ml centrifuge tube and treated with 5 ml of methanol: chloroform: water (60: 25: 15 v/v). The contents were incubated in water bath for 2 h at 60° C. After incubation, 5 ml of distilled water was added and the samples were centrifuged at 10000 rpm for 15 min. Supernatant was used for estimation of total sugars by phenol sulfuric acid method (Dubios *et al.*, 1956).

One ml of supernatant was taken in a test tube to which 1 ml of 5 per cent phenol and 5 ml of concentrated sulfuric acid was added and mixed thoroughly for 10 min. The tubes were incubated in a water bath at 25° C for 20 min. The yellow orange color developed was read at 490 nm against water blank in an UV-Vis spectrophotometer. Simultaneously, a standard curve was prepared using various concentrations of standard 100 ppm glucose solution. The amount of total sugar was calculated from the standard curve and expressed as mg per g dry weight.

3.5.7.1.4 Determination of sodium and potassium

Sodium and potassium contents in wheat plants was estimated at 30 and 60 DAS following the method of Chapman and Pratt (1961).

The oven dried plant sample was ground to a fine powder and 100 mg sample was digested with tri-acid mixture (HNO₃: H₂SO₄: HClO₄ in the proportion of 10:1:4 respectively). The digested sample was filtered through Whatman No.1 filter paper and the volume was made up to 100 ml with distilled water. Sodium and potassium content in the samples was estimated using flame emission spectrophotometer and expressed as percentage.

3.5.7.1.5 Total uptake of nutrients

Nitrogen content of whole plant at harvest was estimated by modified micro-kjeldhal's method as outlined by Jackson (1973) and expressed in percentage. Total phosphorus and potassium contents of whole plant at harvest were extracted by wet ashing method. P content was estimated by vanadomolybdate yellow colour method (Jackson, 1973) while K was determined by photometric method (Jackson, 1973). The total uptake of each nutrient was calculated using the following formula and expressed as kg/ha.

$$\text{Nutrient uptake (kg/ha)} = \frac{\text{Nutrient concentration (\%)} \times \text{Biomass (kg/ha)}}{100}$$

3.5.7.2 Microbiological parameters

Soil samples at 0-15 cm depth were drawn from the experimental site at regular intervals of 30, 45, 60 DAS and at harvest. A total of six samples were drawn for each set of treatments and were mixed and homogenized by compositing. The composite soil was sieved to pass through a 2 mm mesh immediately and stored at 4° C until analysis was done for the estimation of microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) and analyzed for the activities of dehydrogenase, alkaline phosphatase and urease.

3.5.7.2.1 Dehydrogenase

Composite soil samples were analyzed for dehydrogenase activity as per the colorimetric method of Tabatabai (1982). Soil samples (5 g) were treated with 3 per cent 2,3,5 tri-phenyl tetrazolium chloride (TTC) (1 ml) and 1 per cent glucose (1 ml) and 2.5 ml of distilled water and incubated for 24 h. Later, methanol (10 ml) was added and incubated for 5 h. After incubation at room temperature, the contents were filtered through Whatman No.42 filter paper and the colour (reddish) was read at 485 nm against a methanol blank in an UV-Vis spectrophotometer. The concentration of dehydrogenase in the sample was obtained from the standard graph using triphenyl farmazane (TPF) and expressed as µg TPF/g soil/day.

3.5.7.2.2 Urease

Similarly, urease activity was measured as per the method of Tabatabai and Bremner (1972). Initially, soil samples (10 g) were treated with toluene (1.5 ml) and incubated for 15 min. Later, 10 per cent urea solution (10 ml) and phosphate buffer (10 ml) were added, mixed thoroughly, stoppered and incubated at 30° C for 24 h. After incubation, 1N KCl (15 ml) was added and the final volume was made up to 100 ml with distilled water and the contents were filtered through Whatman No. 42 filter paper. The amount of ammonia present in the filtrate was determined by Nesslerization. It involves addition of 10 per cent sodium tartarate (2 ml) and 0.5 ml Nessler's reagent to 1ml filtrate and the volume was made up to 20 ml and incubated for 30 min. Colour (yellow) developed was read at 410 nm against blank (without urea solution). The concentration of urease in the sample was obtained from the standard graph using diammonium sulphate and expressed as µg NH₄-N/g soil/day.

3.5.7.2.3 Phosphatase

Composite samples were analyzed for alkaline phosphatase activity as per the method of Tabatabai and Bremner (1969). One g of soil sample was taken in Erlenmeyer flask to which 0.25 ml toluene, 4 ml universal buffer pH 7.5 and 1 ml of 10 mM p- nitrophenyl phosphate (PNP) were added. The flasks were stoppered and incubated at room temperature for 1h. Later, 1 ml of 0.5 M CaCl₂ and 4 ml of 0.5 M NaOH were added and the contents filtered through Whatman No.42 filter paper. Final volume was made up to 50 ml with distilled water and the intensity of colour was read at 420 nm. Phosphatase activity was expressed as µg pNP/g soil/h.

3.5.7.2.4 Microbial biomass carbon (MBC) and nitrogen (MBN)

Microbial biomass carbon and nitrogen were determined by the chloroform fumigation incubation method of Brookes *et al.* (1985) and Vance *et al.* (1987). In this method, 50 g soil sample was fumigated with chloroform. After fumigation, the samples were inoculated with 1 g of respective un-fumigated moist soil and incubated in the presence of NaOH solution. The CO₂ evolved was trapped in NaOH solution and measured by titrating the NaOH solution against standard HCl. Similarly, CO₂ evolution was also measured in un-fumigated soils. Microbial biomass N was determined by incubating the fumigated and non-fumigated samples in a desiccator at 25° C for 10 days in the dark. After incubation, NH₄⁺-N was extracted with 75 ml of 2 M KCl (soil: KCl solution ratio=1:5) after shaking for 30 min, centrifuging at 1480 rpm for 10 min and filtering the resulting supernatant through a Whatman No. 42 filter paper. The NH₄⁺-N thus extracted was determined by steam distillation (Keeney and Nelson, 1982). Microbial biomass C and N were calculated as follows and expressed as mg/kg soil.

3.5.7.2.4.1 Calculation of biomass C

The amount of CO₂-C produced from fumigated and un-fumigated samples were used to calculate soil microbial biomass C (Jenkinson and Ladd, 1981) using the following expression:

$$MBC = (F_c - U_{fc}) / K_c$$

Where,

F_c = CO₂ flush from fumigated soil samples

U_{fc} = CO₂ flush from un-fumigated soil samples

K_c = Constant value *i.e.* 0.45

3.5.7.2.4.2 Calculations of biomass N

Microbial biomass N was calculated from the amount of mineral N produced in the fumigated and un-fumigated soil samples (Jenkinson, 1988) using the following equation:

$$MBN = (F_n - U_{fn}) / K_n$$

Where,

F_n = flush of NH₄⁺-N in fumigated sample

U_{fn} = NH₄⁺-N produced in the un-fumigated soil.

K_n = Constant value *i.e.*, 0.54

3.6 Genes encoding tolerance/ resistance to salt

Studies were initiated to identify the presence of genes encoding for salt tolerance in agriculturally important microorganisms. A set of four biosynthetic genes *viz.*, *proBA*, *ectABC*, *otsAB* and *gbsTIBA* responsible for biosynthesis of proline, ectoine, trehalose and glycine-betaine respectively, were considered. A total of 21 isolates comprising 6 each of free-living N₂ fixers, PSB and FP and 3 associative N₂ fixers, tolerating high NaCl concentration were screened for the above genes. Following set of degenerate primer pairs were used to identify the presence of genes in the test isolates.

Gene	Primer			Expected amplicon (bp)	Reference
	Name	Sequence	T _m (°C)		
<i>proBA</i>	Pro1 F	5'- gctyctaigaacggraggagaaa -3'	50.5	1100	Chen <i>et al.</i> (2007)
	Pro2 R	5'- cgagcrtcatcigtciaatcytccca -3'	51.2		
<i>ectABC</i>	Ect1 F	5'- ccctiaaytatyggicayaay -3'	56.9	750	Reshetnikov <i>et al.</i> (2006) Zhao <i>et al.</i> (2006)
	Ect2 R	5'- cgraagtgcrrtrtgytcncc-3'	55.3		
<i>otsAB</i>	Ots1 F	5'- gggcatgmgtatgcggaaagcgt -3'	65.3	650	Tzvetkov <i>et al.</i> (2003)
	Ots2 R	5'- ggaagmttrccraaatyacgc -3'	66.2		
<i>gbsTIBA</i>	Gbs1 F	5'- cggkgtytgygsvcagatca -3'	59.6	750	Gu <i>et al.</i> (2008)
	Gbs2 R	5'- cmggwccraadaytcttct -3'	55.8		

3.6.1 Standardization of primer annealing temperature

Annealing temperature for each set of primer pair was standardized to amplify a single sharp and specific amplicon by varying temperature. PCR was performed for each set of primers using mastercycler gradient (Eppendorf) to optimize annealing temperature. Based on this exercise, the standardized annealing temperature of 50.4, 57.6, 66.3 and 60.7° C was used for amplification of *proBA*, *ectABC*, *otsAB* and *gbsTIBA* genes respectively for further studies.

3.6.2 PCR based amplification

Twenty μ l PCR mixture containing 100 ng template, 3 U *Taq* polymerase (Bangalore genei, Bangalore, India), 1.0 mM each of dATP, dCTP, dGTP and dTTP, 10x *Taq* buffer B, 25 mM $MgCl_2$ and 5 pM of gene specific primers were used for amplification. The reactions were carried out with an initial 5 min denaturation step, followed by 35 cycles of 94° C for 1 min, gene specific annealing temperature for 30 sec and 72° C for 2 min, with a final extension at 72° C for 5 min.

3.6.3 Gel-electrophoresis

The size and purity of the reaction products were checked by electrophoresis. About 20 μ l of the reaction mixture from each tube with 3 μ l of loading dye were loaded onto one per cent agarose gel along side λ DNA *EcoRI/HindIII* double digest as molecular weight marker. Electrophoresis was done at 50 V for initial 30 min and then at 70 V for 1 hr. The buffer used was 1x TAE at pH 8.0. The DNA bands in the gel were visualized on a UV-transilluminator and documented using a gel documentation system (Syngene Bioimaging Private Ltd, Gurgaon, India).

3.7 Statistical analysis

3.7.1 Phylogenetic analysis based on biochemical and physiological characteristics

The results of the biochemical and physiological tests were used to construct a binary data matrix for each group of salt-tolerant AIMS. Each character was scored as 1 or 0 based on the result of each test, positive or negative respectively. All the strains characterized by the same combination of haplotypes obtained with a set of biochemical characteristics were grouped together, defining a phylotype. Genetic distance between phylotypes was calculated using the Nei and Li (Nei and Li, 1979) coefficient and the resulting distance matrix was used for the un-weighted paired group arithmetic mean (UPGMA) cluster analysis (Sokal and Sneath, 1963) and the Neighbor-joining mid-point analysis (Saitou and Nei, 1987) using TREECON for Windows (Version 1.2) (Van Der Peer and DeWachter, 1994) and NTSYS-pc version 2.02e (Rohlf, 1993), respectively. The consistency of each node was estimated by bootstrapping (Felsenstein, 1985) using 1000 pseudo-replications.

3.7.2 Analysis of 16S rRNA sequences

The partial rRNA gene sequences of the isolates were preliminarily compared with those present in the basic local alignment search tool (BLAST) search (Altschul *et al.*, 1997) and then aligned using the ClustalW version 1.81 (Higgins *et al.*, 1992) available at the European Bioinformatic Institute website (<http://www.ebi.ac.uk/clustalw/>) with the sequences retrieved from GenBank database (Benson *et al.*, 2003) available at the NCBI website (<http://www.ncbi.nlm.nih.gov/>). From the aligned sequences, UPGMA dendrograms were constructed with the molecular evolutionary genetics analysis (MEGA) software version 5.0 (Tamura *et al.*, 2011) after calculating *p*-distance matrix for the 16S rRNA gene sequences. The robustness of the inferred trees was evaluated by 1000 bootstrap resamplings.

3.7.3 Laboratory and field data analysis

Data obtained from the laboratory and field experimentation were statistically analyzed using a factorial completely randomized design (CRD) and randomized complete block design (RCBD) respectively, as described by Yates (1937). The level of significance used in 'F' and 'T' test was $p=0.01$ and $p=0.05$ for laboratory and field experiments respectively. Critical difference (CD) values were calculated wherever the 'F' test values were significant.

4. EXPERIMENTAL RESULTS

Salt-tolerant strains of agriculturally important microorganisms (AIMs) isolated from the stress ecosystems of Karnataka and maintained in the culture bank of the Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad were characterized for their morphological, physiological and biochemical properties and identified using 16S rRNA sequencing. Changes in the functional properties as well as osmoadaptation of salt-tolerant isolates in response to different concentrations of sodium chloride were studied *in vitro*. A total of nineteen salt tolerant isolates having multiple functions with high salt tolerance were screened for their growth promotional activity in wheat under controlled condition. Potential isolates and their consortia were tested for their field performance and alleviation of salt stress in wheat under graded soil salinity at ARS, Gangavati, UAS, Raichur, Karnataka. Twenty one isolates tolerating higher salt concentration were also analyzed for the presence of genes encoding salt tolerance. The results obtained on these aspects are presented below.

4.1 Characterization and identification of salt-tolerant AIMs

4.1.1 Morphological, physiological and biochemical characterization

Observations on morphological, physiological and biochemical characteristics of each isolate were recorded and presented in Tables 5 to 8. The results of the biochemical and physiological tests were used to construct a binary data matrix for each group of salt-tolerant AIMs. Each character was scored as 1 or 0 based on the result of each test, positive or negative respectively. The un-weighted paired group arithmetic mean (UPGMA) dendrogram was constructed using NTSYS pc version 2.02e software (Fig. 3 and 4).

4.1.1.1 Associative nitrogen fixers (ANF)

All the isolates were initially characterized for gram reaction and subsurface pellicle formation in the semisolid N-free malate medium (NFM). It was found that, all of them were gram negative with rod shaped cells and positive for subsurface pellicle formation (Table 5). Based on above observations, they were assigned to category I -Group 4 (Gram negative, aerobic/ microaerophilic rods and cocci) of the Bergey's Manual of Determinative Bacteriology (BMDB) and characterized for various group specific tests as per the manual and identified tentatively up to species level.

Among several tests performed, all isolates were positive for oxidase and negative for cellulose hydrolysis but vary in terms of their colony size, growth at pH 6.0, urease activity and C source utilization. Out of 36 isolates 19, 14 and 3 of them were found to form small, medium and large sized pale green coloured colonies on N-free malate agar medium respectively. Twenty two of them were found to grow at pH 6.0 and 19 were positive for urease activity. Among the isolates tested, 11 were found to utilize glucose and sucrose while 3 were able to utilize inositol as C source. While 12 of them were tentatively identified as *Azospirillum brasilense*, 16 as *A. irakense* and 8 as *A. halopraeferens*.

The dendrogram depicted in Fig. 3a is structured in two main clusters with seven sub-clusters, diverged at a similarity coefficient of 0.64. Cluster I again diverged into 3 sub clusters covering 9 ANF isolates with a similarity value of 0.78 and cluster II diverged into 2 sub and four sub-sub clusters covering 27 ANF isolates with a similarity value of 0.82.

4.1.1.2 Free-living nitrogen fixers (FNF)

All 43 isolates were characterized for gram reaction and cell shape. It was found that, all of them were gram negative and 18 of them had rod shaped cells and 25 isolates with oval shaped cells (Table 6). Based on above observations, they were assigned to category I-Group 4 of BMDB and characterized for various group specific tests as per the manual and identified tentatively up to species level.

All test isolates were positive for cyst formation and catalase activity but negative for acid production from glucose and growth at 60° C. The results of colony size, shape and colour of the isolates indicated that 10, 15 and 18 of them were found to form small, medium and large sized colonies respectively. Twenty five isolates had irregular colony shape and remaining isolates were round. Observations on the colony colour indicated that 17 isolates had pale white and cream coloured colonies while 9 of them formed watery colonies.

Table 5. Biochemical and physiological characteristics of salt tolerant associative N₂ fixing isolates and their tentative identity

Isolate	Morphological characters		Physiological and biochemical characters*										Tentative identity
	Colony morphology	Gram reaction and cell shape	1	2	3	4	5	6	7	C source utilization			
										8	9	10	
ASANFII S22E	Medium, pale green	G -ve rod	+	-	+	+	+	+	-	-	-	-	<i>Azospirillum brasilense</i>
ASANFII S26E	Medium, pale green	G -ve rod	+	-	+	+	+	+	-	-	-	-	<i>A. halopraeferens</i>
ASANFIV S40E	Small, pale green	G -ve rod	+	+	+	+	+	-	-	+	+	-	<i>A. irakense</i>
ASANFIV S61E	Small, pale green	G -ve rod	+	+	+	+	+	-	-	+	+	-	<i>A. irakense</i>
ASANFIV S63E	Medium, pale green	G -ve rod	+	-	+	+	+	+	-	-	-	-	<i>A. halopraeferens</i>
ASANFIV S78E	Large, pale green	G -ve rod	+	+	+	+	+	-	-	-	-	-	<i>A. brasilense</i>
ASANFIV S83E	Small, pale green	G -ve rod	+	+	+	+	+	+	-	+	+	+	<i>A. irakense</i>
ASANFIV Dr8E	Medium, pale green	G -ve rod	+	+	+	+	+	+	-	-	-	-	<i>A. brasilense</i>
ASANFVDr18bE	Large, pale green	G -ve rod	+	+	+	+	+	-	-	-	-	-	<i>A. brasilense</i>
ASANFV WL12E	Medium, pale green	G -ve rod	+	-	+	+	+	+	-	-	-	-	<i>A. halopraeferens</i>
ASANFIX S121E	Small, pale green	G -ve rod	+	+	+	+	+	-	-	-	-	-	<i>A. brasilense</i>
ASAZOIX S125E	Medium, pale green	G -ve rod	+	+	+	+	+	+	-	-	-	-	<i>A. brasilense</i>
ASANFIX S132E	Small, pale green	G -ve rod	+	+	+	+	+	-	-	-	-	-	<i>A. brasilense</i>
ASANFX S135E	Small, pale green	G -ve rod	+	+	+	+	+	+	-	+	+	+	<i>A. irakense</i>
ASANFX S137E	Small, pale green	G -ve rod	+	-	+	+	+	+	-	-	-	-	<i>A. halopraeferens</i>
ASANFX S138E	Medium, pale green	G -ve rod	+	-	+	+	+	+	-	+	+	-	<i>A. irakense</i>
ASANFX S141E	Small, pale green	G -ve rod	+	+	+	+	+	-	-	-	-	-	<i>A. irakense</i>
ASANFX S144E	Small, pale green	G -ve rod	+	-	+	+	+	+	-	+	+	-	<i>A. irakense</i>
ASANFX S145E	Medium, pale green	G -ve rod	+	-	+	+	+	+	-	-	-	-	<i>A. irakense</i>

Contd....

(Table 5 contd....)

Isolate	Morphological characters		Physiological and biochemical characters*										Tentative identity
	Colony morphology	Gram reaction and cell shape	1	2	3	4	5	6	7	C source utilization			
										8	9	10	
ASANFX S148E	Medium, pale green	G -ve rod	+	+	+	+	+	+	-	-	-	-	<i>A. brasilense</i>
ASANFX S149E	Small, pale green	G -ve rod	+	-	+	+	+	-	-	-	-	-	<i>A. halopraeferens</i>
ASANFX S150E	Medium, pale green	G -ve rod	+	+	+	+	+	-	-	-	-	-	<i>A. brasilense</i>
ASANFX S154E	Small, pale green	G -ve rod	+	+	+	+	+	-	-	+	+	-	<i>A. irakense</i>
ASANFX Dr29aE	Medium, pale green	G -ve rod	+	+	+	+	+	-	-	-	-	-	<i>A. irakense</i>
ASANFX Dr31E	Small, pale green	G -ve rod	+	-	+	+	+	+	-	-	-	-	<i>A. halopraeferens</i>
ASANFX WL13E	Medium, pale green	G -ve rod	+	-	+	+	+	-	-	-	-	-	<i>A. halopraeferens</i>
ASANFX WL15E	Medium, pale green	G -ve rod	+	+	+	+	+	+	-	+	+	+	<i>A. irakense</i>
ASANFXI S158E	Small, pale green	G -ve rod	+	-	+	+	+	+	-	-	-	-	<i>A. halopraeferens</i>
ASANFXI S160E	Small, pale green	G -ve rod	+	+	+	+	+	-	-	+	+	-	<i>A. irakense</i>
ASANFXI S167E	Small, pale green	G -ve rod	+	+	+	+	+	+	-	-	-	-	<i>A. brasilense</i>
ASANFXI S170E	Large, pale green	G -ve rod	+	+	+	+	+	-	-	-	-	-	<i>A. brasilense</i>
ASANFXI S173E	Medium, pale green	G -ve rod	+	-	+	+	+	+	-	-	-	-	<i>A. irakense</i>
ASANFXI S182E	Small, pale green	G -ve rod	+	-	+	+	+	+	-	+	+	-	<i>A. irakense</i>
ASANFXI S187E	Small, pale green	G -ve rod	+	+	+	+	+	-	-	-	-	-	<i>A. irakense</i>
ASANFXI WL17E	Small, pale green	G -ve rod	+	+	+	+	+	-	-	-	-	-	<i>A. brasilense</i>
ASANFXI WL19E	Small, pale green	G -ve rod	+	+	+	+	+	-	-	+	+	-	<i>A. irakense</i>

+ : POSITIVE FOR THE TEST/ GROWTH

- : NEGATIVE FOR THE TEST/ NO GROWTH

G-VE: GRAM NEGATIVE

*** Physiological and biochemical tests**

1. Motility (Screw cap)

2. Growth at pH 6.0

3. Growth in the presence of 3% NaCl

4. Subsurface pellicle

5. Oxidase

6. Urease

7. Cellulose hydrolysis

8. Glucose

9. Sucrose

10. Inositol

Table 6. Biochemical and physiological characteristics of salt tolerant free-living N₂ fixing isolates and their tentative identity

Isolate code no.	Morphological characters		Physiological and biochemical characters*										Tentative identity
	Colony Morphology	Gram reaction and cell shape	1	2	3	4	5	6	7	C source utilization			
										8	9	10	
ASFNFI S17(2)S	Large, pale white and irregular	G -ve, rod	+	+	-	-	+	-	+	+	+	+	<i>Azotobacter vinelandii</i>
ASFNFI S19(2)S	Small, pale white and regular	G -ve, oval	+	-	-	-	+	-	-	+	-	+	<i>A. chroococcum</i>
ASFNFI Dr4(1)	Medium, creamy and irregular	G -ve, oval	+	-	-	+	+	-	-	+	-	+	<i>A. chroococcum</i>
ASFNFI Dr2(5)	Medium, pale white and regular	G -ve, oval	+	-	-	+	+	-	-	-	+	+	<i>A. chroococcum</i>
ASFNFI Dr3(3)	Small, pale white and regular	G -ve, rod	+	-	-	+	+	-	-	+	-	-	<i>A. nigricans</i>
ASFNFII S22S	Large, pale white and irregular	G -ve, oval	+	-	-	+	+	-	+	+	-	+	<i>A. chroococcum</i>
ASFNFII S22R	Large, watery and irregular	G -ve, rod	+	-	-	-	+	-	-	-	-	-	<i>A. paspali</i>
ASFNFIII S32S	Large, creamy and irregular	G -ve, rod	+	+	-	+	+	-	-	+	+	+	<i>A. vinelandii</i>
ASFNFIII S31R	Small, watery and regular	G -ve, rod	+	-	-	+	+	-	-	+	-	-	<i>A. nigricans</i>
ASFNFIII S33E	Large, creamy and irregular	G -ve, rod	+	+	-	-	+	-	+	+	+	+	<i>A. vinelandii</i>
ASFNFIV S36(1R)	Medium, colourless and regular	G -ve, oval	+	-	-	+	+	-	+	+	-	+	<i>A. chroococcum</i>
ASFNFIV S37R	Medium, watery and irregular	G -ve, oval	+	-	-	-	+	-	-	+	+	+	<i>A. chroococcum</i>
ASFNFIV S40(1)R	Medium, creamy and irregular	G -ve, oval	+	-	-	+	+	-	-	-	-	+	<i>A. chroococcum</i>
ASFNFIV S40(2)R	Large, creamy and regular	G -ve, rod	+	-	-	+	+	-	-	-	-	-	<i>A. paspali</i>
ASFNFIV S43(2)R	Small, creamy and regular	G -ve, rod	+	-	-	+	+	-	-	+	-	-	<i>A. nigricans</i>
ASFNFIV S44R	Large, creamy and irregular	G -ve, rod	+	+	-	-	+	-	+	+	+	+	<i>A. vinelandii</i>
ASFNFIV S45(1)R	Medium, watery and regular	G -ve, rod	+	-	-	+	+	-	-	-	+	-	<i>A. paspali</i>
ASFNFIV S57(2)R	Large, creamy and regular	G -ve, rod	+	-	-	+	+	-	-	-	-	-	<i>A. paspali</i>
ASFNFIV S60(1)S	Large, pale white and irregular	G -ve, rod	+	+	-	-	+	-	+	+	+	+	<i>A. vinelandii</i>
ASFNFIV S61(1)S	Large, creamy and irregular	G -ve, rod	+	-	-	+	+	-	-	-	-	-	<i>A. paspali</i>
ASFNFIV S62E	Large, pale white and irregular	G -ve, rod	+	+	-	-	+	-	+	+	+	+	<i>A. vinelandii</i>
ASFNFIV S63(1)R	Medium, creamy and irregular	G -ve, oval	+	-	-	-	+	-	-	+	-	+	<i>A. chroococcum</i>
ASFNFIV S66R	Large, creamy and irregular	G -ve, rod	+	+	-	-	+	-	+	+	+	+	<i>A. vinelandii</i>

Contd....

(Table 6 contd....)

Isolate code no.	Morphological characters		Physiological and biochemical characters*										Tentative identity
	Colony Morphology	Gram reaction and cell shape	1	2	3	4	5	6	7	C source utilization			
										8	9	10	
ASFNFIV S75(1)R	Medium, pale white and irregular	G -ve, rod	+	-	-	+	+	-	-	-	-	-	<i>A. paspali</i>
ASFNFIV S75(2)R	Medium, creamy and irregular	G -ve, rod	+	+	-	+	+	-	-	+	+	+	<i>A. vinelandii</i>
ASFNFIV S75E	Medium, pale white and irregular	G -ve, oval	+	-	-	+	+	-	-	+	-	+	<i>A. chroococcum</i>
ASFNFIV S80(1)R	Medium, watery and regular	G -ve, rod	+	-	-	+	+	-	-	-	-	-	<i>A. paspali</i>
ASFNFIV Dr8R	Small, watery and regular	G -ve, oval	+	-	-	-	+	-	-	+	+	+	<i>A. chroococcum</i>
ASFNFIV WL3S	Small, watery and regular	G -ve, oval	+	-	-	-	+	-	-	+	-	+	<i>A. chroococcum</i>
ASFNFIV WL4(1)R	Small, pale white and regular	G -ve, oval	+	-	-	+	+	-	-	-	+	+	<i>A. chroococcum</i>
ASFNFIV WL5(1)R	Medium, pale white and irregular	G -ve, oval	+	-	-	+	+	-	+	+	-	+	<i>A. chroococcum</i>
ASFNFIV WL5(2)R	Medium, creamy and irregular	G -ve, rod	+	-	-	-	+	-	-	-	-	-	<i>A. paspali</i>
ASFNFIV WL6R	Large, watery and regular	G -ve, oval	+	-	-	-	+	-	-	+	+	+	<i>A. chroococcum</i>
ASFNFIV WL7(2)R	Medium, watery and irregular	G -ve, oval	+	-	-	+	+	-	+	+	-	+	<i>A. chroococcum</i>
ASFNFV S85R	Large, pale white and irregular	G -ve, oval	+	-	-	+	+	-	+	+	-	+	<i>A. chroococcum</i>
ASFNFI S127R	Large, creamy and irregular	G -ve, rod	+	+	-	-	+	-	+	+	+	+	<i>A. vinelandii</i>
ASFNFX Dr31R	Medium, creamy and regular	G -ve, rod	+	-	-	+	+	-	-	+	-	-	<i>A. nigricans</i>
ASFNFXI S161E	Small, pale white and regular	G -ve, oval	+	-	-	+	+	-	+	+	-	+	<i>A. chroococcum</i>
ASFNFXI S174E	Large, creamy and irregular	G -ve, rod	+	+	-	-	+	-	+	+	+	+	<i>A. vinelandii</i>
ASFNFXI WL16E	Small, pale white and regular	G -ve, rod	+	-	-	-	+	-	-	-	-	-	<i>A. paspali</i>
ASFNFXII S178E	Large, pale white and irregular	G -ve, rod	+	+	-	-	+	-	+	+	+	+	<i>A. vinelandii</i>
ASFNFXII S190S	Large, pale white and irregular	G -ve, oval	+	-	-	-	+	-	-	+	+	+	<i>A. chroococcum</i>
ASFNFXII S190E	Small, pale white and regular	G -ve, rod	+	-	-	-	+	-	-	-	-	-	<i>A. paspali</i>

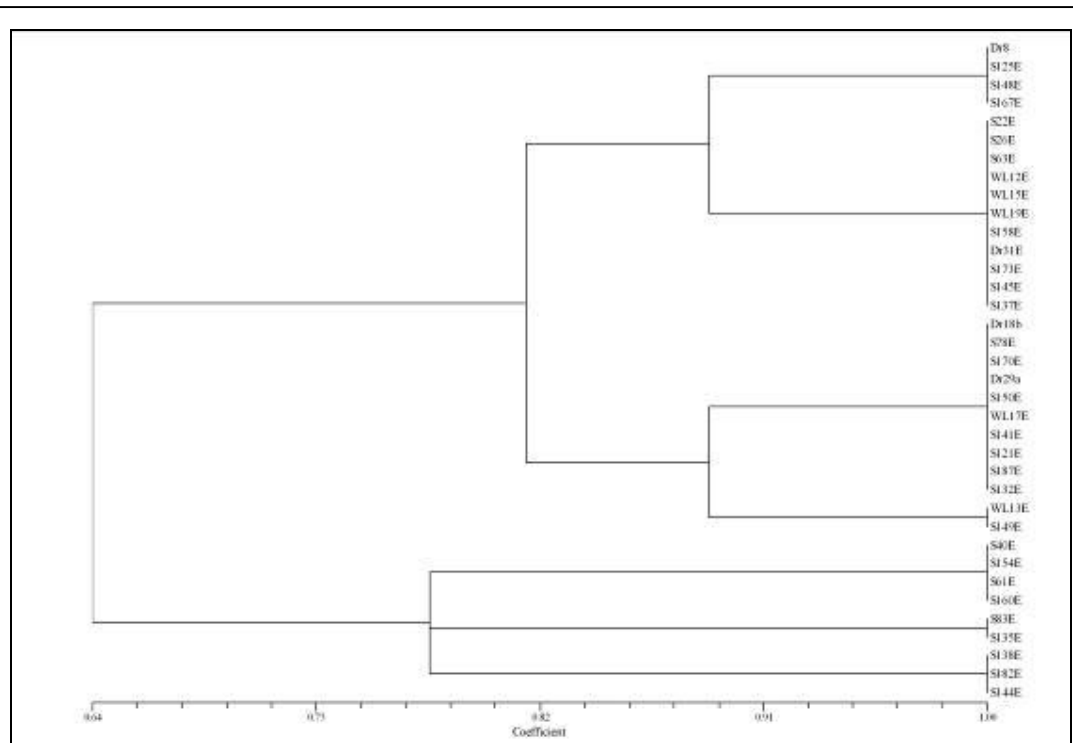
+ : POSITIVE FOR THE TEST/ GROWTH

- : NEGATIVE FOR THE TEST/ NO GROWTH

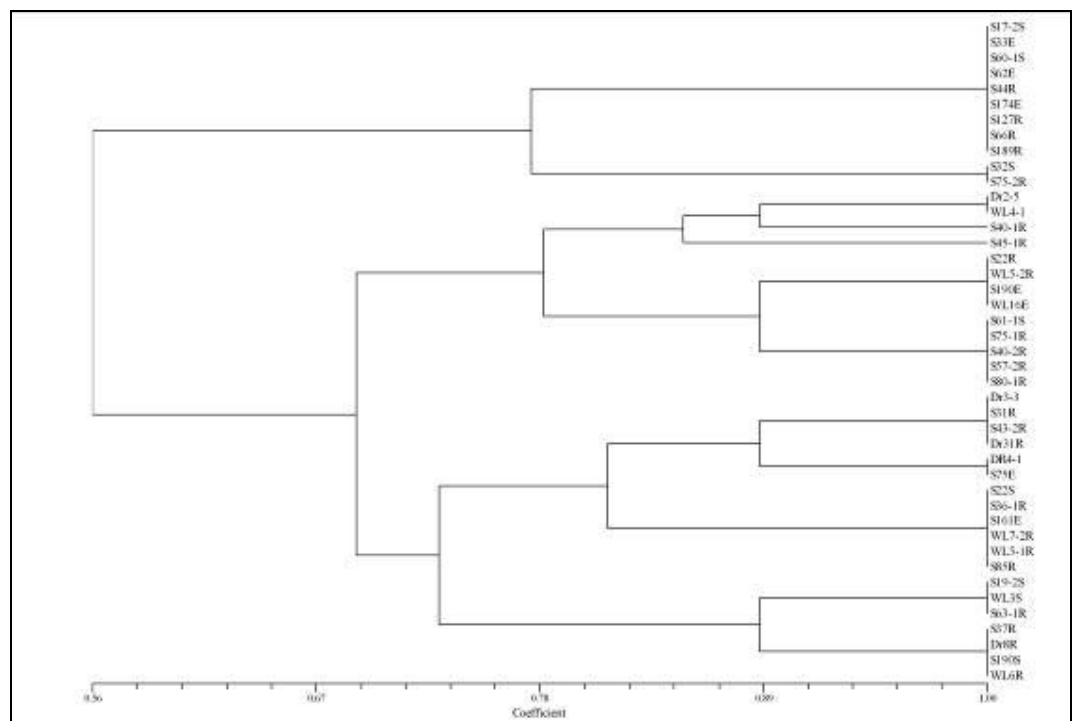
G-VE: GRAM NEGATIVE

*** Physiological and biochemical tests**

- | | |
|------------------------|----------------------|
| 1. Cyst formation | 6. Acid from glucose |
| 2. Fluorescent pigment | 7. Indole production |
| 3. Growth at 60°C | 8. Rhamnose |
| 4. Oxidase | 9. Meso-inositol |
| 5. Catalase | 10. Mannitol |



a. Salt-tolerant associative dinitrogen fixers (36)



b. Salt-tolerant free-living N_2 fixers (43)

Fig. 3. UPGMA dendrogram showing the similarity values among salt-tolerant AIMS

Among the isolates tested, 11, 23 and 15 were found positive for fluorescent pigment production, oxidase activity and indole production respectively. All the isolates showed variation in carbon source utilization. Out of 43 isolates 30, 18 and 29 isolates were able to utilize rhamnose, inositol and mannitol as C source respectively. Fifteen of them utilized all 3 carbon sources, 13 utilized 2 carbon sources and the remaining 15 isolates were able to utilize only one of the 3 carbon sources studied.

Out of 43 isolates 18, 11, 10 and 4 isolates were tentatively identified as *Azotobacter chroococcum*, *A. vinelandii*, *A. paspali* and *A. nigricans* respectively.

The dendrogram constructed for FNF isolates is depicted in Fig. 3b. All 43 isolates found to diverge into 2 main clusters with a similarity coefficient of 0.56 covering 6 sub-clusters. Cluster I included 32 isolates with a similarity value of 0.70 and cluster II included 11 isolates with a similarity value of 0.78. All the isolates were grouped into 9 sub-sub clusters with a similarity coefficient of 1.0.

4.1.1.3 Phosphate solubilizing bacteria (PSB)

All the 55 PSB isolates forming zone of solubilization on Pikovskaya's agar were subjected to morphological, biochemical and physiological characterization. It was observed that, 33 isolates were found to be gram-negative and 22 to be gram-positive (Table 7). Five out of 55 isolates were found to have cocci shaped cells and remaining isolates had rod shaped cells. Based on these results, 28, 5, 5 and 17 out of 55 isolates were assigned to category I-group 4, category I-group 5 (facultative anaerobic gram negative rods), category II-group 17 (gram positive cocci) and category II-group 18 (endospore forming gram positive rods and cocci) of BMDB respectively, for further characterization and tentative identification up to genera.

Variation in pigmentation, colony size and shape was observed among the isolates. All the isolates were positive for catalase activity. Out of 55 isolates 28, 2 and 24 of them were positive for lipid, starch and casein hydrolysis while 30, 5, 3 and 33 isolates positive for oxidase, M-R, V-P and urease tests respectively. All isolates except S150R and S144(1)S were found to produce acid from glucose. Similarly, all isolates except S129R, S138(1)S and Dr27(1)S were negative for denitrification. Among the isolates 7, 13 and 31 were found positive for indole, hydrogen sulfide and acid (from glucose) production. While 6 isolates showed gelatin liquefaction, 6 were negative for citrate utilization test.

Based on these tests, 28 isolates belonging to category I-group 4 of BMDB were tentatively identified up to genera. Out of 28 isolates, 14 of them were identified as *Pseudomonas*; 4 as *Aminobacter*; 2 each as *Glucanobacter* and *Acetobacter* and one each as *Alcaligenes*, *Flavimonas*, *Rhizomonas*, *Rhizobacter*, *Xanthobacter* and *Xanthomonas*. Similarly, out of 5 isolates belonging to category I-group 5 of BMDB, 3 were tentatively identified as *Escherichia* and 2 as *Enterobacter*. Five isolates belonging to category II-group 17 of BMDB were tentatively identified as *Stapylococcus* while 17 isolates belonging to category II-group 18 of BMDB were identified tentatively as *Bacillus*.

All the isolates were found to diverge with a similarity coefficient of 0.62 into 2 major clusters (Fig. 4a). Out of 55 PSB isolates, ASPSBIX 129R alone represented the cluster I while 54 grouped into cluster II. Wide variation among the isolates belonging to cluster II was observed. All the 54 isolates of the cluster II were again distributed into 32 sub-sub cluster with a similarity of 0.92.

4.1.1.4 Fluorescent pseudomonads (FP)

Based on the gram reaction and cell shape, all 61 FP isolates were assigned to category I-group 4 of BMDB. All of them were gram-negative with rod shaped cells (Table 8). Out of 61, 50 isolates produced bright green coloured colonies while remaining 11 showed golden yellow coloured colonies. The isolates displayed wide variation in the intensity of fluorescence. Out of 61 isolates, 31, 23 and 7 showed high, medium and light fluorescence on King's B agar medium respectively. Twenty seven isolates showed growth at 41° C whereas 34 isolates were able to grow at 4° C.

Table 7. Biochemical and physiological characteristics of salt tolerant phosphate solubilizing bacteria (PSB) and their tentative identity

Isolate	Morphological characters		Physiological and biochemical characters*															Tentative identity
	Colony Morphology	Gram reaction and cell shape	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
ASPSBIX S122R	Medium, creamy, irregular and opaque	G +ve, rod	+	-	+	-	+	-	-	+	+	-	-	-	-	+	+	<i>Bacillus</i>
ASPSBIX S122(1)R	Medium, light green, regular and opaque	G -ve, rod	+	-	-	-	+	-	-	+	+	-	-	+	-	+	+	<i>Pseudomonas</i>
ASPSBIX S124(1)R	Medium, yellow, regular and opaque	G +ve, cocci	-	-	-	-	+	-	-	+	+	-	-	+	-	+	-	<i>Staphylococcus</i>
ASPSBIX S124(2)S	Small, white, regular and flat	G -ve, rod	-	-	-	+	+	-	-	-	+	-	-	-	-	+	+	<i>Alcaligenes</i>
ASPSBIX S124E	Small, pale white, round and flat	G +ve, rod	-	-	-	-	+	-	+	+	+	-	-	-	-	+	-	<i>Bacillus</i>
ASPSBIX S125(6)S	Small, yellow, irregular and flat	G -ve, rod	-	-	-	-	+	+	-	-	+	-	-	-	-	+	+	<i>Flavimonas</i>
ASPSBIX S125R	Medium, pale white, irregular and flat	G -ve, rod	-	-	-	-	+	+	-	-	+	-	-	-	-	+	+	<i>Enterobacter</i>
ASPSBIX S125E	Large, light green, regular and opaque	G -ve, rod	+	-	+	+	+	-	-	-	+	+	-	-	-	+	-	<i>Pseudomonas</i>
ASPSBIX S129R	Medium, white, round and flat	G +ve, rod	+	-	+	+	+	-	-	+	+	+	+	+	-	+	+	<i>Bacillus</i>
ASPSBIX S129(1)R	Medium, creamy, irregular and opaque	G -ve, rod	+	-	+	-	+	-	-	-	+	-	-	-	-	+	-	<i>Aminobacter</i>
ASPSBIX S132(2)S	Medium, light yellow, regular and opaque	G -ve, rod	+	-	-	-	+	-	-	+	+	-	-	-	-	+	+	<i>Gluconobacter</i>
ASPSBX S135S	Medium, creamy, regular and flat	G -ve, rod	-	-	-	-	+	-	-	-	+	-	-	-	-	+	+	<i>Acetobacter</i>
ASPSBX S135R	Medium, creamy, irregular and flat	G -ve, rod	+	-	+	+	+	-	-	+	+	-	-	-	+	+	+	<i>Enterobacter</i>
ASPSBX S137(1)S	Small, light green, regular and opaque	G -ve, rod	+	-	+	-	+	-	-	-	+	-	-	-	-	+	-	<i>Pseudomonas</i>
ASPSBX S138(1)S	Big, creamy, regular and opaque	G -ve, rod	-	-	-	-	+	-	-	+	+	-	-	-	+	+	+	<i>Gluconobacter</i>
ASPSBX S138(1)S	Medium, greenish, regular and opaque	G -ve, rod	+	-	+	-	+	-	-	-	+	-	+	-	-	+	+	<i>Pseudomonas</i>
ASPSBX S138R	Medium, golden yellow, irregular and opaque	G -ve, rod	+	-	+	+	+	-	-	-	+	+	-	-	-	+	+	<i>Pseudomonas</i>
ASPSBX S139(1)S	Medium, creamy, regular and opaque	G -ve, rod	+	-	+	+	+	+	-	+	+	-	-	-	-	+	-	<i>Escherichia</i>
ASPSBX S139R	Big, irregular, white and flat	G +ve, rod	+	-	-	+	+	-	+	+	+	-	-	-	-	+	-	<i>Bacillus</i>
ASPSBX S140S	Medium, creamy, irregular and opaque	G +ve, rod	+	-	+	+	+	-	-	-	+	-	-	+	+	+	-	<i>Bacillus</i>
ASPSBX S141(1)S	Medium, light green, irregular and opaque	G -ve, rod	+	-	+	-	+	-	-	+	+	-	-	+	+	+	+	<i>Pseudomonas</i>
ASPSBX S141R	Big, light green, irregular and opaque	G -ve, rod	-	-	-	+	+	-	-	+	+	-	-	-	-	+	+	<i>Aminobacter</i>
ASPSBX S141E	Medium, white, irregular and opaque	G +ve, rod	-	-	-	-	+	-	-	-	+	-	-	-	-	+	+	<i>Bacillus</i>
ASPSBX S142(2)S	Big, white, irregular and flat	G +ve, rod	-	-	+	+	+	-	-	+	+	-	-	-	-	+	-	<i>Bacillus</i>
ASPSBX S144(1)S	Small, white, round and flat	G +ve, rod	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	<i>Bacillus</i>
ASPSBX S145(1)S	Medium, creamy, regular and opaque	G +ve, cocci	+	+	+	-	+	-	-	+	+	-	-	+	+	+	-	<i>Staphylococcus</i>
ASPSBX S147S	Medium, light green, irregular and opaque	G -ve, rod	-	-	-	-	+	-	-	+	+	-	-	-	-	+	+	<i>Xanthomonas</i>
ASPSBX S150S	Medium, light green, regular and opaque	G -ve, rod	+	-	+	-	+	-	-	+	+	-	-	+	+	+	+	<i>Pseudomonas</i>

Contd...

(Table 7 contd....)

Isolate	Morphological characters		Physiological and biochemical characters*															Tentative identity
	Colony Morphology	Gram reaction and cell shape	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
ASPSBX S150R	Medium, creamy, irregular and flat	G +ve, cocci	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	<i>Staphylococcus</i>
ASPSBX Dr27S	Big, creamy, irregular and flat	G -ve, rod	-	-	-	+	+	-	-	+	+	-	-	-	-	+	+	<i>Aminobacter</i>
ASPSBX Dr27(1)S	Medium, white, irregular and flat	G +ve, rod	+	-	+	+	+	-	-	+	+	-	+	-	-	+	-	<i>Bacillus</i>
ASPSBX Dr29aS	Medium, light green, irregular and opaque	G -ve, rod	+	+	+	+	+	-	-	+	+	-	-	-	+	+	-	<i>Pseudomonas</i>
ASPSBX Dr29aR	Big, irregular, white and flat	G +ve, rod	-	-	-	-	+	-	-	+	+	-	-	-	+	+	+	<i>Bacillus</i>
ASPSBX Dr31(1)S	Medium, pale white, irregular and flat	G -ve, rod	-	-	-	-	+	-	-	-	+	-	-	-	-	+	+	<i>Acetobacter</i>
ASPSBX Dr32S	Big, light green, irregular and opaque	G -ve, rod	+	-	-	+	+	-	-	-	+	-	-	-	-	+	-	<i>Rhizomonas</i>
ASPSBX WL15S	Small, light green, regular and opaque	G -ve, rod	+	-	+	+	+	-	-	+	+	-	-	-	-	+	-	<i>Pseudomonas</i>
ASPSBX WL15(3)S	Medium, white, irregular and flat	G +ve, rod	-	-	-	-	+	-	-	+	+	-	-	-	+	+	+	<i>Bacillus</i>
ASPSBXI S156R	Medium, creamy, regular and flat	G +ve, cocci	-	-	+	+	+	-	-	+	+	-	-	-	-	+	-	<i>Staphylococcus</i>
ASPSBXI S158S	Small, creamy regular and flat	G -ve, rod	-	-	-	+	+	-	-	-	-	-	-	-	-	+	+	<i>Aminobacter</i>
ASPSBXI S160S	Medium, green, regular and opaque	G -ve, rod	+	-	+	+	+	-	-	+	+	-	-	-	-	+	-	<i>Pseudomonas</i>
ASPSBXI S160(1)E	Big, irregular, white and flat	G +ve, rod	-	-	-	-	+	-	-	-	+	-	-	-	-	+	+	<i>Bacillus</i>
ASPSBXI S160(2)E	Big, irregular, white and flat	G +ve, rod	-	-	-	+	+	-	-	+	+	+	-	-	+	+	+	<i>Bacillus</i>
ASPSBXI S169(1)R	Medium, creamy, irregular and opaque	G +ve, rod	-	-	-	-	+	-	-	-	+	-	-	-	-	+	-	<i>Bacillus</i>
ASPSBXI S172(2)S	Medium, light green, irregular and opaque	G -ve, rod	-	-	-	+	+	-	-	+	+	-	-	-	-	+	+	<i>Pseudomonas</i>
ASPSBXI S175E	Medium, light green, regular and opaque	G -ve, rod	+	-	-	+	+	-	-	-	-	-	-	-	-	+	-	<i>Pseudomonas</i>
ASPSBXI S176S	Small, golden yellow, regular and opaque	G -ve, rod	+	-	+	+	+	-	-	+	+	-	-	-	-	+	+	<i>Pseudomonas</i>
ASPSBXI S177E	Small, light yellow, regular and flat	G -ve, rod	+	-	+	+	+	-	-	-	+	-	-	-	+	+	-	<i>Rhizobacter</i>
ASPSBXI S181R	Big, irregular, white and flat	G +ve, rod	-	-	-	+	+	-	-	+	+	+	-	-	+	+	+	<i>Bacillus</i>
ASPSBXI S187(2)S	Medium, creamy, regular and opaque	G -ve, rod	+	-	+	+	+	+	-	+	+	-	-	-	-	+	-	<i>Escherichia</i>
ASPSBXI WL18E	Medium, white, irregular and opaque	G +ve, rod	+	-	+	-	+	-	+	+	+	-	-	-	+	+	-	<i>Bacillus</i>
ASPSBXI WL19E	Medium, creamy, regular and opaque	G -ve, rod	+	-	+	+	+	+	-	+	+	-	-	-	-	+	-	<i>Escherichia</i>
ASPSBXI WL20S	Small, creamy, irregular and flat	G -ve, rod	-	-	-	+	+	-	-	+	+	-	-	-	-	+	+	<i>Xanthobacter</i>
ASPSBXII S189E	Small, light yellow, regular and flat	G +ve, cocci	-	-	-	+	+	-	-	-	-	-	-	-	-	+	+	<i>Staphylococcus</i>
ASPSBXII S190S	Medium, white, irregular and flat	G +ve, rod	+	-	+	+	+	-	-	-	+	+	-	-	-	+	+	<i>Bacillus</i>
ASPSBXII Dr33S	Medium, light green, irregular and opaque	G -ve, rod	-	-	-	+	+	-	-	-	-	-	-	-	-	+	+	<i>Pseudomonas</i>

+ : POSITIVE FOR THE TEST/ GROWTH

- : NEGATIVE FOR THE TEST/ NO GROWTH

G-VE: GRAM NEGATIVE

G+VE: GRAM POSITIVE

* Physiological and biochemical tests

1. Lipid hydrolysis
2. Starch hydrolysis
3. Casein hydrolysis
4. Oxidation test

5. Catalase test
6. Methyl red test
7. Voges Prokauer test
8. Urease test

9. Citrate utilization
10. Gelatin liquefaction
11. Denitrofication
12. Indole production

13. H₂S Production
14. Acid production
15. Gas production

Table 8. Biochemical and physiological characteristics of salt tolerant fluorescent pseudomonads and their tentative identity

Isolate	Morphological characters			Physiological and biochemical characters*																					Tentative identity
	Colony colour	Fluore-scence	Gram reaction and cell shape	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
ASFPI S3(1)S	Green	++	G -ve, rod	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>Pseudomonas mendocina</i>
ASFPI S3(2)S	Green	+++	G -ve, rod	-	+	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. fluorescens</i>
ASFPI S4(1)S	Green	++	G -ve, rod	-	+	+	+	-	-	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	<i>P. fluorescens</i>
ASFPI S6(1)S	Green	+++	G -ve, rod	-	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. fluorescens</i>
ASFPI S9(1)S	Green	+++	G -ve, rod	-	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. fluorescens</i>
ASFPI S14(3)S	Green	++	G -ve, rod	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>P. mendocina</i>
ASFPI S15(1)S	Green	+++	G -ve, rod	+	-	+	+	+	-	-	+	+	+	-	+	-	+	+	+	+	+	+	+	+	<i>P. aeruginosa</i>
ASFPI Dr6(8)S	Green	+++	G -ve, rod	+	-	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+	<i>P. aeruginosa</i>
ASFPV S35R	Green	+++	G -ve, rod	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. aeruginosa</i>
ASFPV S85R	Green	++	G -ve, rod	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. pseudomallei</i>
ASFPV S94(2)E	Green	+++	G -ve, rod	-	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. fluorescens</i>
ASFPV S97R	Green	++	G -ve, rod	+	-	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. aeruginosa</i>
ASFPV S100E	Green	+++	G -ve, rod	-	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. fluorescens</i>
ASFPV S112(a)R	Green	++	G -ve, rod	-	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. fluorescens</i>
ASFPV S158(2)R	Green	++	G -ve, rod	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	<i>P. pseudomallei</i>
ASFPV Dr10R	Green	+++	G -ve, rod	-	+	+	+	-	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	<i>P. fluorescens</i>
ASFPV Dr10E	Yellow	+	G -ve, rod	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	<i>P. aureofasciens</i>
ASFPV Dr16E	Yellow	+++	G -ve, rod	-	+	+	+	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	<i>P. aureofasciens</i>
ASFPX S121(3)S	Yellow	+++	G -ve, rod	-	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. aureofasciens</i>
ASFPX S121R	Yellow	+++	G -ve, rod	-	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	<i>P. aureofasciens</i>
ASFPX S131S	Green	++	G -ve, rod	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>P. mendocina</i>
ASFPX S134(3)S	Green	++	G -ve, rod	-	+	+	+	-	+	+	+	+	+	+	-	-	+	-	+	+	+	-	+	-	<i>P. aeruginosa</i>
ASFPX S149S	Green	+	G -ve, rod	+	-	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	<i>P. aeruginosa</i>
ASFPX S155S	Green	++	G -ve, rod	+	-	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	<i>P. pseudomallei</i>
ASFPX S156R	Yellow	+++	G -ve, rod	+	-	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	<i>P. aureofasciens</i>
ASFPX S156E	Green	++	G -ve, rod	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	<i>P. pseudomallei</i>
ASFPX S157(1)S	Green	++	G -ve, rod	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	<i>P. aeruginosa</i>
ASFPX S157(2)R	Green	+++	G -ve, rod	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. aeruginosa</i>
ASFPX S158(1)R	Green	+++	G -ve, rod	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	<i>P. aeruginosa</i>
ASFPX S161(1)R	Green	+++	G -ve, rod	-	+	+	+	+	-	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	<i>P. fluorescens</i>
ASFPX S161(2)R	Green	++	G -ve, rod	+	-	+	+	+	+	+	-	+	-	+	-	-	+	+	+	+	+	+	+	-	<i>P. vesicularis</i>
ASFPX S163S	Green	+++	G -ve, rod	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	<i>P. aeruginosa</i>

Contd...

(Table 8 contd....)

Isolate	Morphological characters			Physiological and biochemical characters*																					Tentative identity
	Colony colour	Fluorescence	Gram reaction and cell shape	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
ASFPXI S164S	Green	+++	G -ve, rod	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. aeruginosa</i>
ASFPXI S164(b)E	Green	+++	G -ve, rod	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. aeruginosa</i>
ASFPXI S167(2)S	Yellow	+++	G -ve, rod	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. aureofasciens</i>
ASFPXI S168R	Green	+++	G -ve, rod	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. fluorescens</i>
ASFPXI S169(2)S	Green	+++	G -ve, rod	-	+	+	+	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	-	<i>P. fluorescens</i>
ASFPXI S170R	Green	+++	G -ve, rod	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>P. aeruginosa</i>
ASFPXI S172R	Green	+++	G -ve, rod	-	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. fluorescens</i>
ASFPXI S173S	Yellow	+++	G -ve, rod	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. aureofasciens</i>
ASFPXI S174S	Green	+	G -ve, rod	-	+	+	+	+	-	+	+	+	+	+	-	+	-	+	-	-	+	+	+	-	<i>P. aeruginosa</i>
ASFPXI S174(1)R	Green	++	G -ve, rod	-	+	+	+	-	-	+	-	-	-	+	-	-	-	+	+	+	-	+	-	-	<i>P. fluorescens</i>
ASFPXI S174(2)R	Green	++	G -ve, rod	+	-	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	<i>P. pseudomallei</i>
ASFPXI S176S	Green	++	G -ve, rod	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	-	-	+	+	+	+	<i>P. aeruginosa</i>
ASFPXI S178(1)S	Green	++	G -ve, rod	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. aeruginosa</i>
ASFPXI S178(2)S	Green	++	G -ve, rod	-	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. fluorescens</i>
ASFPXI S178R	Green	+	G -ve, rod	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. pseudomallei</i>
ASFPXI S178E	Yellow	+++	G -ve, rod	-	+	+	+	-	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. aureofasciens</i>
ASFPXI S180S	Green	++	G -ve, rod	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	<i>P. aeruginosa</i>
ASFPXI S180E	Yellow	+++	G -ve, rod	-	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. aureofasciens</i>
ASFPXI S181R	Green	++	G -ve, rod	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. fluorescens</i>
ASFPXI S183R	Green	+++	G -ve, rod	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	<i>P. aeruginosa</i>
ASFPXI S183E	Green	+++	G -ve, rod	-	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. aeruginosa</i>
ASFPXI S184R	Green	+	G -ve, rod	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>P. aeruginosa</i>
ASFPXI S186S	Green	++	G -ve, rod	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	<i>P. pseudomallei</i>
ASFPXI S187S	Green	+	G -ve, rod	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. putida</i>
ASFPXI WL16R	Green	++	G -ve, rod	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. aeruginosa</i>
ASFPXI WL16(1)R	Yellow	+++	G -ve, rod	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. aureofasciens</i>
ASFPXI WL17R	Green	++	G -ve, rod	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. aeruginosa</i>
ASFPXI S190R	Green	+	G -ve, rod	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. aeruginosa</i>
ASFPXI Dr33R	Yellow	+++	G -ve, rod	-	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	<i>P. aureofasciens</i>

+++ : HIGH FLUORESCENCE

++ : MEDIUM FLUORESCENCE

+ : LIGHT FLUORESCENCE/ POSITIVE FOR THE TEST/ GROWTH

- : NEGATIVE FOR THE TEST/

NO GROWTH

G-VE: GRAM NEGATIVE

*** Physiological and biochemical tests**

1. Growth at 41°C

5. Starch hydrolysis

9. Glucose

13. Valine

17. Rhamnose

21. Maltose

2. Growth at 4°C

6. Denitrification

10. Trehalose

14. Alanine

18. Sucrose

3. Arginine hydrolysis

7. Gelatin liquefaction

11. Inositol

15. Xylose

19. Lactose

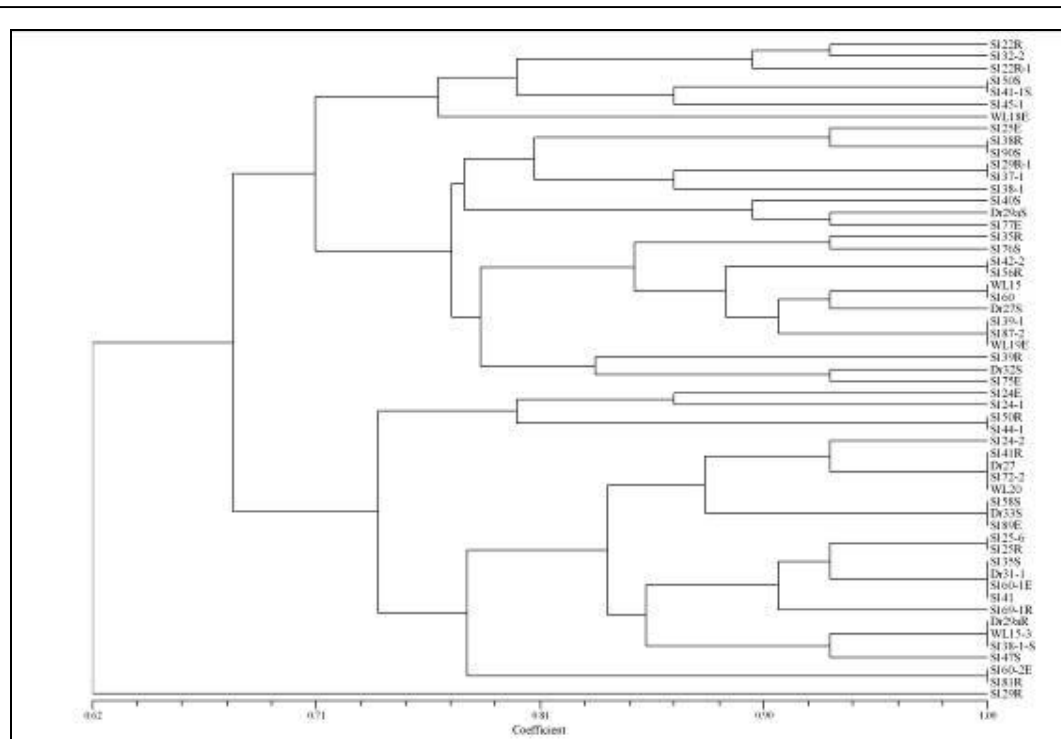
4. Oxidase test

8. NO₃ as N source

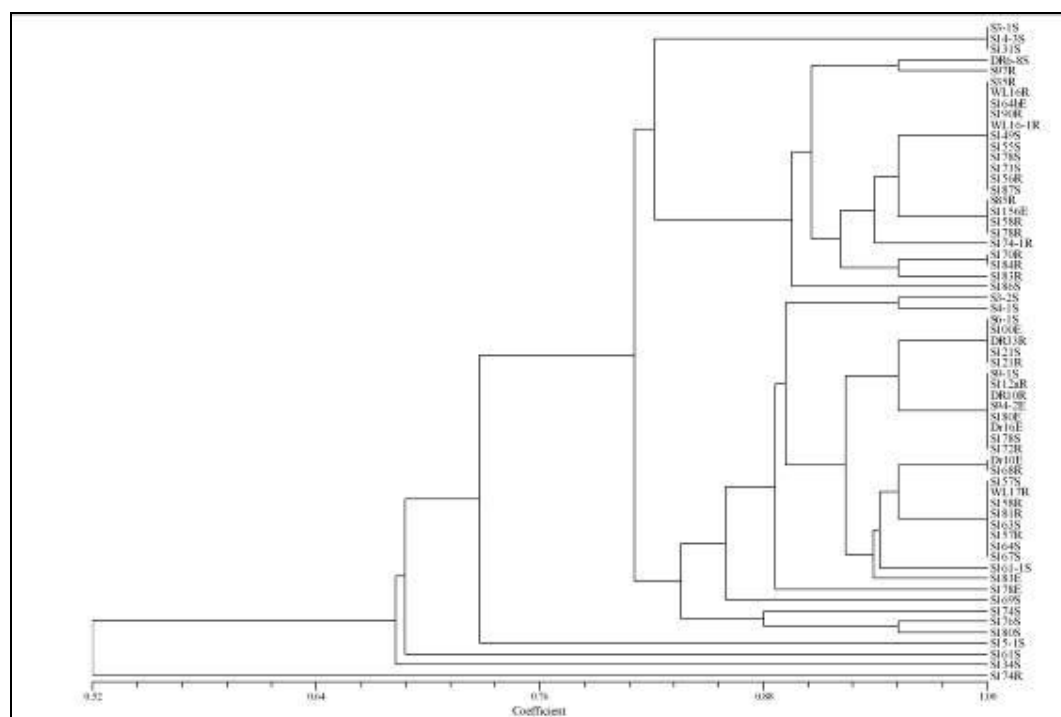
12. Geraniol

16. Ribose

20. Mannitol



a. Salt-tolerant P-solubilizing bacteria (55)



b. Salt-tolerant fluorescent pseudomonads (61)

Fig. 4. UPGMA dendrogram showing the similarity values among salt-tolerant AIMS

All isolates were found to be positive for arginine hydrolysis and urease activity. Similarly, 39 isolates were found positive for starch hydrolysis whereas 18 were positive for denitrification. All the isolates except S161(2)R, S174(1)R and S178E were found to utilize nitrate as N source while nine out of 61 isolates were found negative for gelatin liquefaction.

Among the C sources tested, xylose and lactose were used as C sources by all the isolates whereas only seven isolates were found to utilize geraniol as C source. Among the isolates, S174(1)R failed to utilize glucose and mannitol as C sources while two isolates each were unable to utilize ribose and sucrose as C sources. Similarly, three isolates were found negative for trehalose utilization, 4 isolates each for inositol, valine, alanine and rhamnose whereas six isolates were negative for utilization of maltose as C source.

Considering the morphological and biochemical characteristics, ability of the isolates to utilize different carbon sources and their ability to grow at 4°C and 41°C temperatures, 15 isolates were tentatively identified as *Pseudomonas fluorescens*, 23 as *P. aeruginosa*, 11 as *P. aureofaciens*; seven as *P. pseudomallei*; three as *P. mendocina* and one each as *P. putida* and *P. vesicularis*.

Phylogenetic tree constructed for 61 salt-tolerant isolates revealed that, all the isolates were found to diverge into 2 major clusters with similarity coefficient of 0.52. Out of 61 FP isolates, ASFPSXI S174(2)R alone represented the cluster I while 60 grouped into cluster II. All the 60 isolates of the cluster II were again distributed into 24 sub clusters with a similarity coefficient of nearly 0.68 (Fig. 4b).

4.1.2 Molecular characterization

A total of 91 salt-tolerant AIMs were subjected for molecular identification using 16S rRNA gene sequencing technique. Initially, the genomic DNA of all the isolates was examined for PCR amplification of 16S rRNA gene using the universal primer pair (F/R) qualitatively in a 20 µl reaction mixture (Plate 1). The expected amplicon size of 1450 bp was observed in all the isolates. Later, the 16S rRNA gene from all isolates was amplified in bulk (200 µl), gel eluted, cloned and transformed into *Escherichia coli* DH-5α (Plate 1). Recombinant confirmed plasmids carrying the inserts were sequenced at Applied Bio-Systems Private Ltd., Hyderabad.

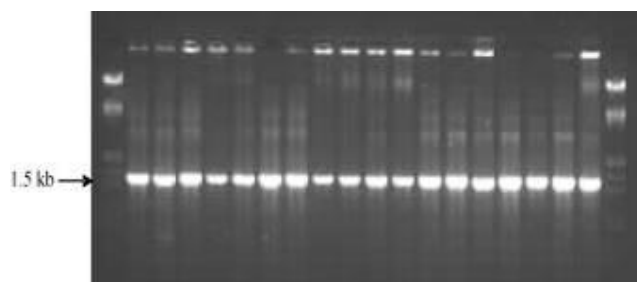
4.1.2.1 Identity of salt-tolerant AIMs and their taxonomic affiliation

Ninety one salt-tolerant isolates consisting of 18 each of ANF and FNF; 28 PSB and 27 FP were selected for 16S rRNA gene sequencing so as to retrieve sequence homology and bacterial identity from NCBI database. The closest identities of the isolates were retrieved from NCBI and presented in Table 9a.

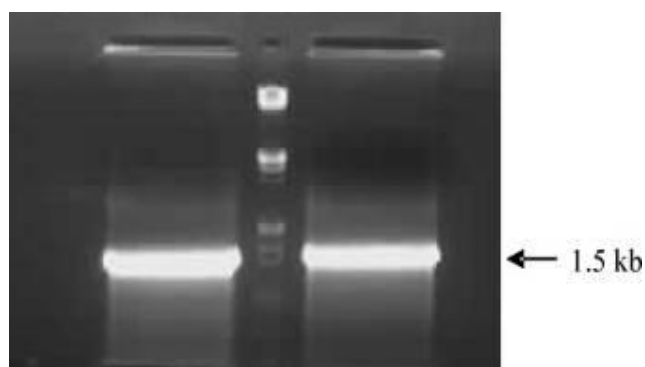
Out of 91 isolates, 77 were identified up to species level whereas the remaining 14 were identified only up to genus. Identity of all the isolates revealed their representation to any of the six family viz., Rhodospirillaceae and Rhizobiaceae of the Class α-Proteobacteria, Pseudomonadaceae and Enterobacteriaceae of Class γ-Proteobacteria and, Bacillaceae and Staphylococcaceae of Phylum Firmicutes (Class-Bacilli). Out of 91 isolates, 69 were grouped in Phylum Proteobacteria while 22 in Firmicutes. All the isolates were found to represent any of the three class viz., α- and γ-Proteobacteria and Bacilli. Out of 69 proteobacteria, eight isolates represented α-Proteobacteria while 61 represented γ-Proteobacteria (Table 9b).

BLAST analysis of 16S rRNA sequence of 18 salt-tolerant ANF isolates revealed that, only six isolates showed their closest affiliation to α-proteobacteria and were identified as *Azospirillum* (3 as *A. irakense* and 1 each as *A. brasilense*, *A. halopraeferens* and *Azospirillum* sp.). The remaining 12 isolates were found to represent the class γ-proteobacteria and all of them were identified as *Pseudomonas nitroreducens* (Table 9b).

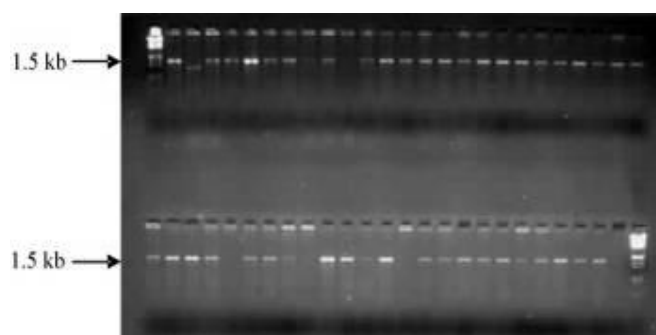
The microbial identity of 18 salt-tolerant FNF isolates was recorded through 16S rRNA gene sequencing followed by BLAST analysis indicated that six each were identified as *Azotobacter* (3 as *A. vinelandii*, 2 as *A. chroococcum* and one as *A. salinestris*) and *Enterobacter* (5 as *E. aerogenes* and one as *E. oryzae*), four as *Klebsiella* (two as *K. oxytoca* and one each as *K. pneumoniae* and *Klebsiella* sp.) and two as *Agrobacterium tumefaciens* (Table 9b).



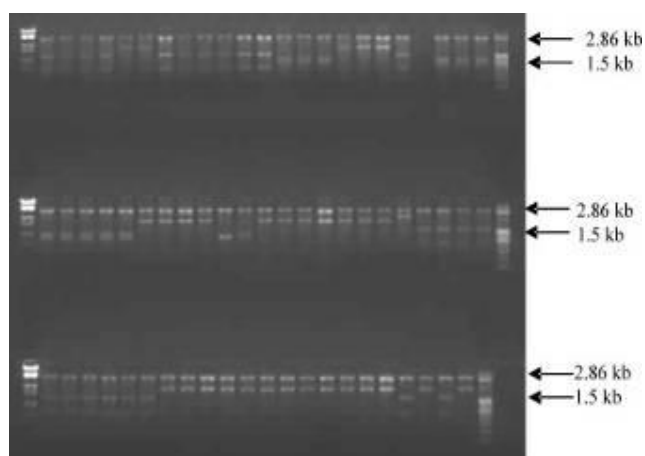
PCR amplification of 16S rRNA



Amplification of 16S rRNA in bulk



PCR Confirmation using M13 Primers



Restriction confirmation of insert using XbaI and BamHI

Plate 1. Steps used for identification of salt tolerant AIM's using 16S rDNA sequencing

Similarly, out of 28 salt-tolerant FP isolates identified using 16S rRNA sequencing, 12 isolates showed closest affiliation to *Pseudomonas aeruginosa*, 7 to *Pseudomonas* sp., 3 each to *Pseudomonas putida* and *Pseudomonas chlororaphis* sub sp. *aureofaciens* and one each to *Pseudomonas monteilii* and *P. fluorescens* (Table 9b).

Based on the 16S rRNA sequence homology and BLAST results, the microbial identity for 28 salt-tolerant PSB isolates were also recorded (Table 9b). Based on their closest affiliation to the 16S rRNA sequences deposited in the NCBI database, 17 isolates were identified as *Bacillus* (9 as *B. endophyticus*, 5 as *B. megaterium* and 2 as *B. subtilis*), five as *Staphylococcus* sp., four as *Pseudomonas aeruginosa* and two as *Enterobacter cloacae*.

4.1.2.2 Phylogenetic analysis

Taxonomic specific 16S rRNA gene sequences of the salt-tolerant AIMs were subjected for multiple alignments using BioEdit version 7.13 software. From the sequences aligned, UPGMA dendrograms were constructed with the MEGA software version 5.0. The robustness of the inferred trees was evaluated by 1000 bootstrap resamplings. The relatedness between isolate and type strain sequences are depicted in Figs. 4, 5 and 6.

4.1.2.2.1 Alphaproteobacteria

Phylogenetic tree was constructed using 18 strains including six salt-tolerant ANF isolates and the 12 type strains belonging to 3 species of *Azospirillum* of the family Rhodospirillaceae of class α -Proteobacteria. Isolates ASANFXI WL19E, S145E and S173E constituted a separate branch within a lineage including four species of *Azospirillum irakense* and were distinct from other species of *Azospirillum*. While, one isolate each viz., ASANFX S148E, Dr31E and S141E formed a separate branch within the lineage containing *A. brasilense*, *A. halopraeferens* and *Azospirillum* sp. respectively (Fig. 5a).

Similarly, dendrogram was also constructed using seven strains including two salt-tolerant FNF isolates and the 5 type strains belonging to 2 species of *Agrobacterium* of family Rhizobiaceae of class α -Proteobacteria. It was observed that, both isolates formed a separate cluster within a lineage *Agrobacterium tumefaciens* (Fig. 5b).

4.1.2.2.2 Gammaproteobacteria

UPGMA dendrogram was constructed using 85 strains including 49 salt-tolerant AIMs and the 36 type strains belonging to different species of *Azotobacter* and *Pseudomonas* of family Pseudomonadaceae of class γ -Proteobacteria. It was observed that, 12 salt-tolerant FP and 4 PSB isolates formed a distinct branch within a lineage including nine strains of *Pseudomonas aeruginosa*. While 12 strains showed its distinctness within a lineage of *Pseudomonas nitroreducens* but bootstrap values among the isolates was recorded to be low. Two isolates viz., ASFPXI S174S and S100E had a distinct branch within a lineage of *Pseudomonas* sp. Similarly, three isolates viz., ASFPXI S167(2)S, Dr16E and S156R were found to have separate branch within a lineage comprising two strains of *Pseudomonas chlororaphis* sub sp. *aureofaciens* while the isolate ASFPXI S181R alone was found to form distinct branch within a lineage consisting of type strain *Pseudomonas fluorescens* (Fig. 6a).

Out of six salt-tolerant FNF isolates, two viz., ASFNFIV WL7(2)R and S62E formed a separate branch within a lineage including five strains of *Azotobacter chroococcum* while three isolates viz., ASFNFIV S61(1)S, WL5(2)R and S40(1)R formed two separate branches within a lineage including type strain *Azotobacter vinelandii* while isolate ASFNFIV S43(2)R was found to have its closest lineage with *Azotobacter salinestris*.

The 16S rRNA gene sequences of 31 strains including 14 salt-tolerant isolates (12 FNF and 2 PSB) and 19 type strains of different species of *Enterobacter* and *Klebsiella* belonging to family Enterobacteriaceae of class γ -Proteobacteria were used to construct the UPGMA dendrogram. Two PSB isolates (ASPSBIX S125R and S135R) formed a distinct cluster within a lineage consisting of five strains of *Enterobacter cloacae*. However, all the 12 FNF isolates were found to form three distinct branches. Two FNF isolates (S45(1)R and S37R) formed the first branch followed by second branch formed by ASFNFI Dr4(1) and S75(1)R isolates within a lineage consisting of 3 species of *Klebsiella* type strains. The third distinct branch was formed by a group of six FNF isolates (ASFNFIV S36(1)R, S60(1)S, S63(1)R, S75E, WL4(1)R and S43(2)R) within a lineage consisting of *Enterobacter aerogenes* and *E. oryzae* type strains (Fig. 6b).

Table 9a. Identity of salt tolerant isolates with accession numbers and similarity

Isolate code	GenBank accession number	Closest NCBI strain with accession number	Similarity (%)
ASFPI S3(1)S	HQ018734	<i>Pseudomonas putida</i> strain WH3; FJ262368	99.4
ASFPI S4(1)S	HQ018735	<i>Pseudomonas</i> sp. RP7; GU979228	99.2
ASFPSXI 187S	HQ018736	<i>Pseudomonas putida</i> strain JM7; FJ472859	99.1
ASFPI S9(1)S	HQ018737	<i>Pseudomonas</i> sp. TO7; HQ457044	99.3
ASFPXI S167(2)S	HQ018738	<i>Pseudomonas chlororaphis</i> subsp. <i>aurantiaca</i> strain ST-1; GU947817	98.4
ASFPV Dr16E	HQ018739	<i>Pseudomonas chlororaphis</i> subsp. <i>aureofaciens</i> NBRC 3921; AB680175	99.5
ASFPXI S156R	HQ018740	<i>Pseudomonas chlororaphis</i> subsp. <i>aureofaciens</i> NBRC 3921; AB680175	99.0
ASFPXI WL17R	HQ018741	<i>Pseudomonas aeruginosa</i> strain AS2; GU447238	99.5
ASFPV S94(2)E	HQ018742	<i>Pseudomonas putida</i> strain A1w4; AY512610	99.0
ASFPXI S164bE	HQ018743	<i>Pseudomonas aeruginosa</i> strain RI-1; JQ773431	99.5
ASFPV Dr10E	HQ018744	<i>Pseudomonas</i> sp. DYJK4-9; HQ843845	99.2
ASFPV S97R	HQ018745	<i>Pseudomonas aeruginosa</i> strain AS2; GU447238	99.7
ASFNFIV WL7(2)R	HQ018746	<i>Azotobacter chroococcum</i> strain H-A; FJ032009	98.9
ASFNFIV S61(1)S	HQ018747	<i>Azotobacter vinelandii</i> strain ISSDS-386; EF620439	99.1
ASFNFIV S62E	HQ018748	<i>Azotobacter chroococcum</i> strain ISSDS-347; EF620427	98.8
ASFNFIV S43(2)R	HQ018749	<i>Azotobacter salinestris</i> strain NBRC 102611; AB681885	99.2
ASFNFIV WL5(2)R	HQ018750	<i>Azotobacter vinelandii</i> strain LEP21; AB623045	99.8
ASFNFIV S40(1)R	HQ018751	<i>Azotobacter vinelandii</i> ; L40329	99.1
ASANFX S141E	HQ018752	<i>Azospirillum</i> sp. YM 195; GU396257	99.2
ASANFXI WL19E	HQ018753	<i>Azospirillum irakense</i> KBC1; NR044949	99.4
ASANFX Dr31E	HQ018754	<i>Azospirillum halopraeferens</i> strain Au4; NR044859	98.8
ASANFX S145E	HQ018755	<i>Azospirillum irakense</i> KBC1; NR044949	99.1
ASANFX S148E	HQ018756	<i>Azospirillum brasilense</i> strain 42M; HM002662	99.3
ASANFXI S173E	HQ018757	<i>Azospirillum irakense</i> strain DSM 11586; GU256440	99.2
ASPSBIX S129R	JF513122	<i>Bacillus endophyticus</i> strain A6; HQ323418	98.9
ASPSBX S139R	JF513123	<i>Bacillus endophyticus</i> strain A6; HQ323418	98.7
ASPSBIX S122R	JF513124	<i>Bacillus endophyticus</i> strain XJSL4-6; GQ903415	99.3
ASFPV S112(a)R	JF513125	<i>Pseudomonas</i> sp. IM4; FJ211165	99.5
ASFPI S14(3)S	JF513126	<i>Pseudomonas monteilii</i> strain SB 3067; GU191931	99.6
ASPSBXI S189E	JF513127	<i>Staphylococcus</i> sp. TUT1203; AB188210	99.7
ASPSBX S150R	JF513128	<i>Staphylococcus</i> sp. TUT1203; AB188210	99.1
ASPSBX S144(1)	JF513129	<i>Bacillus endophyticus</i> strain A6; HQ323418	99.4
ASPSBX Dr29aR	JF513130	<i>Bacillus endophyticus</i> strain A6; HQ323418	99.3
ASPSBX S142(2)	JF513131	<i>Bacillus endophyticus</i> strain A6; HQ323418	98.8
ASPSBX S140S	JF513132	<i>Bacillus endophyticus</i> strain A6; HQ323418	99.5
ASFPXI S158(2)R	JF513133	<i>Pseudomonas aeruginosa</i> strain AS2; GU447238	99.7
ASFPI S15(1)S	JF513134	<i>Pseudomonas aeruginosa</i> strain ANSC; GU296674	99.6
ASFPV S35R	JF513135	<i>Pseudomonas aeruginosa</i> strain AS2; GU447238	99.7
ASANFIX S132E	JF513136	<i>Pseudomonas nitroreducens</i> strain 0802; AF494091	99.4
ASPSBIX S125R	JF513137	<i>Enterobacter cloacae</i> strain C111; HQ407286	99.6
ASFPXI S181R	JF513138	<i>Pseudomonas fluorescens</i> strain ost5; DQ439976	99.7
ASPSBX S145(1)	JF513139	<i>Staphylococcus</i> sp. SW80; HQ202805	99.6
ASFPV S85R	JF513140	<i>Pseudomonas aeruginosa</i> strain AS2; GU447238	99.6

Contd...

(Table 9a contd....)

Isolate code	GenBank accession number	Closest NCBI strain with accession number	Similarity (%)
ASFPV Dr10R	JF513141	<i>Pseudomonas aeruginosa</i> strain AS2; GU447238	99.6
ASFPXI S134(3)S	JF513142	<i>Pseudomonas aeruginosa</i> strain AS2; GU447238	99.1
ASPSBX Dr29aS	JF513143	<i>Pseudomonas aeruginosa</i> strain AS2; GU447238	99.5
ASFPXI S157(1)S	JF513144	<i>Pseudomonas aeruginosa</i> strain RI-1; JQ773431	99.5
ASFPXI S163S	JF513145	<i>Pseudomonas aeruginosa</i> strain AS2; GU447238	99.7
ASPSBXI S172(2)S	JF513146	<i>Pseudomonas aeruginosa</i> strain AS2; GU447238	99.8
ASPSBXII Dr33S	JF513147	<i>Pseudomonas aeruginosa</i> strain AS2; GU447238	99.7
ASFPXI S174S	JF513148	<i>Pseudomonas</i> sp. BWDY-42; DQ213044	99.7
ASFPV S100E	JF513149	<i>Pseudomonas</i> sp. BWDY-42; DQ213044	99.7
ASFPXI S178S	JF513150	<i>Pseudomonas</i> sp. MBR; EU307111	99.8
ASANFXI WL17E	JF513151	<i>Pseudomonas nitroreducens</i> strain 0802; AF494091	99.5
ASFPXI S6(1)S	JF513152	<i>Pseudomonas aeruginosa</i> strain ANSC; GU296674	99.7
ASFPXI S180S	JF513153	<i>Pseudomonas aeruginosa</i> strain AS2; GU447238	99.6
ASANFX S138E	JF513154	<i>Pseudomonas nitroreducens</i> strain 0802; AF494091	99.2
ASANFX WL13E	JF513155	<i>Pseudomonas nitroreducens</i> strain 0802; AF494091	99.5
ASANFX S150E	JF513156	<i>Pseudomonas nitroreducens</i> strain 0802; AF494091	99.6
ASANFXI S160E	JF513157	<i>Pseudomonas nitroreducens</i> strain 0802; AF494091	99.6
ASANFX S135E	JF513158	<i>Pseudomonas nitroreducens</i> strain 0802; AF494091	99.4
ASANFX WL15E	JF513159	<i>Pseudomonas nitroreducens</i> strain 0802; AF494091	99.4
ASPSBX S135R	JF513160	<i>Enterobacter cloacae</i> subsp. <i>cloacae</i> ATCC 13047; CP001918	99.1
ASPSBXI S169(1)R	JF513161	<i>Bacillus megaterium</i> strain HDDMG02; EU723818	99.3
ASPSBXII S190S	JF513162	<i>Bacillus megaterium</i> isolate TS IW 36; FM164631	99.3
ASPSBIX S124(1)R	JF513163	<i>Staphylococcus</i> sp. SW80; HQ202805	99.8
ASPSBXI S156R	JF513164	<i>Staphylococcus</i> sp. SW80; HQ202805	99.7
ASPSBXI WL18E	JF513165	<i>Bacillus megaterium</i> strain HDDMG02; EU723818	99.0
ASPSBX S141E	JF513166	<i>Bacillus megaterium</i> strain HDDMG02; EU723818	99.3
ASPSBX WL15(3)S	JF513167	<i>Bacillus megaterium</i> strain HDDMG02; EU723818	99.5
ASPSBX Dr27S	JF513168	<i>Bacillus megaterium</i> strain HDDMG02; EU723818	99.3
ASPSBIX S124E	JF513169	<i>Bacillus subtilis</i> isolate C14-1; EU257452	99.6
ASPSBXI S181R	JF513170	<i>Bacillus subtilis</i> isolate C14-1; EU257452	99.3
ASFNFI Dr4(1)	JF513171	<i>Klebsiella</i> sp. D3S; GU259534	99.7
ASFNFIV S75(1)R	JF513172	<i>Klebsiella pneumoniae</i> 342; CP000964	99.9
ASFNFIV S45(1)R	JF513173	<i>Klebsiella oxytoca</i> strain 5; AB353045	99.6
ASFNFIV S37R	JF513174	<i>Klebsiella oxytoca</i> KCTC 1686; CP003218	99.7
ASFNFIX S190E	JF513175	<i>Agrobacterium tumefaciens</i> strain AFM2; EU592041	99.5
ASFNFIX S178E	JF513176	<i>Agrobacterium tumefaciens</i> strain AFM2; EU592041	99.7
ASFNFIV S36(1)R	JF513177	<i>Enterobacter aerogenes</i> strain LSRC164; JF772083	99.4
ASFNFIV S60(1)S	JF513178	<i>Enterobacter aerogenes</i> strain LSRC164; JF772083	99.7
ASFNFIV S75(2)R	JF513179	<i>Enterobacter oryzae</i> strain AB285; HQ706110	99.3
ASFNFIV S63(1)R	JF513180	<i>Enterobacter aerogenes</i> strain LSRC164; JF772083	99.1
ASFNFIV S75E	JF513181	<i>Enterobacter aerogenes</i> strain LSRC164; JF772083	99.1
ASFNFIV WL4(1)R	JF513182	<i>Enterobacter aerogenes</i> strain LSRC164; JF772083	99.3
ASPSBXI S160(1)E	JF513183	<i>Bacillus endophyticus</i> strain A6; HQ323418	99.2
ASPSBS160(2)E	JF513184	<i>Bacillus endophyticus</i> strain A6; HQ323418	99.7
ASANFX S154E	JF513185	<i>Pseudomonas nitroreducens</i> strain 0802; AF494091	99.4
ASANFXI S170E	JF513186	<i>Pseudomonas nitroreducens</i> strain 0802; AF494091	99.3
ASANFIX S125E	JF513187	<i>Pseudomonas nitroreducens</i> strain 0802; AF494091	99.5
ASANFX S149E	JF513188	<i>Pseudomonas nitroreducens</i> strain 0802; AF494091	99.5
ASPSFWL9S	JN043475	<i>Aspergillus niger</i> strain PIAL16; JN672584	99.3

Table 9b. Taxonomic classification of salt-tolerant AIMS identified using 16S rRNA gene sequencing

Taxonomy	Microbial identity	Isolates	
		Number	Details
Class: Alphaproteobacteria Order: Rhodospirillales Family: Rhodospirillaceae	<i>Azospirillum irakense</i>	3	ASANFXI WL19E, ASANFX S145E and ASANFXI S173E
	<i>Azospirillum brasilense</i>	1	ASANFX S148E
	<i>Azospirillum halopraeferens</i>	1	ASANFX Dr31E
	<i>Azospirillum</i> sp.	1	ASANFX S141E
	<i>Agrobacterium tumefaciens</i>	2	ASFNFXXII S190E and ASFNFXXII S178E
Class: Gammaproteobacteria Order: Pseudomonadales Family: Pseudomonadaceae	<i>Pseudomonas aeruginosa</i>	16	ASFPXI S158(2)R, ASFPI S15(1)S, ASFPV S35R, ASFPXI WL17R, ASFPXI S164bE, ASFPV S97R, ASFPV S85R, ASFPV Dr10R, ASFPIX S134(3)S, ASPSBX Dr29aS, ASFPXI S157(1)S, ASFPXI S163S, ASPSBXI S172(2)S, ASPSBXII Dr33S, ASFPI S6(1)S and ASFPXI S180S
	<i>Pseudomonas nitroreducens</i>	12	ASANFIX S132E, ASANFXI WL17E, ASANFX S138E, ASANFX WL13E, ASANFX S150E, ASANFXI S160E, ASANFX S135E, ASANFX WL15E, ASANFX S154E, ASANFXI S170E, ASANFIX S125E and ASANFX S149E
	<i>Pseudomonas putida</i>	3	ASFPXI S3(1)S, ASFPSXI 187S and ASFPV S94(2)E
	<i>Pseudomonas chlororaphis</i>	3	ASFPXI S167(2)S, ASFPV Dr16E and ASFPXI S156R
	<i>Pseudomonas monteilii</i>	1	ASFPXI S14(3)S
	<i>Pseudomonas fluorescens</i>	1	ASFPXI S181R
	<i>Pseudomonas</i> sp.	7	ASFPXI S4(1)S, ASFPI S9(1)S, ASFPV Dr10E, ASFPV S112(a)R, ASFPXI S174S, ASFPV S100E, ASFPXI S178S
	<i>Azotobacter vinelandii</i>	3	ASFNFIV S61(1)S, ASFNFIV WL5(2)R and ASFNFIV S40(1)R
	<i>Azotobacter chroococcum</i>	2	ASFNFIV WL7(2)R and ASFNFIV S62E
	<i>Azotobacter salinestris</i>	1	ASFNFIV S43(2)R
Class: Gammaproteobacteria Order: Enterobacteriales Family: Enterobacteriaceae	<i>Enterobacter aerogenes</i>	5	ASFNFIV S36(1)R, ASFNFIV S60(1)S, ASFNFIV S63(1)R, ASFNFIV S75E and ASFNFIV WL4(1)R
	<i>Enterobacter cloacae</i>	2	ASPSBIX S125R and ASPSBX S135R
	<i>Enterobacter oryzae</i>	1	ASFNFIV S75(2)R
	<i>Klebsiella oxytoca</i>	2	ASFNFIV S45(1)R and ASFNFIV S37R
	<i>Klebsiella</i> sp.	1	ASFNFI Dr4(1)
	<i>Klebsiella pneumoniae</i>	1	ASFNFIV S75(1)R
Class: Bacilli Order: Bacillales Family: Bacillaceae	<i>Bacillus endophyticus</i>	9	ASPSBIX S129R, ASPSBX S139R, ASPSBIX S122R, ASPSBX S144(1), ASPSBX Dr29aR, ASPSBX S142(2), ASPSBX S140S, ASPSBXI S160(1)E and ASPSBS160(2)E
	<i>Bacillus megaterium</i>	6	ASPSBIX S169(1)R, ASPSBXII S190S, ASPSBXI WL18E, ASPSBX S141E, ASPSBX WL15(3)S and ASPSBX Dr27S
	<i>Bacillus subtilis</i>	2	ASPSBIX S124E & ASPSBXI S181R
Class: Bacilli Order: Bacillales Family: Staphylococcaceae	<i>Staphylococcus</i>	5	ASPSBIX S189E, ASPSBX S150R, ASPSBX S145(1), ASPSBIX S124(1)R and ASPSBXI S156R

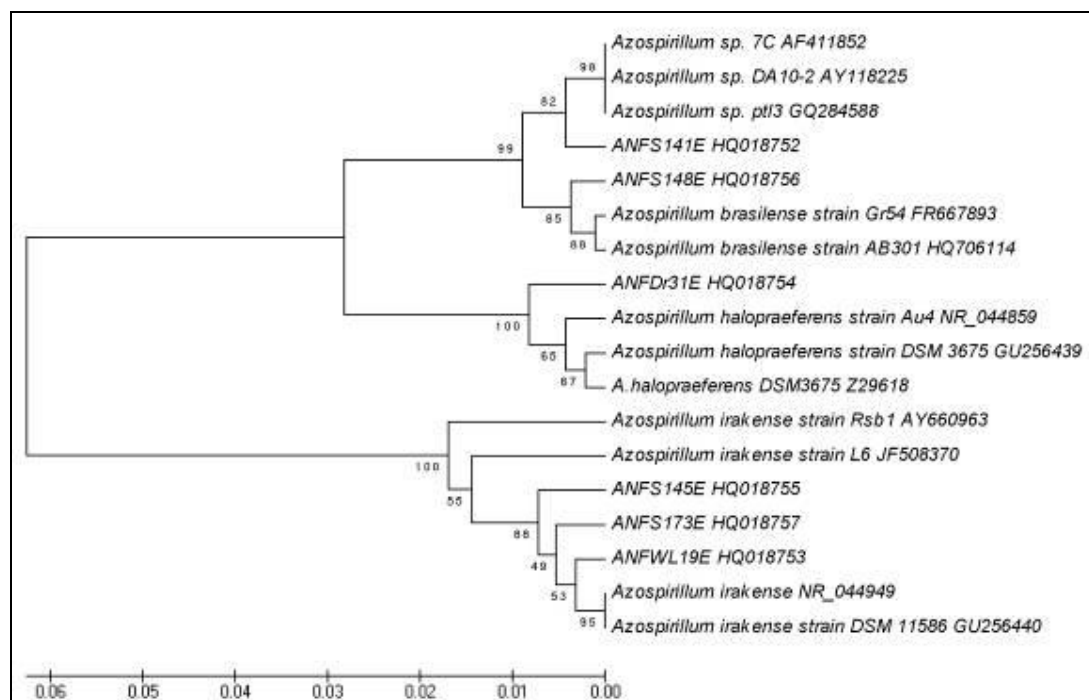


Fig. 5a. UPGMA Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of salt-tolerant *AIMs* to other *Azospirillum* species and related bacteria belonging to family Rhodospirillaceae of alpha-proteobacteria class. Significant bootstrap probability values are indicated at the branching point

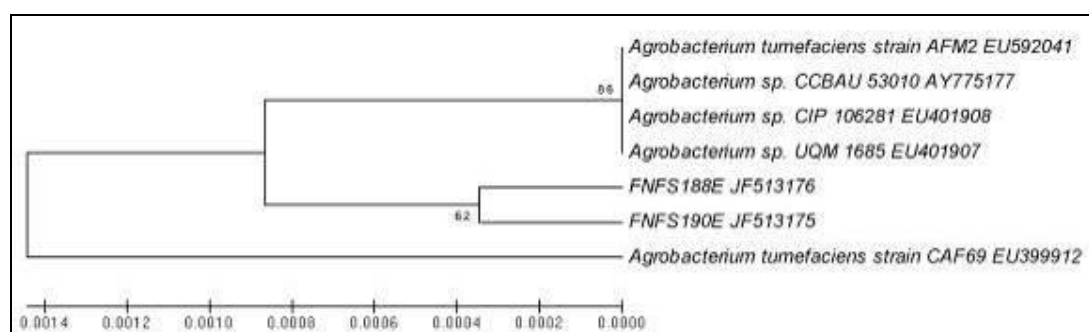


Fig. 5b. UPGMA Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of salt-tolerant *AIMs* to other *Agrobacterium* species and related bacteria belonging to family Rhizobiaceae of class alpha-proteobacteria. Significant bootstrap probability values are indicated at the branching point

4.1.2.2.3 Firmicutes

A total of 22 salt-tolerant isolates were found to represent Phylum Firmicutes. Phylogenetic tree was constructed using 12 strains including 5 salt-tolerant PSB isolates and seven type strains of *Staphylococcus* belonging to family Staphylococcaceae of class Bacilli. The results of the study indicated that, all the five PSB isolates were distributed into two distinct branches within a lineage consisting of 7 type strains of *Staphylococcus* sp. The first branch was formed by 3 PSB isolates [ASPSBX S145(1), S124(1)R and S156R] and second by 2 isolates viz., ASPSBXI S189E and S150R (Fig. 7a).

Gene sequences of 17 salt-tolerant PSB isolates along with 29 type strains of *Bacillus* belonging to family Bacillaceae were subjected for phylogenetic analysis. All the isolates were found to form two distinct main branches. First branch comprised of 9 PSB isolates [ASPSBIX S129R, S139R, S122R, S144(1), Dr29aR, S142(2), S140S, S160(1)E and S160(2)E] and was found to have its closest lineage with 13 *Bacillus endophyticus* type strains. Eight PSB isolates [ASPSBXI S169(1)R, S190S, WL18E, S141E, WL15(3)S, Dr27S, S124E and S181R] formed the second main branch within a lineage consisting of *Bacillus megaterium* and *B. subtilis* type strains (Fig. 7b).

4.2 Functional characterization

4.2.1 Dinitrogen fixation

A total of 79 isolates consisting of 36 ANF and 43 FNF were analyzed for dinitrogen (N_2) fixation in response to changes in the medium salt concentration. The results obtained from the study are presented in Tables 10a and 10b.

4.2.1.1 Associative nitrogen fixers (ANF)

All the 36 isolates were tested for their ability to fix nitrogen in presence of different concentrations of NaCl in N-free semi-solid malate medium. The amount of N_2 fixed by the isolates in general was found to decrease with increase in NaCl concentration. All 36 isolates showed N_2 fixation at 5% NaCl which ranged from 30.1 to 110.1 μg N_2 per mg protein (Table 10a). At 7.5% NaCl 18 out of 36 isolates were able to fix N_2 whereas at 10% NaCl only 3 isolates showed N_2 fixation. The amount of N_2 fixed by the isolates at 7.5 and 10% NaCl ranged from 0.00 to 112.1 μg and 0.00 to 93.1 μg N_2 per mg protein respectively. N_2 fixation by all the isolates in the normal medium (without added NaCl) ranged from 59.2 to 136.2 μg N_2 per mg protein.

Among the isolates, strain ASANFX S145E showed the highest mean N_2 fixation (105.1 μg per mg protein) followed by ASANFX S148E (97.4 μg N_2 per mg protein) and ASANFXI S173E (95.1 μg N_2 per mg protein) all of which were on par with each other but significantly superior over other isolates (Table 10a). Among different salt concentrations tested, the maximum N_2 fixation of 90.5 μg N_2 per mg protein was recorded in the normal medium which was significantly superior over all NaCl concentrations tested. The interaction effect between the isolates and NaCl concentrations were significant wherein the strain ASANFX S145E recorded maximum N_2 fixation of 122.5 μg N_2 per mg protein in control medium which was significantly superior over rest of the combinations.

4.2.1.2 Free-living nitrogen fixers (FNF)

A total of 43 FNF isolates were tested for their ability to fix nitrogen in presence of different concentrations of NaCl in Norris N-free broth. The amount of N_2 fixation in general was found to decrease with increase in NaCl concentration. While all 43 isolates fixed N_2 at 5% NaCl, only 14, 6, 2 and one isolate showed N_2 fixation at 7.5, 10, 12.5 and 15 per cent NaCl in the medium respectively (Table 10b). The amount of N_2 fixed by the isolates under salt stress ranged from 51 to 508 μg , 0.00 to 671 μg , 0.00 to 526 μg and 0.00 to 500 μg and 0.00 to 447 μg N_2 per mg protein in the presence of 5, 7.5, 10, 12.5 and 15 per cent NaCl respectively as compared to 66 to 541 μg N_2 per mg protein in control broth (without added NaCl).

Among the isolates, strain ASFNFIV S75(1)R showed highest mean N_2 fixation (431.8 μg per mg protein) and was significantly superior over all other isolates.

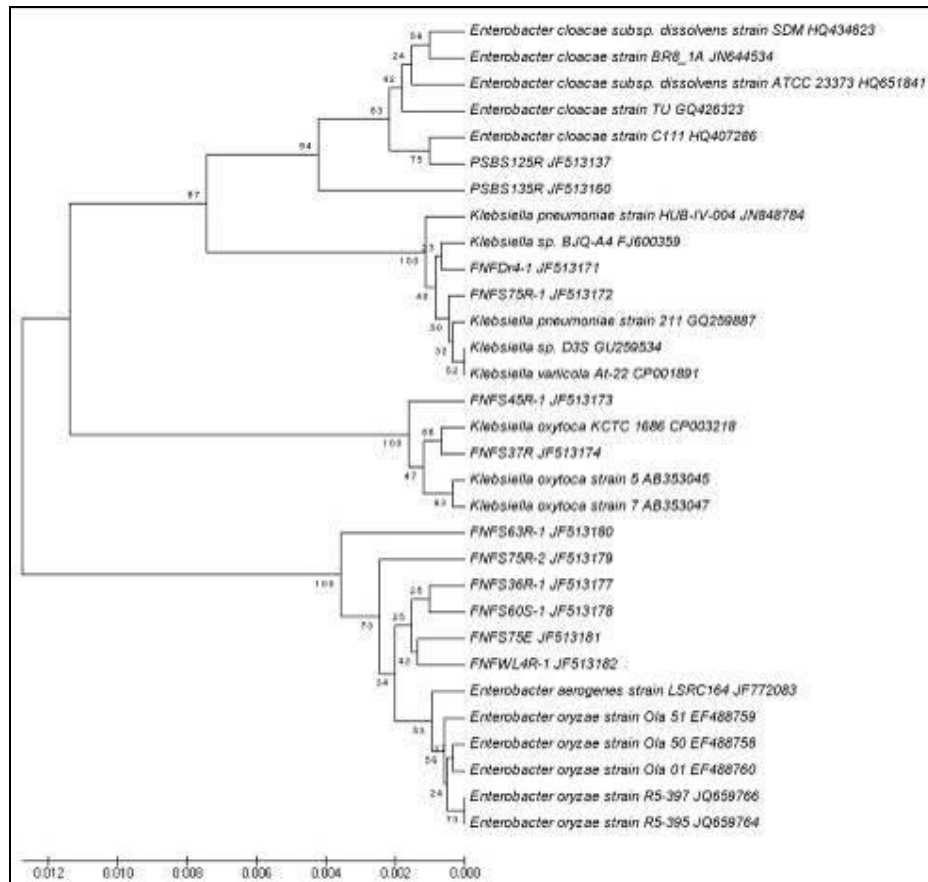


Fig. 6b. UPGMA Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of salt-tolerant AIMs to other related species of bacteria belonging to family Enterobacteriaceae of class alpha-proteobacteria. Significant bootstrap probability values are indicated at the branching point

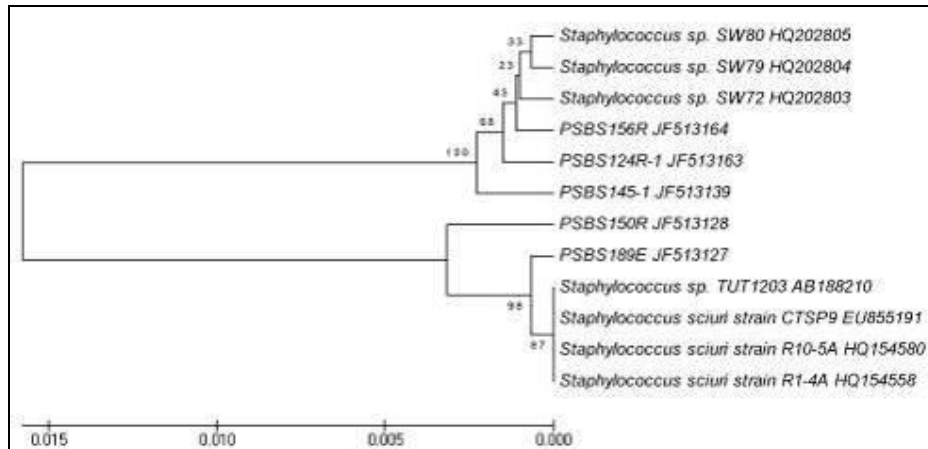


Fig. 7a. UPGMA Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of salt-tolerant AIMs to other related species of bacteria belonging to family Staphylococcaceae of class Bacilli. Significant bootstrap probability values are indicated at the branching point

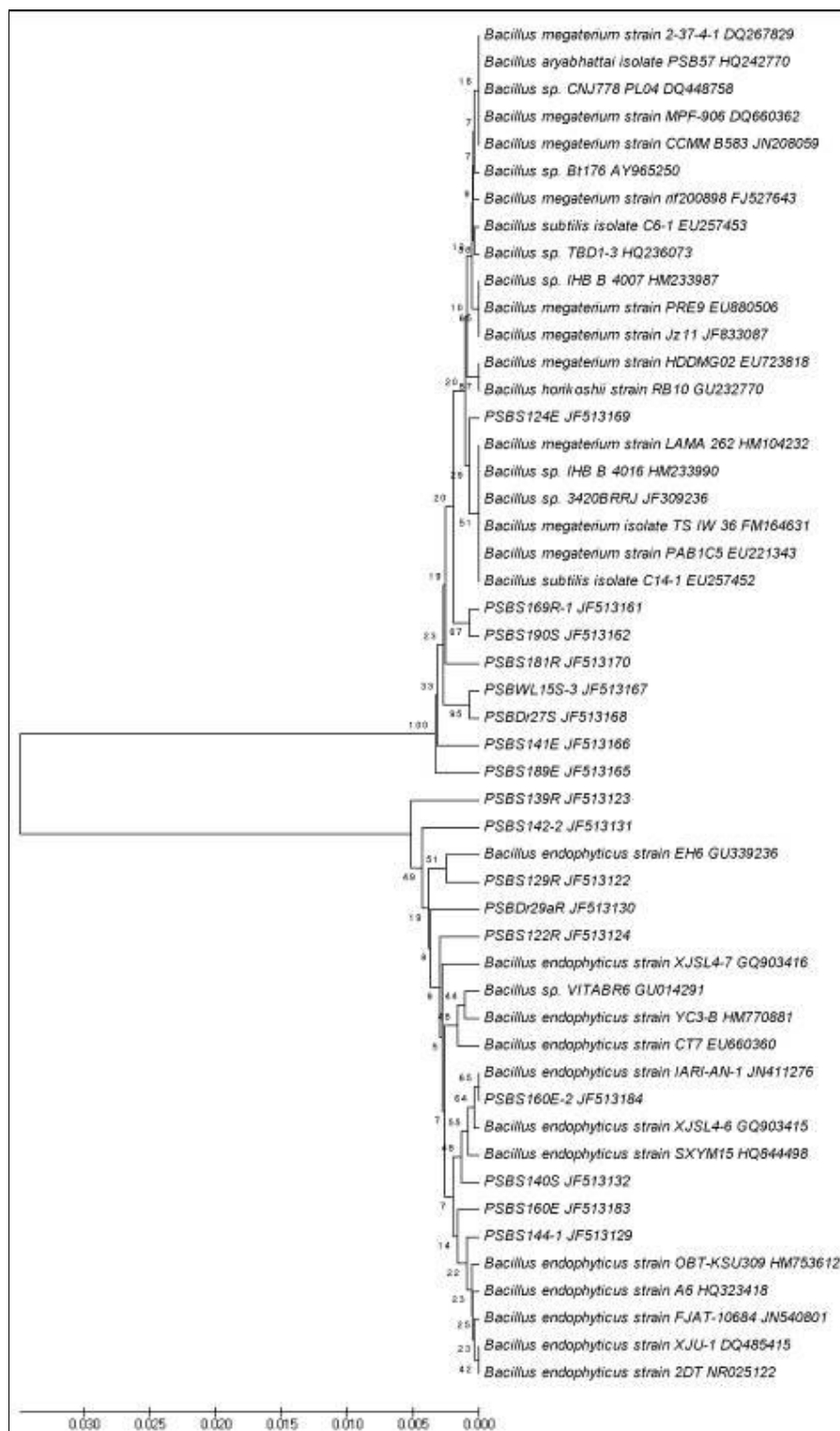


Fig. 7b. UPGMA Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of salt-tolerant AIMs to other related species of bacteria belonging to family Bacillaceae of class Bacilli. Significant bootstrap probability values are indicated at the branching point

Table 10a. Nitrogen fixation ($\mu\text{g}/\text{mg}$ protein) by salt tolerant associative N_2 fixing isolates at different salt concentrations

Isolate	NaCl concentrations (%)				Mean
	0.002	5	7.5	10	
ASANFX S145E	122.5	92.9	112.1	93.1	105.1
ASANFX S148E	116.4	92.9	103.5	76.6	97.4
ASANFXI S173E	114.8	92.1	101.1	72.5	95.1
ASANFIX S125E	113.1	90.1	80.6	0.0	70.9
ASANFXI WL19E	107.9	104.4	58.9	0.0	67.8
ASANFX Dr31E	111.5	83.5	71.1	0.0	66.5
ASANFIX S132E	85.4	65.6	38.7	0.0	47.4
ASANFX S135E	110.9	110.1	60.1	0.0	70.3
ASANFX S138E	59.8	64.7	45.9	0.0	42.6
ASANFX S141E	87.3	59.3	30.4	0.0	44.2
ASANFX S149E	71.9	65.7	30.1	0.0	41.9
ASANFX S150E	66.5	60.9	24.3	0.0	37.9
ASANFX S154E	70.9	66.2	41.9	0.0	44.8
ASANFX WL13E	98.1	109.1	70.8	0.0	69.5
ASANFX WL15E	96.2	92.7	52.8	0.0	60.4
ASANFXI S160E	74.9	68.7	52.4	0.0	49.0
ASANFXI S170E	59.2	49.3	33.2	0.0	35.4
ASANFXI WL17E	86.0	85.3	48.8	0.0	55.0
ASANFII S22E	103.7	83.7	0.0	0.0	46.8
ASANFIV S40E	109.2	99.3	0.0	0.0	52.1
ASANFIV S61E	109.3	80.2	0.0	0.0	47.4
ASANFIV S78E	85.7	94.3	0.0	0.0	45.0
ASANFV Dr18bE	136.2	98.7	0.0	0.0	58.7
ASANFX Dr29aE	96.8	58.4	0.0	0.0	38.8
ASANFIV Dr8E	66.0	30.1	0.0	0.0	24.0
ASANFII S26E	77.6	52.6	0.0	0.0	32.5
ASANFIV S63E	81.2	58.7	0.0	0.0	35.0
ASANFIV S83E	83.2	59.5	0.0	0.0	35.7
ASANFV WL12E	93.5	73.6	0.0	0.0	41.8
ASANFIX S121E	91.5	73.4	0.0	0.0	41.2
ASANFX S137E	69.6	43.7	0.0	0.0	28.3
ASANFX S144E	70.9	41.6	0.0	0.0	28.1
ASANFXI S158E	87.0	66.6	0.0	0.0	38.4
ASANFXI S167E	62.7	32.7	0.0	0.0	23.9
ASANFXI S182E	89.7	71.0	0.0	0.0	40.2
ASANFXI S187E	92.0	75.2	0.0	0.0	41.8
Mean	90.5	73.5	29.4	6.7	
	NaCl (S)		Isolate (I)		S x I
S.Em. $\pm$	1.3		3.8		7.7
C.D.(0.01)	4.7		14.2		28.3

Lsulates ASFNFI Dr4(1) and ASFNFIV S63(1)R showed high N₂ fixation with mean N₂ fixation of 349 and 339.8 µg N₂ per mg protein respectively and both of them were found to be on par with each other but significantly superior over most other isolates (Table 10b). Among different salt concentrations tested, the maximum mean N₂ fixation of 275.5 µg per mg protein was recorded in the control medium which was significantly superior over all NaCl concentrations tested. The interaction effect was found to be significant wherein the strain ASFNFIV S75(1)R recorded maximum N₂ fixation of 671 µg per mg protein in the presence of 7.5 per cent NaCl which was significantly superior over rest of the combinations.

4.2.2 Phosphate solubilization

A total of 81 isolates positive for P-solubilization (55 PSB, 23 FP and 3 PSF) were examined for their ability to release Pi from TCP in broth supplemented with different concentrations of NaCl. The results obtained from the study are presented in Tables 11a, b and c.

4.2.2.1 Phosphate solubilizing bacteria (PSB)

All 55 PSB isolates were tested for their ability to solubilize TCP in presence of different concentrations of NaCl in Pikovskaya's broth. The amount of Pi released, irrespective of isolates, was found to increase with increase in NaCl concentration up to 7.5 per cent and declined thereafter (Table 11a). While all 55 isolates showed P-solubilization in the presence of up to 10% NaCl, 23 isolates released Pi in the broth with 12.5% NaCl and only six isolates performed the function at 15% NaCl. However, the isolate ASPSBIX S122R showed increased Pi release even up to 12.5 per cent NaCl. The amount of Pi released by the isolates under salt stress ranged from 3.5 to 21.7, 3.2 to 19.9, 3.1 to 16.4, 0.0 to 19 and 0.0 to 13.4 per cent in the presence of 5, 7.5, 10, 12.5 and 15 per cent NaCl respectively as compared to 3.2 to 22.3 per cent in normal broth (without added NaCl).

Among all the isolates, ASPSBIX S125R found to be significantly superior over all other strains and recorded the highest mean Pi release of 17.4 per cent (Table 11a). Four isolates viz., ASPSBIX S124E, ASPSBIX S122R, ASPSBIX S156R and ASPSBIX Dr29aR were found to release 13.1, 12.5, 12.1 and 11.4 per cent Pi respectively and all of them were found to be on par with each other. Among different salt concentrations tested, the maximum Pi release of 10.8 per cent was recorded in the medium supplemented with 5 per cent NaCl which was significantly superior over all other NaCl concentrations and also over control. The interaction effect was found to be significant wherein the strain ASPSBIX S125R recorded maximum Pi release of 22.3 per cent in the control broth and was significantly superior over rest of the combinations (Table 11a).

4.2.2.2 P-solubilizing fluorescent pseudomonads

Out of 61 FP isolates, 23 found to be positive for P-solubilization in the qualitative test at 5 and 7.5% NaCl were examined for quantitative Pi release. The amount of Pi released by the isolates ranged from 1.32 to 15.64 and 0.00 to 12.39 per cent in the presence of 5 and 7.5 per cent NaCl respectively as compared to 4.37 to 18.34 per cent in control broth (Table 11b).

Irrespective of NaCl concentrations, maximum Pi release was recorded by the isolate ASFPV Dr10E (13.23%) which was significantly superior over all other isolates except ASFPV Dr10R (12.70%) with which it was on par. Isolates ASFPXI S174S (11.62%), ASFPI S6(1)S (11.51%), ASFPI DR6(8)S (10.82%) and ASFPI S3(2)S (10.54%) also recorded significantly higher Pi release over several other isolates. In general, the amount of Pi release was found to decrease with increase in NaCl concentration (Table 11b).

Among all the treatment combinations, ASFPI DR6(8)S without any added NaCl recorded maximum Pi release of 18.34 per cent and was significantly superior over all other treatment combinations except ASFPXI S174S (17.71%) and ASFPV Dr10R (16.04%) both in control medium with which it was on par (Table 11b).

4.2.2.3 P-solubilizing fungi (PSF)

Out of 18 PSF isolates, only three were found to be positive for P-solubilization at 5% NaCl in qualitative test and were further examined for quantitative estimation with 5% NaCl. The amount of Pi release was drastically reduced at 5% NaCl (Table 11c).

Table 10b: Nitrogen fixation (µg/mg protein) by salt tolerant free-living N₂ fixing isolates at different salt concentrations

Isolate	NaCl concentrations (%)						Mean
	0.02	5	7.5	10	12.5	15.0	
ASFNFIV S63(1)R	314	216	347	310	405	447	339.8
ASFNFIV S75(1)R	472	439	671	509	500	0	431.8
ASFNFIV S37R	516	349	297	315	0	0	246.2
ASFNFIV WL4(1)R	533	508	524	365	0	0	321.7
ASFNFI Dr4(1)	541	456	571	526	0	0	349.0
ASFNFIV S45(1)R	454	355	611	469	0	0	314.8
ASFNFIV S36(1)R	405	340	255	0	0	0	166.7
ASFNFIV S40(1)R	359	272	159	0	0	0	131.7
ASFNFIV S43(2)R	506	469	409	0	0	0	230.7
ASFNFIV S60(1)S	406	350	280	0	0	0	172.7
ASFNFIV S61(1)S	454	397	311	0	0	0	193.7
ASFNFIV S75(2)R	276	200	119	0	0	0	99.2
ASFNFIV S75E	279	188	82	0	0	0	91.5
ASFNFIV WL5(2)R	369	310	242	0	0	0	153.5
ASFNFI S127R	340	292	0	0	0	0	105.3
ASFNFX Dr31R	66	303	0	0	0	0	61.5
ASFNFXI S174E	144	210	0	0	0	0	59.0
ASFNFXI WL16E	220	154	0	0	0	0	62.3
ASFNFXI S161E	144	155	0	0	0	0	49.8
ASFNFXII S190S	149	169	0	0	0	0	53.0
ASFNFXII S178E	215	120	0	0	0	0	55.8
ASFNFXII S190E	239	116	0	0	0	0	59.2
ASFNFI S17(2)S	175	100	0	0	0	0	45.8
ASFNFI Dr2(5)	218	107	0	0	0	0	54.2
ASFNFI Dr3(3)	77	74	0	0	0	0	25.2
ASFNFI S19(2)S	315	235	0	0	0	0	91.7
ASFNFII S22S	203	131	0	0	0	0	55.7
ASFNFII S22R	161	120	0	0	0	0	46.8
ASFNFIII S32S	150	99	0	0	0	0	41.5
ASFNFIII S31R	211	174	0	0	0	0	64.2
ASFNFIII S33E	251	220	0	0	0	0	78.5
ASFNFIV WL3S	170	128	0	0	0	0	49.7
ASFNFIV S40(2)R	201	173	0	0	0	0	62.3
ASFNFIV S62E	364	354	0	0	0	0	119.7
ASFNFIV S44R	277	254	0	0	0	0	88.5
ASFNFIV S57(2)R	94	51	0	0	0	0	24.2
ASFNFV S85R	111	69	0	0	0	0	30.0
ASFNFIV S66R	146	103	0	0	0	0	41.5
ASFNFIV S80(1)R	207	171	0	0	0	0	63.0
ASFNFIV WL5(1)R	318	297	0	0	0	0	102.5
ASFNFIV WL6R	304	279	0	0	0	0	97.2
ASFNFIV WL7(2)R	340	324	0	0	0	0	110.7
ASFNFIV Dr8R	152	111	0	0	0	0	43.8
Mean	275.5	231.2	113.4	58.0	21.0	10.4	
	NaCl (S)		Isolate (I)			S x I	
S.Em.±	1.8		4.8			11.7	
C.D.(0.01)	6.5		17.5			42.8	

Table 11a. Per cent Pi released from TCP in broth by P-solubilizing bacteria at different salt concentrations

Isolate	NaCl concentrations (%)						Mean
	0	5	7.5	10	12.5	15.0	
ASPSBIX S122R	6.5	7.4	12.5	16.4	19.0	13.4	12.5
ASPSBIX S124E	16.0	15.4	14.3	13.3	10.2	9.4	13.1
ASPSBIX S125R	22.3	21.7	19.9	16.0	15.1	9.5	17.4
ASPSBXI S156R	18.9	18.2	16.5	11.0	5.7	2.5	12.1
ASPSBXI S181R	9.6	8.2	6.4	4.0	1.9	1.6	5.3
ASPSBXII S189E	8.1	7.4	5.2	3.4	2.0	1.0	4.5
ASPSBIX S129R	10.2	12.5	10.5	8.1	5.1	0.0	7.7
ASPSBX S135R	11.6	14.1	12.3	10.2	6.9	0.0	9.2
ASPSBX S139R	9.8	12.5	10.1	8.1	5.1	0.0	7.6
ASPSBX S150R	10.2	12.5	10.1	8.3	5.6	0.0	7.8
ASPSBX Dr29aR	14.6	17.2	14.6	12.6	9.6	0.0	11.4
ASPSBX S142(2)S	13.1	15.9	14.0	12.1	9.1	0.0	10.7
ASPSBX S140S	10.2	12.6	10.1	8.6	5.3	0.0	7.8
ASPSBX S144(1)S	11.6	14.1	11.6	9.3	6.2	0.0	8.8
ASPSBX S145(1)S	8.1	11.2	9.1	7.1	4.1	0.0	6.6
ASPSBX Dr29aS	10.2	12.6	10.5	8.6	5.6	0.0	7.9
ASPSBX WL15(3)S	11.6	14.6	11.9	10.1	7.2	0.0	9.2
ASPSBX Dr27S	13.1	16.2	13.9	12.0	8.9	0.0	10.7
ASPSBX S141E	12.6	15.6	13.2	11.3	8.1	0.0	10.1
ASPSBXI S169(1)R	11.6	14.5	12.4	10.5	7.3	0.0	9.4
ASPSBXI S172(2)S	8.1	11.2	8.5	6.3	3.2	0.0	6.2
ASPSBXII Dr33S	10.2	13.1	10.8	8.6	5.5	0.0	8.0
ASPSBXII S190S	7.8	10.9	8.6	6.3	3.2	0.0	6.1
ASPSBIX S122(1)R	7.8	9.1	10.3	6.2	0.0	0.0	5.6
ASPSBIX S124(1)R	8.6	10.1	11.2	7.1	0.0	0.0	6.2
ASPSBIX S124(2)S	7.8	9.2	10.3	6.2	0.0	0.0	5.6
ASPSBIX S125E	9.3	10.5	12.2	7.9	0.0	0.0	6.7
ASPSBIX S125(6)S	8.6	9.6	11.6	7.6	0.0	0.0	6.2
ASPSBIX S129(1)R	10.2	11.3	12.8	8.9	0.0	0.0	7.2
ASPSBIX S132(2)S	11.6	12.8	14.2	10.3	0.0	0.0	8.2

Contd...

(Table 11a contd....)

Isolate	NaCl concentrations (%)						Mean
	0	5	7.5	10	12.5	15.0	
ASPSBX S138R	8.6	10.0	11.3	6.9	0.0	0.0	6.1
ASPSBX S141R	9.2	10.3	12.0	7.8	0.0	0.0	6.6
ASPSBX S150S	7.8	9.7	10.8	6.3	0.0	0.0	5.8
ASPSBX S138(1)S	5.6	7.2	11.1	4.8	0.0	0.0	4.8
ASPSBX S135S	6.3	7.8	12.6	5.6	0.0	0.0	5.4
ASPSBX S137(1)S	5.6	7.1	7.2	5.9	0.0	0.0	4.3
ASPSBX Dr31(1)S	6.5	7.5	7.8	5.8	0.0	0.0	4.6
ASPSBX S147S	6.4	7.6	6.4	6.0	0.0	0.0	4.4
ASPSBX Dr32S	6.7	6.2	5.2	4.8	0.0	0.0	3.8
ASPSBX WL15(2)	6.4	7.4	6.0	4.8	0.0	0.0	4.1
ASPSBX Dr27(1)S	5.7	5.9	6.3	4.8	0.0	0.0	3.8
ASPSBX S138(1)	5.6	7.2	11.1	4.8	0.0	0.0	4.8
ASPSBX S139(1)S	6.6	4.8	4.1	3.3	0.0	0.0	3.1
ASPSBX S141(1)S	4.2	3.5	3.2	3.2	0.0	0.0	2.4
ASPSBXI S158S	5.2	4.2	3.4	3.3	0.0	0.0	2.7
ASPSBXI S160(2)E	15.7	16.7	17.0	8.1	0.0	0.0	9.6
ASPSBXI S177E	6.3	5.6	4.8	4.3	0.0	0.0	3.5
ASPSBXI WL18E	3.2	6.5	4.0	3.1	0.0	0.0	2.8
ASPSBXI S187(2)	6.9	15.0	4.9	4.6	0.0	0.0	5.2
ASPSBXI WL19E	9.5	14.3	15.9	13.6	0.0	0.0	8.9
ASPSBXI S160(1)E	6.3	6.5	7.2	4.9	0.0	0.0	4.2
ASPSBXI WL20S	3.9	7.1	7.5	5.6	0.0	0.0	4.0
ASPSBXI S160S	12.7	17.0	14.9	13.3	0.0	0.0	9.7
ASPSBXI S175E	7.5	9.8	15.4	6.4	0.0	0.0	6.5
ASPSBXI S176S	6.5	8.2	5.9	3.2	0.0	0.0	4.0
Mean	9.2	10.8	10.3	7.7	2.9	0.7	
	NaCl (S)		Isolate (I)			S x I	
S.Em.±	0.13		0.39			0.95	
C.D.(0.01)	0.47		1.42			3.49	

Table 11b. Per cent Pi released from TCP in broth by fluorescent pseudomonads at different salt concentrations

Isolate	NaCl concentrations (%)			Mean
	0	5	7.5	
ASFPI S3(1)S	4.37	8.89	7.78	7.01
ASFPI S3(2)S	14.77	10.96	5.88	10.54
ASFPI S4(1)S	5.64	7.70	9.92	7.75
ASFPI S6(1)S	10.80	15.64	8.10	11.51
ASFPI S14(3)S	5.64	1.32	0.00	2.32
ASFPI DR6(8)S	18.34	9.53	4.60	10.82
ASFPV S35R	5.40	7.07	3.97	5.48
ASFPV S85R	5.64	7.38	3.81	5.61
ASFPV S112(a)R	11.51	9.21	7.30	9.34
ASFPV Dr10R	16.04	13.81	8.26	12.70
ASFPV Dr10E	11.67	15.64	12.39	13.23
ASFPXI S134(3)S	4.37	2.13	0.00	2.17
ASFPXI S155S	5.60	2.13	0.00	2.58
ASFPXI S156E	8.93	3.36	0.00	4.10
ASFPXI S158(1)R	10.20	2.13	0.00	4.11
ASFPXI S161(2)R	10.20	4.60	0.00	4.93
ASFPXI S163S	8.89	4.37	0.00	4.42
ASFPXI S172R	10.20	3.36	0.00	4.52
ASFPXI S174S	17.71	11.67	5.48	11.62
ASFPXI S180E	6.83	2.13	0.00	2.99
ASFPXI S183R	5.60	2.13	0.00	2.58
ASFPXI S186S	6.83	4.60	0.00	3.81
ASFPXI DR33R	4.53	7.38	4.76	5.56
Mean	9.12	6.83	3.58	
	NaCl (S)	Isolate (I)		S x I
S.Em.±	0.13	0.37		0.64
C.D.(0.01)	0.50	1.39		2.41

Table 11c. Per cent Pi released from TCP in broth by P-solubilizing fungi at different salt concentrations

Isolate	NaCl concentrations (%)	
	0	5
PSF WL9S	25.41	19.21
PSF Dr12bR	17.55	7.86
PSF S114S	19.13	9.61

Among all the PSF isolates, PSF WL9S showed maximum P-solubilization of 25.4 and 19.2 per cent with and without added NaCl respectively followed by PSF S114S which recorded Pi release of 19.1 and 9.6 per cent in the control and at 5 per cent NaCl respectively.

4.2.3 Antagonistic activity against plant pathogenic fungi

All the 61 fluorescent pseudomonad isolates were examined for their potential to inhibit five fungal plant pathogens under *in-vitro* conditions. Out of 61 isolates, 18 exhibited inhibition potential against one or the other pathogen while the remaining 43 isolates did not show inhibitory activity against any of the pathogens tested (Table 12). Out of these 18 antagonistic isolates, 6 were inhibitory to *Alternaria carthami*, 17 to *Fusarium oxysporum* f. sp. *carthami*, 11 to *Sclerotium rolfsii*, 5 to *Rhizoctonia bataticola* and 11 to *Pyricularia oryzae*. Fifteen isolates showed inhibition potential against more than one pathogen whereas 3 isolates had inhibitory activity against only one of the five pathogens tested (Plate 2). Of the 18 isolates showing antagonistic activity, five isolates (ASFPV S94(2)E, ASFPXI S134(3)S, ASFPXI S158(1)R, ASFPXI S174(1)R and ASFPXI S187S) showed inhibition potential against all the five pathogens tested. While two isolates were inhibitory to three pathogens each and 8 inhibited two pathogens each.

The data on zone of inhibition (ZOI) of various fungal plant pathogens by the fluorescent pseudomonad isolates are shown in Table 12. Among the isolates inhibitory to *A. carthami*, ASFPV S94(2)E was highly inhibitory recording the inhibition zone of 5.2 cm followed by ASFPXI S158(1)R which recorded ZOI of 4.5 cm and both of them were found to be superior over rest of the isolates. Of the 17 isolates antagonistic towards *F. oxysporum* f. sp. *carthami*, six isolates viz., ASFPI S3(1)S (5.0 cm), ASFPI S15(1)S (5.0 cm), ASFPV S94(2)E (5.1 cm), ASFPV S100E (6.7 cm), ASFPXI S174(1)R (5.8 cm) and ASFPXI S178(1)S (6.1 cm) showed greater inhibitory activity over other isolates (inhibition zone ranging from 3.6 to 4.9 cm). Similarly, out of 11 isolates antagonistic towards *S. rolfsii*, two isolates ASFPV S94(2)E and ASFPXI S158(1)R recorded the zone of inhibition of 5.2 and 5.7 cm respectively and the remaining 9 isolates were observed to have inhibition zone ranging from 2.3 to 4.8 cm.

Among five isolates found inhibitory to *R. bataticola*, ASFPV S94(2)E recorded the maximum ZOI (4.1 cm) as compared to other isolates. Similarly, out of 11 isolates inhibitory to *Pyricularia oryzae*, two isolates ASFPXI S169(2)S and ASFPXI S158(1)R were found to have maximum ZOI of 3.2 and 2.9 cm respectively when compared to other isolates whose ZOI ranged from 1.2 to 2.7 cm.

4.2.4 Phytohormone production

A total of 195 isolates consisting of 36 ANF, 43 FNF, 55 PSB and 61 FP isolates were subjected for indole 3-acetic acid (IAA) and gibberlic acid (GA) production in the presence of different concentrations of NaCl. The results obtained from the study are presented in Tables 13 (a to d) and 14 (a to d).

4.2.4.1 Indole 3-acetic acid (IAA)

4.2.4.1.1 Associative nitrogen fixers (ANF)

All the 36 isolates found positive for IAA production in the qualitative test were further examined for the amount of IAA produced in presence of different concentrations of NaCl in Czepak's broth after 7 days of incubation. The amount of IAA produced in general was found to be higher with 5% NaCl as compared to other concentrations and control. While all the isolates produced IAA in the presence of 5% NaCl, only 18 and 3 isolates produced IAA at 7.5 and 10% NaCl respectively. The amount of IAA produced by the isolates under salt stress ranged from 3.5 to 12.7 µg, 0.0 to 10.4 µg and 0.0 to 5.3 µg per mg protein in the presence of 5, 7.5 and 10 per cent NaCl respectively as compared to 4.3 to 10.1 µg per mg protein in control (without added NaCl) (Table 13a).

Irrespective of NaCl concentrations, two isolates viz., ASANFX S148E and ASANFX S138E produced maximum IAA of 7.7 µg per mg protein in broth followed two other isolates viz., ASANFX S149E and ASANFX S160E (7.5 µg per mg protein in broth) and all the four isolates were found to be on par with each other (Table 13a).

Table 12. Inhibitory activity of salt-tolerant fluorescent pseudomonads against plant pathogenic fungi

Isolate	<i>Alternaria carthami</i>	<i>F. oxysporum f.sp. carthami</i>	<i>Sclerotium rolfsii</i>	<i>Rhizoctonia bataticola</i>	<i>Pyricularia oryzae</i>
ASFPI S3(1)S	-	5.0*	3.4	-	-
ASFPI S3(2)S	-	-	-	-	-
ASFPI S4(1)S	-	3.6	-	-	1.5
ASFPI S6(1)S	-	-	-	-	-
ASFPI S9(1)S	-	4.5	-	-	-
ASFPI S14(3)S	-	-	-	-	-
ASFPI S15(1)S	-	5.0	-	-	2.7
ASFPI Dr6(8)S	-	-	-	-	-
ASFPV S35R	-	-	-	-	-
ASFPV S85R	-	-	-	-	-
ASFPV S94(2)E	5.2	5.1	5.2	4.1	1.7
ASFPV S97R	-	-	-	-	-
ASFPV S100E	-	6.7	4.3	-	1.2
ASFPV S112(a)R	-	-	-	-	-
ASFPV S158(2)R	-	-	-	-	-
ASFPV Dr10R	-	-	-	-	-
ASFPV Dr10E	-	-	-	-	-
ASFPV Dr16E	-	-	-	-	-
ASFPIX S121(3)S	-	-	-	-	-
ASFPIX S121R	-	-	-	-	-
ASFPIX S131S	-	-	-	-	-
ASFPIX S134(3)S	3.1	4.9	4.2	3.5	2.7
ASFPX S149S	-	3.8	-	-	-
ASFPXI S155S	-	-	-	-	-
ASFPXI S156R	-	-	-	-	-
ASFPXI S156E	-	-	-	-	-
ASFPXI S157(1)S	-	-	-	-	-
ASFPXI S157(2)R	-	-	-	-	-
ASFPXI S158(1)R	4.5	4.9	5.7	3.5	2.9
ASFPXI S161(1)R	-	-	-	-	-
ASFPXI S161(2)R	-	-	-	-	-
ASFPXI S163S	-	4.1	-	-	-
ASFPXI S164S	-	-	-	-	-
ASFPXI S164(b)E	-	-	-	-	-
ASFPXI S167(2)S	-	-	-	-	-

Contd...

(Table 12 contd....)

Isolate	<i>Alternaria carthami</i>	<i>F. oxysporum f.sp. carthami</i>	<i>Sclerotium rolfsii</i>	<i>Rhizoctonia bataticola</i>	<i>Pyricularia oryzae</i>
ASFPXI S168R	-	-	-	-	-
ASFPXI S169(2)S	-	3.9	-	-	3.2
ASFPXI S170R	-	-	-	-	-
ASFPXI S172R	-	4.9	-	-	2.6
ASFPXI S173S	-	-	-	-	-
ASFPXI S174S	-	-	-	-	-
ASFPXI S174(1)R	2.8	5.8	3.6	2.6	1.7
ASFPXI S174(2)R	-	-	-	-	-
ASFPXI S176S	-	-	-	-	-
ASFPXI S178(1)S	-	6.1	4.8	-	2.2
ASFPXI S178(2)S	-	-	-	-	-
ASFPXI S178R	-	-	-	-	-
ASFPXI S178E	-	-	-	-	-
ASFPXI S180S	-	-	-	-	-
ASFPXI S180E	-	-	-	-	-
ASFPXI S181R	-	-	-	-	-
ASFPXI S183R	8.0	-	9.0	-	-
ASFPXI S183E	-	4.6	3.7	-	-
ASFPXI S184R	-	-	-	-	-
ASFPXI S186S	-	-	-	-	-
ASFPXI S187S	2.4	4.3	3.9	3.9	1.6
ASFPXI WL16R	-	-	-	-	-
ASFPXI WL16(1)R	-	-	-	-	-
ASFPXI WL17R	-	3.6	3.0	-	-
ASFPXII S190R	-	-	-	-	-
ASFPXII Dr33R	-	-	-	-	-

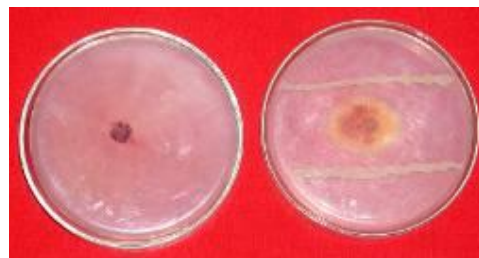
* Inhibition zone in cm
- No inhibition



Rhizoctonia bataticola



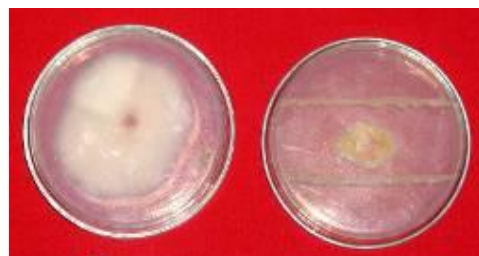
Sclerotium rolfsii



Pyricularia oryzae



Alternaria carthanuii



Fusarium oxysporium

Plate 2. Inhibition of fungal plant pathogens by salt tolerant fluorescent pseudomonad isolate ASFPVS94(2)E

Table 13a. Amount of IAA ($\mu\text{g}/\text{mg}$ protein) produced by salt tolerant associative N_2 fixers at different salt concentrations

Isolate	NaCl concentrations (%)				Mean
	0	5	7.5	10	
ASANFX S145E	7.7	8.4	7.0	5.0	7.0
ASANFX S148E	8.6	9.4	7.4	5.3	7.7
ASANFXI S173E	6.9	7.4	7.3	5.3	6.7
ASANFIX S125E	8.4	9.3	8.6	0.0	6.6
ASANFXI WL19E	9.1	10.2	5.0	0.0	6.1
ASANFX Dr31E	10.1	11.7	5.2	0.0	6.7
ASANFIX S132E	6.4	6.8	9.0	0.0	5.5
ASANFX S135E	7.4	8.0	9.7	0.0	6.3
ASANFX S138E	9.6	10.8	10.4	0.0	7.7
ASANFX S141E	9.3	10.3	8.6	0.0	7.1
ASANFX S149E	9.2	10.4	10.4	0.0	7.5
ASANFX S150E	8.5	9.6	6.8	0.0	6.2
ASANFX S154E	6.5	6.9	5.0	0.0	4.6
ASANFX WL13E	7.9	8.8	2.8	0.0	4.9
ASANFX WL15E	7.8	8.6	3.5	0.0	5.0
ASANFXI S160E	10.1	11.6	8.4	0.0	7.5
ASANFXI S170E	7.4	8.0	2.6	0.0	4.5
ASANFXI WL17E	5.9	6.2	4.6	0.0	4.2
ASANFII S22E	7.1	10.3	0.0	0.0	4.3
ASANFIV S40E	7.1	9.6	0.0	0.0	4.2
ASANFIV S61E	6.8	10.6	0.0	0.0	4.3
ASANFIV S78E	7.9	12.7	0.0	0.0	5.1
ASANFV Dr18bE	6.4	8.3	0.0	0.0	3.7
ASANFX Dr29aE	6.2	6.2	0.0	0.0	3.1
ASANFIV Dr8E	4.8	3.6	0.0	0.0	2.1
ASANFII S26E	5.0	4.6	0.0	0.0	2.4
ASANFIV S63E	5.1	4.9	0.0	0.0	2.5
ASANFIV S83E	5.3	5.1	0.0	0.0	2.6
ASANFV WL12E	5.8	5.9	0.0	0.0	2.9
ASANFIX S121E	5.5	5.6	0.0	0.0	2.8
ASANFX S137E	4.6	4.0	0.0	0.0	2.1
ASANFX S144E	4.8	4.1	0.0	0.0	2.2
ASANFXI S158E	5.4	5.3	0.0	0.0	2.7
ASANFXI S167E	4.3	3.5	0.0	0.0	1.9
ASANFXI S182E	5.4	5.5	0.0	0.0	2.7
ASANFXI S187E	5.4	5.6	0.0	0.0	2.7
Mean	6.9	7.7	3.4	0.4	
	NaCl (S)		Isolate (I)		S x I
S.Em. $\pm$	0.1		0.3		0.6
C.D.(0.01)	0.4		1.1		2.2

Table 13b. Amount of IAA ($\mu\text{g}/\text{mg}$ protein) produced by salt tolerant free-living N_2 fixers at different salt concentrations

Isolate	NaCl concentrations (%)						Mean
	0	5	7.5	10	12.5	15.0	
ASFNFIV S63(1)R	6.7	6.6	12.7	8.8	8.2	7.6	8.4
ASFNFIV S75(1)R	7.7	7.0	8.9	9.0	7.1	0.0	6.6
ASFNFIV S37R	5.2	4.3	7.5	6.6	0.0	0.0	3.9
ASFNFIV WL4(1)R	7.4	6.1	8.1	9.0	0.0	0.0	5.1
ASFNFI Dr4(1)	11.6	6.6	10.0	11.8	0.0	0.0	6.7
ASFNFIV S45(1)R	9.6	7.8	12.9	12.6	0.0	0.0	7.2
ASFNFIV S36(1)R	7.9	5.6	2.6	0.0	0.0	0.0	2.7
ASFNFIV S40(1)R	8.3	4.7	2.8	0.0	0.0	0.0	2.6
ASFNFIV S43(2)R	12.3	7.6	7.5	0.0	0.0	0.0	4.6
ASFNFIV S60(1)S	8.5	5.4	5.8	0.0	0.0	0.0	3.3
ASFNFIV S61(1)S	6.5	4.4	4.1	0.0	0.0	0.0	2.5
ASFNFIV S75(2)R	9.1	4.5	2.2	0.0	0.0	0.0	2.6
ASFNFIV S75E	7.2	7.5	5.4	0.0	0.0	0.0	3.4
ASFNFIV WL5(2)R	8.3	7.6	5.2	0.0	0.0	0.0	3.5
ASFNFI S127R	5.7	4.5	0.0	0.0	0.0	0.0	1.7
ASFNFX Dr31R	6.2	4.9	0.0	0.0	0.0	0.0	1.9
ASFNFXI S174E	7.2	6.1	0.0	0.0	0.0	0.0	2.2
ASFNFXI WL16E	5.9	4.3	0.0	0.0	0.0	0.0	1.7
ASFNFXI S161E	6.0	4.7	0.0	0.0	0.0	0.0	1.8
ASFNFXII S190S	4.7	3.2	0.0	0.0	0.0	0.0	1.3
ASFNFXII S178E	5.7	4.4	0.0	0.0	0.0	0.0	1.7
ASFNFXII S190E	5.4	4.0	0.0	0.0	0.0	0.0	1.6
ASFNFI S17(2)S	5.8	4.6	0.0	0.0	0.0	0.0	1.7
ASFNFI Dr2(5)	6.2	5.1	0.0	0.0	0.0	0.0	1.9
ASFNFI Dr3(3)	6.1	4.9	0.0	0.0	0.0	0.0	1.8
ASFNFI S19(2)S	6.7	5.5	0.0	0.0	0.0	0.0	2.0
ASFNFII S22S	6.0	4.6	0.0	0.0	0.0	0.0	1.8
ASFNFII S22R	6.2	4.9	0.0	0.0	0.0	0.0	1.9
ASFNFIII S32S	7.1	5.8	0.0	0.0	0.0	0.0	2.2
ASFNFIII S31R	6.9	5.6	0.0	0.0	0.0	0.0	2.1
ASFNFIII S33E	6.2	4.7	0.0	0.0	0.0	0.0	1.8
ASFNFIV WL3S	5.4	3.7	0.0	0.0	0.0	0.0	1.5
ASFNFIV S40(2)R	4.2	2.6	0.0	0.0	0.0	0.0	1.1
ASFNFIV S62E	5.1	3.1	0.0	0.0	0.0	0.0	1.4
ASFNFIV S44R	6.0	4.6	0.0	0.0	0.0	0.0	1.8
ASFNFIV S57(2)R	5.4	4.0	0.0	0.0	0.0	0.0	1.6
ASFNFV S85R	4.7	3.2	0.0	0.0	0.0	0.0	1.3
ASFNFIV S66R	5.1	3.5	0.0	0.0	0.0	0.0	1.4
ASFNFIV S80(1)R	5.1	3.3	0.0	0.0	0.0	0.0	1.4
ASFNFIV WL5(1)R	7.1	5.7	0.0	0.0	0.0	0.0	2.1
ASFNFIV WL6R	6.4	4.7	0.0	0.0	0.0	0.0	1.9
ASFNFIV WL7(2)R	5.2	3.0	0.0	0.0	0.0	0.0	1.4
ASFNFIV Dr8R	4.9	3.3	0.0	0.0	0.0	0.0	1.4
Mean	6.6	4.9	2.2	1.3	0.4	0.2	
	NaCl (S)		Isolate (I)			S x I	
S.Em. $\pm$	0.1		0.2			0.5	
C.D.(0.01)	0.3		0.7			1.7	

Among different salt concentrations tested, the maximum IAA production of 7.7 µg per mg protein was recorded at 5 per cent NaCl and was significantly superior over all other NaCl concentrations and also over control. The interaction between the isolates and NaCl concentrations were significant wherein the isolate ASANFIV S78E recorded maximum IAA production of 12.7 µg per mg protein at 5 per cent NaCl which was significantly superior over rest of the combinations.

4.2.4.1.2 Free-living nitrogen fixers (FNF)

All the 43 isolates found positive for IAA production in the initial test were further assessed quantitatively in presence of different concentrations of NaCl in Czepak's broth after 7 days of incubation. The amount of IAA produced in general was found to decrease with increase in NaCl concentration. While all the isolates produced IAA in the presence of 5% NaCl, only 14, 6, 2 and one isolates produced IAA at 7.5, 10, 12.5 and 15% NaCl respectively. The amount of IAA produced by the isolates under salt stress ranged from 2.6 to 7.8, 0.0 to 12.9, 0.0 to 12.6 and 0.0 to 8.2 and 0.0 to 7.6 µg per mg protein in the presence of 5, 7.5, 10, 12.5 and 15 per cent NaCl respectively as compared to control wherein the IAA production ranged from 4.2 to 12.3 µg per mg protein (Table 13b).

Among all the isolates, irrespective of NaCl concentrations, ASFNFIV S63(1)R was found to be significantly superior over all other isolates and produced maximum IAA of 8.4 µg per mg protein while three isolates viz., ASFNFIV S75(1)R, ASFNFI Dr4(1) and ASFNFIV S45(1)R produced 6.6, 6.7 and 7.2 µg IAA per mg protein respectively and all of them were found to be on par with each other but significantly superior over other isolates (Table 13b). Among different salt concentrations tested, the maximum IAA production of 6.6 µg per mg protein was recorded in the control medium (without added NaCl) and was significantly superior over all NaCl concentrations. The interaction effects between the isolates and NaCl concentrations were significant wherein the isolate ASFNFIV S45(1)R (12.9 µg) followed by ASFNFIV S63(1)R (12.9 µg) recorded maximum IAA production at 7.5 per cent NaCl and both were significantly superior over rest of the combinations.

4.2.4.1.3 Phosphate solubilizing bacteria (PSB)

The amount of IAA produced in general was found to increase with increase in NaCl concentration up to 10 per cent and declined thereafter (Table 13c). While all the isolates produced IAA in the presence of 10% NaCl, only 23 and 6 isolates produced IAA at 12.5 and 15% NaCl respectively. It was observed that, IAA production by two isolates ASPSBIX S122R and ASPSBIX S124E was found to increase even up to 12.5 per cent NaCl. Six isolates viz., ASPSBIX S122R, ASPSBIX S124E, ASPSBIX S125R, ASPSBIX S156R, ASPSBIX S181R and ASPSBIX S189E produced considerable amounts of IAA (7.6 to 9.1 µg per mg protein) even at 15% NaCl.

The amount of IAA produced by the isolates under salt stress ranged from 4.3 to 9.4, 4.5 to 10.4, 4.2 to 10.0, 0.0 to 10.7 and 0.0 to 9.9 µg per mg protein in the presence of 5, 7.5, 10, 12.5 and 15 per cent NaCl respectively as compared to control wherein the IAA production ranged from 4.4 to 7.3 µg per mg protein. Among the isolates, irrespective of NaCl concentrations, ASPSBIX S189E recorded the highest IAA production (9.1 µg in broth) and was significantly superior over all other isolates except ASPSBIX S125R (8.4 µg in broth), ASPSBIX S122R and ASPSBIX S181R (8.3 µg in broth) with which it was on par.

Among different salt concentrations tested, treatment receiving 7.5 per cent NaCl recorded the maximum mean IAA production (7.0 µg per mg protein) and was significantly superior over other NaCl concentrations and control (Table 13c). The interaction effect was found to be significant wherein the isolate ASPSBIX S124E recorded the highest IAA production (10.7 µg per mg protein) followed by ASPSBIX S122R (10.3 µg per mg protein) at 12.5 per cent NaCl and both were significantly superior over rest of the combinations.

4.2.4.1.4 Fluorescent pseudomonads (FP)

All the 61 FP isolates were analyzed for IAA production in the presence different NaCl concentrations and the results are presented in Table 13d. The amount of IAA produced in general was found to increase with increase in NaCl concentration up to 7.5 per cent and declined thereafter.

Table 13c. Amount of IAA (µg/mg protein) produced by salt tolerant P-solubilizers at different salt concentrations

Isolate	NaCl concentrations (%)						Mean
	0	5	7.5	10	12.5	15.0	
ASPSBIX S122R	5.4	7.3	8.3	9.9	10.3	8.8	8.3
ASPSBIX S124E	6.4	7.3	8.2	8.3	10.7	6.0	7.8
ASPSBIX S125R	6.7	8.2	9.5	9.5	9.1	7.1	8.4
ASPSBXI S156R	5.3	7.2	8.1	8.9	8.2	7.6	7.6
ASPSBXI S181R	7.3	7.6	8.5	8.4	8.1	9.9	8.3
ASPSBXII S189E	7.2	9.4	10.4	10.0	8.4	9.0	9.1
ASPSBIX S129R	5.9	6.0	6.8	8.1	8.5	0.0	5.9
ASPSBX S135R	6.0	6.0	6.7	7.5	6.5	0.0	5.4
ASPSBX S139R	5.5	5.5	6.1	6.6	4.9	0.0	4.8
ASPSBX S150R	5.2	5.2	5.6	5.9	3.8	0.0	4.3
ASPSBX Dr29aR	6.2	6.6	7.4	8.8	9.4	0.0	6.4
ASPSBX S142(2)S	6.2	6.5	7.4	8.2	8.3	0.0	6.1
ASPSBX S140S	6.1	6.3	7.2	7.7	8.2	0.0	5.9
ASPSBX S144(1)S	6.3	6.3	7.3	8.0	6.7	0.0	5.7
ASPSBX S145(1)S	5.5	5.4	6.1	6.5	4.4	0.0	4.7
ASPSBX Dr29aS	5.2	5.1	5.6	5.6	4.5	0.0	4.3
ASPSBX WL15(3)S	5.0	4.9	5.4	5.5	2.7	0.0	3.9
ASPSBX Dr27S	5.3	5.3	5.8	6.1	3.4	0.0	4.3
ASPSBX S141E	5.0	4.9	5.4	5.5	2.7	0.0	3.9
ASPSBXI S169(1)R	4.9	4.8	5.3	5.3	3.1	0.0	3.9
ASPSBXI S172(2)S	5.2	5.2	5.6	5.8	4.0	0.0	4.3
ASPSBXII Dr33S	5.5	5.4	5.9	6.2	4.5	0.0	4.6
ASPSBXII S190S	6.1	6.0	6.9	7.8	5.5	0.0	5.4
ASPSBIX S122(1)R	5.7	5.7	6.5	7.1	0.0	0.0	4.2
ASPSBIX S124(1)R	6.5	6.6	7.7	8.9	0.0	0.0	4.9
ASPSBIX S124(2)S	6.9	7.1	8.3	9.7	0.0	0.0	5.3
ASPSBIX S125E	6.3	6.4	7.2	8.1	0.0	0.0	4.7
ASPSBIX S125(6)S	6.2	6.2	6.9	7.8	0.0	0.0	4.5
ASPSBIX S129(1)R	4.7	4.6	4.9	4.9	0.0	0.0	3.2
ASPSBIX S132(2)S	4.4	4.3	4.5	4.3	0.0	0.0	2.9

Contd....

(Table 13c contd....)

Isolate	NaCl concentrations (%)						Mean
	0	5	7.5	10	12.5	15.0	
ASPSBX S138R	5.0	4.9	5.4	5.4	0.0	0.0	3.4
ASPSBX S141R	5.0	4.9	5.3	5.3	0.0	0.0	3.4
ASPSBX S150S	6.5	6.7	7.7	9.3	0.0	0.0	5.0
ASPSBX S138(1)S	6.3	6.5	7.3	8.5	0.0	0.0	4.8
ASPSBX S135S	6.5	6.6	7.5	8.6	0.0	0.0	4.8
ASPSBX S137(1)S	6.7	7.0	7.9	9.1	0.0	0.0	5.1
ASPSBX Dr31(1)S	5.5	5.6	6.3	6.9	0.0	0.0	4.1
ASPSBX S147S	5.9	6.0	6.8	7.6	0.0	0.0	4.4
ASPSBX Dr32S	4.8	4.7	5.1	5.3	0.0	0.0	3.3
ASPSBX WL15(2)	5.8	5.8	6.4	6.9	0.0	0.0	4.1
ASPSBX Dr27(1)S	6.2	6.0	6.7	7.2	0.0	0.0	4.3
ASPSBX S138(1)	6.4	6.4	7.2	8.0	0.0	0.0	4.7
ASPSBX S139(1)S	5.5	5.3	5.7	5.8	0.0	0.0	3.7
ASPSBX S141(1)S	5.5	5.4	5.9	6.2	0.0	0.0	3.8
ASPSBXI S158S	5.0	4.8	5.2	5.2	0.0	0.0	3.4
ASPSBXI S160(2)E	4.9	4.7	5.0	5.0	0.0	0.0	3.3
ASPSBXI S177E	4.5	4.3	4.5	4.2	0.0	0.0	2.9
ASPSBXI WL18E	5.4	5.4	5.9	6.3	0.0	0.0	3.8
ASPSBXI S187(2)	5.7	5.7	6.4	7.1	0.0	0.0	4.2
ASPSBXI WL19E	5.7	5.6	6.1	6.6	0.0	0.0	4.0
ASPSBXI S160(1)E	5.9	5.8	6.2	6.8	0.0	0.0	4.1
ASPSBXI WL20S	5.8	5.8	6.4	7.1	0.0	0.0	4.2
ASPSBXI S160S	4.9	4.8	5.3	5.5	0.0	0.0	3.4
ASPSBXI S175E	5.1	5.0	5.6	6.0	0.0	0.0	3.6
ASPSBXI S176S	5.3	5.2	5.8	6.4	0.0	0.0	3.8
Mean	5.7	5.9	6.5	7.0	2.7	0.9	
	NaCl (S)		Isolate (I)			S x I	
S.Em.±	0.1		0.3			0.7	
C.D.(0.01)	0.3		1.0			2.4	

Table 13d. Amount of IAA ($\mu\text{g}/\text{mg}$ protein) produced by salt tolerant fluorescent pseudomonads at different salt concentrations

Isolate	NaCl concentrations (%)							Mean
	0	5	7.5	10	12.5	15.0	17.5	
ASFPI S3(1)S	4.6	6.3	5.0	5.1	7.2	6.5	6.3	5.8
ASFPI S4(1)S	5.0	7.4	5.8	4.3	6.4	8.1	6.3	6.2
ASFPI S6(1)S	4.0	5.1	3.8	3.8	4.4	5.5	4.2	4.4
ASFPV Dr16E	5.3	7.8	6.5	6.0	5.0	4.4	3.6	5.8
ASFPV S94(2)E	3.7	5.4	4.1	4.4	7.6	6.2	4.5	5.2
ASFPV S100E	3.2	3.7	2.5	2.9	3.4	2.5	2.3	3.0
ASFPI S9(1)S	3.7	4.5	2.9	2.5	2.7	1.2	0.0	2.9
ASFPI S14(3)S	3.5	4.1	2.7	2.7	2.3	2.2	0.0	2.9
ASFPV Dr10E	4.1	6.6	4.9	2.8	2.2	2.0	0.0	3.8
ASFPV S97R	4.7	7.0	5.6	4.3	4.9	3.5	0.0	5.0
ASFPV S112(a)R	3.8	5.3	3.7	2.3	3.1	0.0	0.0	3.0
ASFPV S158(2)R	4.4	5.8	4.4	3.4	4.3	0.0	0.0	3.7
ASFPV S35R	4.5	7.0	7.3	7.2	0.0	0.0	0.0	4.3
ASFPV S85R	4.8	7.5	8.1	8.6	0.0	0.0	0.0	4.8
ASFPV Dr10R	4.4	6.5	6.6	6.1	0.0	0.0	0.0	3.9
ASFPI S15(1)S	5.0	8.3	9.9	12.9	0.0	0.0	0.0	6.0
ASFPIX S134(3)S	5.0	8.9	12.0	29.2	0.0	0.0	0.0	9.2
ASFPX S149S	4.6	7.1	7.5	7.6	0.0	0.0	0.0	4.5
ASFPXI S157(1)S	4.3	6.7	6.8	6.2	0.0	0.0	0.0	4.0
ASFPXI S163S	3.9	5.8	5.4	4.3	0.0	0.0	0.0	3.2
ASFPXI S164S	4.8	7.4	7.9	8.2	0.0	0.0	0.0	4.7
ASFPXI S167(2)S	4.2	6.2	6.0	5.3	0.0	0.0	0.0	3.6
ASFPXI S169(2)S	3.9	5.5	5.1	4.1	0.0	0.0	0.0	3.1
ASFPXI S174S	3.9	6.1	5.8	4.5	0.0	0.0	0.0	3.4
ASFPXI S178(2)S	4.2	6.3	6.2	5.5	0.0	0.0	0.0	3.7
ASFPXI S180S	5.7	9.9	13.7	28.4	0.0	0.0	0.0	9.6
ASFPXI S186S	4.5	7.0	7.3	7.2	0.0	0.0	0.0	4.3
ASFPXI S187S	5.3	8.8	10.8	15.1	0.0	0.0	0.0	6.7
ASFPXI S156R	3.7	5.4	4.8	3.4	0.0	0.0	0.0	2.9
ASFPXI S157(2)R	4.0	5.7	5.3	4.4	0.0	0.0	0.0	3.2
ASFPXI S158(1)R	3.7	5.2	4.6	3.6	0.0	0.0	0.0	2.9
ASFPXI S168R	3.7	5.0	4.4	3.6	0.0	0.0	0.0	2.8

Contd.....

(Table 13d contd....)

Isolate	NaCl concentrations (%)							Mean
	0	5	7.5	10	12.5	15.0	17.5	
ASFPXI S170R	4.6	7.1	7.6	7.6	0.0	0.0	0.0	4.5
ASFPXI S172R	4.2	6.1	5.9	5.1	0.0	0.0	0.0	3.6
ASFPXI S178R	4.3	7.0	7.4	7.4	0.0	0.0	0.0	4.4
ASFPXI S181R	3.0	4.0	3.1	1.9	0.0	0.0	0.0	2.0
ASFPXI S183R	3.5	4.9	4.2	2.8	0.0	0.0	0.0	2.6
ASFPXI S164(b)E	3.4	4.8	4.1	2.7	0.0	0.0	0.0	2.5
ASFPXI S183E	3.9	5.7	5.3	4.2	0.0	0.0	0.0	3.2
ASFPXI WL17R	4.7	7.3	7.8	8.1	0.0	0.0	0.0	4.7
ASFPXI WL16R	3.5	4.6	4.0	3.0	0.0	0.0	0.0	2.5
ASFPI S3(2)S	4.5	13.0	23.2	0.0	0.0	0.0	0.0	6.8
ASFPI Dr6(8)S	4.1	9.6	11.4	0.0	0.0	0.0	0.0	4.2
ASFPIX S121(3)S	3.7	7.6	7.7	0.0	0.0	0.0	0.0	3.2
ASFPIX S131S	3.8	10.3	14.6	0.0	0.0	0.0	0.0	4.8
ASFPIX S121R	3.7	7.7	7.8	0.0	0.0	0.0	0.0	3.2
ASFPXI S155S	3.4	7.7	7.9	0.0	0.0	0.0	0.0	3.2
ASFPXI S176S	3.8	10.5	15.1	0.0	0.0	0.0	0.0	4.9
ASFPXI S178(1)S	4.0	11.4	18.3	0.0	0.0	0.0	0.0	5.6
ASFPXI S173S	3.0	6.1	5.3	0.0	0.0	0.0	0.0	2.4
ASFPXI S161(1)R	3.3	6.3	5.8	0.0	0.0	0.0	0.0	2.6
ASFPXI S161(2)R	3.7	7.5	7.6	0.0	0.0	0.0	0.0	3.1
ASFPXI S174(1)R	4.8	13.3	22.2	0.0	0.0	0.0	0.0	6.7
ASFPXI S174(2)R	4.1	9.7	11.6	0.0	0.0	0.0	0.0	4.2
ASFPXI S184R	3.6	7.1	6.9	0.0	0.0	0.0	0.0	2.9
ASFPXI S156E	3.8	8.5	9.2	0.0	0.0	0.0	0.0	3.6
ASFPXI S178E	3.0	5.6	4.7	0.0	0.0	0.0	0.0	2.2
ASFPXI S180E	3.7	10.1	13.8	0.0	0.0	0.0	0.0	4.6
ASFPXI WL16(1)R	3.7	9.0	10.5	0.0	0.0	0.0	0.0	3.9
ASFPXII S190R	3.6	7.3	7.2	0.0	0.0	0.0	0.0	3.0
ASFPXII Dr33R	3.5	6.0	5.4	0.0	0.0	0.0	0.0	2.5
Mean	4.1	7.0	7.5	4.8	1.0	0.8	0.5	
	NaCl (S)			Isolate (I)			S x I	
S.Em.±	0.2			0.6			1.6	
C.D.(0.01)	0.8			2.2			5.9	

While all the isolates produced IAA in the presence of 7.5% NaCl, only 41, 12, 10 and 6 isolates produced IAA at 10, 12.5, 15 and 17.5% NaCl respectively. It was interesting to note that, seven isolates were found to produce increased amounts of IAA with increase in NaCl concentration up to 10 per cent. The amount of IAA produced by the isolates under salt stress ranged from 3.7 to 13.3, 2.5 to 23.2, 0.0 to 29.2, 0.0 to 7.6, 0.0 to 8.1 and 0.0 to 6.3 μg per mg protein in the presence of 5, 7.5, 10, 12.5, 15 and 17.5 per cent NaCl respectively as compared to control wherein the IAA production ranged from 3.0 to 5.7 μg per mg protein.

Among all the isolates irrespective of NaCl concentrations, isolate ASFPXI S180S followed by ASFPIX S134(3)S produced maximum IAA (9.6 and 9.2 μg per mg protein respectively) and were found to be on par with each other but significantly superior over remaining isolates (Table 13d). Among different NaCl concentrations tested, supplementation of 7.5 per cent NaCl to the growth medium resulted in maximum mean IAA production (7.5 μg per mg protein) which was on par with 5 per cent NaCl treatment (7.0 μg per mg protein) and both were significantly superior over other NaCl concentrations and control. The interaction effect between the isolates and NaCl concentrations was found to be significant wherein three isolates ASFPIX S134(3)S, ASFPXI S180S and ASFPI S3(2)S with 10 per cent NaCl recorded maximum IAA production of 29.2, 28.4 and 23.2 μg per mg protein respectively, which were significantly superior over rest of the combinations.

4.2.4.2 Gibberlic acid (GA)

4.2.4.2.1 Associative nitrogen fixers (ANF)

Data pertaining to amount of GA produced by salt-tolerant ANF isolates are presented in Table 14a. All the 36 isolates found positive for GA production under qualitative test were assessed for quantitative GA production in presence of different concentrations of NaCl. The amount of GA produced in general across the isolates was found to decrease with increase in NaCl concentration. While all the isolates produced GA in the presence of 5% NaCl, only 18 and 3 isolates produced GA at 7.5 and 10% NaCl respectively. The amount of GA produced by the isolates under salt stress ranged from 0.46 to 4.58, 0.00 to 3.02 and 0.00 to 1.99 μg per mg protein in the presence of 5, 7.5 and 10 per cent NaCl respectively as compared to control (without added NaCl) which recorded GA in the range of 0.59 to 3.43 μg per mg protein.

Among the isolates, irrespective of NaCl concentrations, ASANFX S145E, ASANFX S148E, ASANFXI S173E and ASANFX S138E recorded significantly higher GA production (2.21, 2.37, 2.19 and 2.32 μg per mg protein, respectively) and all of them were found to be on par with each other but significantly superior over remaining isolates. Among different salt concentrations tested, the maximum GA production of 2.08 μg per mg protein was recorded in the control treatment and was significantly superior over all NaCl concentrations. The interaction effects between the isolates and NaCl concentrations were significant wherein the strain ASANFIV S78E recorded maximum GA production of 4.58 μg per mg protein at 5 per cent NaCl which was significantly superior over rest of the combinations (Table 14a).

4.2.4.2.2 Free-living nitrogen fixers (FNF)

The effect of changes in medium NaCl concentration on GA production by 43 salt-tolerant FNF isolates was studied *in vitro*. The results of the study indicated that, increase in NaCl concentration resulted in significant reduction in GA production but two isolates viz., ASFNFIV S63(1)R and ASFNFIV S75(1)R produced higher amounts of GA even at 15 and 12.5 per cent NaCl concentration respectively (Table 14b). While all the isolates produced GA in the presence of 5% NaCl, only 14, 6 and 2 isolates produced GA at 7.5, 10, 12.5 and 15% NaCl respectively. The amount of GA produced by the isolates under salt stress ranged from 0.56 to 3.93 μg , 0.00 to 5.35 μg , 0.00 to 8.78 μg , 0.00 to 5.61 μg and 0.00 to 5.26 μg per mg protein in the presence of 5, 7.5, 10, 12.5 and 15 per cent NaCl respectively as compared to control wherein the GA production ranged from 1.42 to 4.22 μg per mg protein.

Among the isolates, ASFNFIV S63(1)R recorded the highest amounts of GA (3.98 μg per mg protein) followed by the isolates ASFNFIV S75(1)R and ASFNFIV S45(1)R (3.77 and 3.67 μg GA per mg protein, respectively) and all the them were found to be on par with each other but significantly superior over other isolates.

Table 14a. Amount of GA ($\mu\text{g}/\text{mg}$ protein) produced by salt tolerant associative N_2 fixers at different salt concentrations

Isolate	NaCl concentrations (%)				Mean
	0	5	7.5	10	
ASANFX S145E	2.65	2.15	2.08	1.97	2.21
ASANFX S148E	2.95	2.49	2.13	1.90	2.37
ASANFXI S173E	2.34	2.23	2.18	1.99	2.19
ASANFIX S125E	2.75	2.63	2.55	0.00	1.98
ASANFXI WL19E	2.62	1.97	1.15	0.00	1.44
ASANFX Dr31E	2.83	2.11	1.35	0.00	1.57
ASANFIX S132E	2.03	1.45	3.02	0.00	1.63
ASANFX S135E	2.39	1.83	1.78	0.00	1.50
ASANFX S138E	3.43	3.00	2.86	0.00	2.32
ASANFX S141E	3.19	2.77	1.80	0.00	1.94
ASANFX S149E	3.00	2.44	2.11	0.00	1.89
ASANFX S150E	2.19	1.52	1.38	0.00	1.27
ASANFX S154E	1.74	1.50	1.40	0.00	1.16
ASANFX WL13E	1.39	1.22	1.17	0.00	0.95
ASANFX WL15E	1.38	1.28	1.24	0.00	0.98
ASANFXI S160E	2.44	2.18	2.11	0.00	1.68
ASANFXI S170E	2.05	1.43	1.04	0.00	1.13
ASANFXI WL17E	1.53	1.04	0.84	0.00	0.85
ASANFII S22E	2.80	3.16	0.00	0.00	1.49
ASANFIV S40E	2.70	3.02	0.00	0.00	1.43
ASANFIV S61E	2.62	3.12	0.00	0.00	1.44
ASANFIV S78E	3.23	4.58	0.00	0.00	1.95
ASANFV Dr18bE	2.00	2.04	0.00	0.00	1.01
ASANFX Dr29aE	2.01	1.56	0.00	0.00	0.89
ASANFIV Dr8E	0.78	0.49	0.00	0.00	0.32
ASANFII S26E	1.26	1.10	0.00	0.00	0.59
ASANFIV S63E	1.40	1.42	0.00	0.00	0.71
ASANFIV S83E	1.53	1.52	0.00	0.00	0.76
ASANFV WL12E	2.00	2.36	0.00	0.00	1.09
ASANFIX S121E	1.83	2.23	0.00	0.00	1.02
ASANFX S137E	0.88	0.56	0.00	0.00	0.36
ASANFX S144E	0.98	0.46	0.00	0.00	0.36
ASANFXI S158E	1.66	1.87	0.00	0.00	0.88
ASANFXI S167E	0.59	0.51	0.00	0.00	0.28
ASANFXI S182E	1.75	2.09	0.00	0.00	0.96
ASANFXI S187E	1.81	2.26	0.00	0.00	1.02
Mean	2.08	1.93	0.89	0.16	
	NaCl (S)		Isolate (I)		S x I
S.Em. $\pm$	0.02		0.06		0.12
C.D.(0.01)	0.07		0.21		0.43

Table 14b. Amount of GA ($\mu\text{g}/\text{mg}$ protein) produced by salt tolerant free-living N_2 fixers at different salt concentrations

Isolate	NaCl concentrations (%)						Mean
	0	5	7.5	10	12.5	15.0	
ASFNFIV S63(1)R	2.21	2.26	4.49	4.41	5.21	5.26	3.98
ASFNFIV S75(1)R	3.14	2.78	5.35	5.74	5.61	0.00	3.77
ASFNFIV S37R	1.96	1.74	3.14	3.01	0.00	0.00	1.64
ASFNFIV WL4(1)R	3.04	2.52	2.86	3.78	0.00	0.00	2.03
ASFNFI Dr4(1)	2.92	2.47	4.19	5.21	0.00	0.00	2.47
ASFNFIV S45(1)R	4.22	3.93	5.09	8.78	0.00	0.00	3.67
ASFNFIV S36(1)R	3.46	1.62	1.24	0.00	0.00	0.00	1.05
ASFNFIV S40(1)R	3.16	1.95	1.78	0.00	0.00	0.00	1.15
ASFNFIV S43(2)R	3.96	1.28	1.43	0.00	0.00	0.00	1.11
ASFNFIV S60(1)S	2.94	1.28	1.55	0.00	0.00	0.00	0.96
ASFNFIV S61(1)S	2.88	1.80	0.99	0.00	0.00	0.00	0.95
ASFNFIV S75(2)R	4.18	1.28	0.90	0.00	0.00	0.00	1.06
ASFNFIV S75E	3.58	1.39	0.99	0.00	0.00	0.00	0.99
ASFNFIV WL5(2)R	3.56	1.83	0.77	0.00	0.00	0.00	1.03
ASFNFI S127R	1.83	1.19	0.00	0.00	0.00	0.00	0.50
ASFNFX Dr31R	1.42	0.56	0.00	0.00	0.00	0.00	0.33
ASFNFXI S174E	2.27	1.62	0.00	0.00	0.00	0.00	0.65
ASFNFXI WL16E	2.55	1.86	0.00	0.00	0.00	0.00	0.73
ASFNFXI S161E	2.53	1.96	0.00	0.00	0.00	0.00	0.75
ASFNFXII S190S	1.85	1.17	0.00	0.00	0.00	0.00	0.50
ASFNFXII S178E	2.08	1.45	0.00	0.00	0.00	0.00	0.59
ASFNFXII S190E	2.40	1.83	0.00	0.00	0.00	0.00	0.70
ASFNFI S17(2)S	2.25	1.67	0.00	0.00	0.00	0.00	0.65
ASFNFI Dr2(5)	1.96	1.34	0.00	0.00	0.00	0.00	0.55
ASFNFI Dr3(3)	1.85	1.17	0.00	0.00	0.00	0.00	0.50
ASFNFI S19(2)S	2.61	2.01	0.00	0.00	0.00	0.00	0.77
ASFNFII S22S	2.58	2.03	0.00	0.00	0.00	0.00	0.77
ASFNFII S22R	2.72	2.20	0.00	0.00	0.00	0.00	0.82
ASFNFIII S32S	3.00	2.46	0.00	0.00	0.00	0.00	0.91
ASFNFIII S31R	2.49	1.83	0.00	0.00	0.00	0.00	0.72
ASFNFIII S33E	2.70	2.06	0.00	0.00	0.00	0.00	0.79
ASFNFIV WL3S	2.15	1.42	0.00	0.00	0.00	0.00	0.60
ASFNFIV S40(2)R	1.66	0.98	0.00	0.00	0.00	0.00	0.44
ASFNFIV S62E	2.51	1.79	0.00	0.00	0.00	0.00	0.72
ASFNFIV S44R	2.70	2.13	0.00	0.00	0.00	0.00	0.80
ASFNFIV S57(2)R	2.41	1.89	0.00	0.00	0.00	0.00	0.72
ASFNFV S85R	2.43	1.90	0.00	0.00	0.00	0.00	0.72
ASFNFIV S66R	2.43	1.82	0.00	0.00	0.00	0.00	0.71
ASFNFIV S80(1)R	2.25	1.56	0.00	0.00	0.00	0.00	0.64
ASFNFIV WL5(1)R	2.84	2.19	0.00	0.00	0.00	0.00	0.84
ASFNFIV WL6R	2.87	2.22	0.00	0.00	0.00	0.00	0.85
ASFNFIV WL7(2)R	3.01	2.42	0.00	0.00	0.00	0.00	0.91
ASFNFIV Dr8R	2.47	1.90	0.00	0.00	0.00	0.00	0.73
Mean	2.65	1.83	0.81	0.72	0.25	0.12	
	NaCl (S)		Isolate (I)			S x I	
S.Em. $\pm$	0.02		0.06			0.16	
C.D.(0.01)	0.09		0.23			0.57	

Table 14c. Amount of GA ($\mu\text{g}/\text{mg}$ protein) produced by salt tolerant P-solubilizers at different salt concentrations

Isolate	NaCl concentrations (%)						Mean
	0	5	7.5	10	12.5	15.0	
ASPSBIX S122R	1.65	2.29	2.41	2.75	4.17	3.07	2.72
ASPSBIX S124E	1.71	1.98	2.05	2.16	3.20	1.68	2.13
ASPSBIX S125R	1.88	2.40	2.52	2.07	3.68	2.49	2.51
ASPSBXI S156R	1.45	1.96	1.99	2.07	3.96	3.37	2.47
ASPSBXI S181R	2.05	2.18	2.30	2.26	3.27	4.69	2.79
ASPSBXII S189E	1.73	2.35	2.56	2.69	3.22	3.59	2.69
ASPSBIX S129R	1.47	1.48	1.59	2.10	4.46	0.00	1.85
ASPSBX S135R	1.73	1.80	1.99	2.47	3.97	0.00	1.99
ASPSBX S139R	1.45	1.51	1.60	1.92	2.87	0.00	1.56
ASPSBX S150R	1.34	1.32	1.35	1.61	1.98	0.00	1.27
ASPSBX Dr29aR	1.59	1.99	2.17	2.79	5.42	0.00	2.33
ASPSBX S142(2)S	1.62	2.00	2.22	2.64	5.00	0.00	2.25
ASPSBX S140S	1.68	2.02	2.35	2.69	5.24	0.00	2.33
ASPSBX S144(1)S	1.74	2.00	2.26	2.73	3.76	0.00	2.08
ASPSBX S145(1)S	1.26	1.22	1.26	1.43	1.72	0.00	1.15
ASPSBX Dr29aS	1.14	1.21	1.28	1.33	2.03	0.00	1.17
ASPSBX WL15(3)S	1.00	0.93	0.92	0.93	0.40	0.00	0.70
ASPSBX Dr27S	1.09	1.07	1.07	1.13	0.71	0.00	0.85
ASPSBX S141E	0.89	0.83	0.81	0.84	0.13	0.00	0.58
ASPSBXI S169(1)R	0.92	0.95	1.00	1.02	0.75	0.00	0.77
ASPSBXI S172(2)S	1.22	1.26	1.30	1.49	1.57	0.00	1.14
ASPSBXII Dr33S	1.38	1.63	1.73	2.04	2.36	0.00	1.52
ASPSBXII S190S	1.68	1.99	2.29	3.00	3.12	0.00	2.01
ASPSBIX S122(1)R	1.51	1.82	2.10	2.59	0.00	0.00	1.34
ASPSBIX S124(1)R	1.76	2.48	2.89	3.83	0.00	0.00	1.83
ASPSBIX S124(2)S	1.88	2.66	3.12	4.18	0.00	0.00	1.97
ASPSBIX S125E	1.72	2.06	2.30	2.96	0.00	0.00	1.51
ASPSBIX S125(6)S	1.51	1.84	1.92	2.51	0.00	0.00	1.30
ASPSBIX S129(1)R	0.90	0.68	0.53	0.59	0.00	0.00	0.45
ASPSBIX S132(2)S	0.76	0.57	0.40	0.29	0.00	0.00	0.34

Contd....

(Table 14c contd....)

Isolate	NaCl concentrations (%)						Mean
	0	5	7.5	10	12.5	15.0	
ASPSBX S138R	0.92	0.82	0.82	0.67	0.00	0.00	0.54
ASPSBX S141R	1.04	0.92	0.92	0.84	0.00	0.00	0.62
ASPSBX S150S	1.55	2.06	2.37	3.24	0.00	0.00	1.54
ASPSBX S138(1)S	1.51	2.00	2.23	3.02	0.00	0.00	1.46
ASPSBX S135S	1.74	2.20	2.50	3.31	0.00	0.00	1.63
ASPSBX S137(1)S	1.69	2.17	2.34	3.03	0.00	0.00	1.54
ASPSBX Dr31(1)S	1.01	0.99	1.05	1.14	0.00	0.00	0.70
ASPSBX S147S	1.25	1.22	1.30	1.50	0.00	0.00	0.88
ASPSBX Dr32S	0.91	0.80	0.82	0.88	0.00	0.00	0.57
ASPSBX WL15(2)	1.40	1.80	1.92	2.39	0.00	0.00	1.25
ASPSBX Dr27(1)S	1.69	1.91	2.08	2.49	0.00	0.00	1.36
ASPSBX S138(1)	1.67	2.26	2.59	3.35	0.00	0.00	1.65
ASPSBX S139(1)S	1.20	1.23	1.17	1.19	0.00	0.00	0.80
ASPSBX S141(1)S	1.24	1.41	1.52	1.70	0.00	0.00	0.98
ASPSBXI S158S	1.02	0.98	0.94	0.96	0.00	0.00	0.65
ASPSBXI S160(2)E	0.91	0.83	0.72	0.69	0.00	0.00	0.53
ASPSBXI S177E	0.84	0.60	0.54	0.41	0.00	0.00	0.40
ASPSBXI WL18E	1.36	1.33	1.29	1.47	0.00	0.00	0.91
ASPSBXI S187(2)	1.42	1.75	1.96	2.52	0.00	0.00	1.28
ASPSBXI WL19E	1.53	1.81	1.94	2.45	0.00	0.00	1.29
ASPSBXI S160(1)E	1.44	1.74	1.78	2.19	0.00	0.00	1.19
ASPSBXI WL20S	1.25	1.19	1.23	1.33	0.00	0.00	0.83
ASPSBXI S160S	0.74	0.50	0.45	0.42	0.00	0.00	0.35
ASPSBXI S175E	0.82	0.69	0.67	0.82	0.00	0.00	0.50
ASPSBXI S176S	0.99	0.76	0.77	0.99	0.00	0.00	0.59
Mean	1.36	1.54	1.64	1.93	1.22	0.34	
	NaCl (S)		Isolate (I)			S x I	
S.Em.±	0.02		0.07			0.16	
C.D.(0.01)	0.08		0.24			0.59	

Among different salt concentrations tested, the maximum GA production of 2.65 µg per mg protein was recorded in the control medium (without added NaCl) and was significantly superior over all NaCl concentrations. The interaction effects between salt-tolerant isolates and NaCl concentrations were found to be significant wherein the isolate ASFNFIV S45(1)R with 10 per cent NaCl recorded the highest GA production (8.78 µg per mg protein) and was significantly superior over rest of the combinations.

4.2.4.2.3 Phosphate solubilizing bacteria (PSB)

All the 55 PSB isolates were examined for the amount of GA produced and in general it was found to increase with increase in NaCl concentration up to 10 per cent and declined thereafter (Table 14c). While all 55 isolates produced GA up to 10% NaCl, 23 and 6 isolates produced GA even at 12.5 and 15% NaCl respectively. It was observed that, 19 and two out of 55 isolates produced higher amounts of GA in the presence of NaCl even up to 12.5 and 15 per cent respectively as compared to control. The amount of GA produced by the isolates under salt stress ranged from 0.50 to 2.66 µg, 0.40 to 3.12 µg, 0.29 to 4.18 µg, 0.00 to 5.42 µg and 0.00 to 4.69 µg per mg protein in the presence of 5, 7.5, 10, 12.5 and 15 per cent NaCl respectively as compared to amount of GA produced in control which was in the range of 0.74 to 2.05 µg per mg protein.

Out of 55 isolates, 8 isolates were found to be on par with each other for their ability to produce GA but were significantly superior over remaining isolates. Among different salt concentrations tested, treatment receiving 10 per cent NaCl recorded the mean maximum GA production of 1.93 µg per mg protein and was significantly superior over other NaCl concentrations and control (without any added salts). The interaction effect was also significant wherein ASPSBX Dr29aR with 12.5 per cent NaCl recorded the highest GA production (5.42 µg per mg protein) which was significantly superior over other treatment combinations (Table 14c).

4.2.4.2.4 Fluorescent pseudomonads (FP)

All the 61 FP isolates were subjected for GA production in the presence of different NaCl concentrations and results indicated that all of them produced GA up to 7.5% NaCl whereas 41, 12, 10 and 6 isolates produced GA up to 10, 12.5, 15 and 17.5% NaCl respectively (Table 14d). The amount of GA produced in general was found to increase with increase in NaCl concentration up to 7.5 per cent and declined thereafter. The amount of GA produced by the isolates under salt stress ranged from 0.63 to 4.07 µg, 0.16 to 8.02 µg, 0.00 to 5.80 µg, 0.00 to 3.55 µg, 0.00 to 3.14 µg and 0.00 to 3.61 µg per mg protein in the presence of 5, 7.5, 10, 12.5, 15 and 17.5 per cent NaCl respectively as compared to amount of GA produced in control which was in the range of 0.49 to 1.89 µg per mg protein.

Among the test isolates, four viz., ASFPI S3(1)S, ASFPI S4(1)S, ASFPV S94(2)E and ASFPXI S180S produced mean maximum GA of 2.37, 2.60, 2.00 and 2.20 µg per mg protein respectively and were found to be on par with each other but significantly superior over remaining isolates (Table 14d). Among different NaCl concentrations tested, supplementation of 7.5 per cent NaCl to the growth medium resulted in mean maximum GA production of 2.01 µg per mg protein and was significantly superior over other NaCl concentrations and control. The interaction effect between the isolates and NaCl concentrations was found to be significant wherein the isolate ASFPXI S174(1)R with 7.5 per cent NaCl recorded maximum GA production of 8.02 µg per mg protein, which was significantly superior over all other treatment combinations.

4.2.4.3 Cytokinin

Twelve functionally important salt-tolerant isolates consisting 3 each of ANF, FNF, PSB and FP isolates were tested for the production of cytokinin (Zeatin) in the presence of 5 and 10% NaCl by high performance liquid chromatography (HPLC) and the results are presented in Table 15 and Fig. 8a and 8b. All the isolates produced cytokinin in the presence of 5 and 10% NaCl though in varying quantities. In general there was significant reduction in cytokinin production with increase in NaCl concentration. The amount of cytokinin produced by the isolates under salt stress ranged from 20.7 to 48.8 and 1.3 to 20.4 µg per l in the presence of 5 and 10 per cent NaCl respectively as compared control which was in the range of 39.3 to 53.7 µg per l.

Table 14d. Amount of GA ($\mu\text{g}/\text{mg}$ protein) produced by salt tolerant fluorescent pseudomonads at different salt concentrations

Isolate	NaCl concentrations (%)							Mean
	0	5	7.5	10	12.5	15.0	17.5	
ASFPI S3(1)S	1.23	1.73	1.82	2.15	2.89	3.14	3.61	2.37
ASFPI S4(1)S	1.74	2.76	3.21	2.56	2.63	2.88	2.39	2.60
ASFPI S6(1)S	0.79	1.00	0.94	1.33	2.20	2.09	2.20	1.51
ASFPV Dr16E	1.11	1.67	1.79	1.82	1.12	0.81	0.50	1.26
ASFPV S94(2)E	1.05	1.42	1.44	1.55	3.55	2.91	2.07	2.00
ASFPV S100E	1.06	1.34	1.34	1.53	1.81	1.15	0.98	1.32
ASFPI S9(1)S	1.29	1.82	1.93	2.02	1.39	1.08	0.00	1.36
ASFPI S14(3)S	1.00	1.32	1.31	1.94	1.75	2.16	0.00	1.35
ASFPV Dr10E	1.19	1.82	1.98	1.46	0.90	1.22	0.00	1.22
ASFPV S97R	1.51	2.25	2.50	1.90	1.39	1.39	0.00	1.56
ASFPV S112(a)R	0.80	1.06	1.01	0.96	0.73	0.00	0.00	0.65
ASFPV S158(2)R	0.93	1.26	1.25	0.91	0.81	0.00	0.00	0.74
ASFPV S35R	1.38	1.83	2.18	1.30	0.00	0.00	0.00	0.96
ASFPV S85R	1.53	2.10	2.65	1.46	0.00	0.00	0.00	1.11
ASFPV Dr10R	1.46	1.92	2.26	1.25	0.00	0.00	0.00	0.98
ASFPI S15(1)S	1.46	2.08	2.78	1.89	0.00	0.00	0.00	1.17
ASFPX S134(3)S	1.27	1.96	3.43	3.68	0.00	0.00	0.00	1.48
ASFPX S149S	1.37	1.82	2.19	1.25	0.00	0.00	0.00	0.95
ASFPXI S157(1)S	1.00	1.23	1.19	0.62	0.00	0.00	0.00	0.58
ASFPXI S163S	0.92	1.07	0.95	0.42	0.00	0.00	0.00	0.48
ASFPXI S164S	1.65	2.27	2.88	1.78	0.00	0.00	0.00	1.23
ASFPXI S167(2)S	1.24	1.57	1.73	0.83	0.00	0.00	0.00	0.77
ASFPXI S169(2)S	1.16	1.41	1.47	0.68	0.00	0.00	0.00	0.67
ASFPXI S174S	0.88	1.13	1.14	0.32	0.00	0.00	0.00	0.50
ASFPXI S178(2)S	1.23	1.56	1.72	0.77	0.00	0.00	0.00	0.75
ASFPXI S180S	1.89	2.93	4.76	5.80	0.00	0.00	0.00	2.20
ASFPXI S186S	1.34	1.77	2.10	1.13	0.00	0.00	0.00	0.91
ASFPXI S187S	1.76	2.60	3.76	3.09	0.00	0.00	0.00	1.60
ASFPXI S156R	0.80	1.00	1.13	0.35	0.00	0.00	0.00	0.47
ASFPXI S157(2)R	1.20	1.48	1.58	0.77	0.00	0.00	0.00	0.72
ASFPXI S158(1)R	1.12	1.34	1.36	0.63	0.00	0.00	0.00	0.64
ASFPXI S168R	1.28	1.54	1.64	0.77	0.00	0.00	0.00	0.75

Contd....

(Table 14d contd....)

Isolate	NaCl concentrations (%)							Mean
	0	5	7.5	10	12.5	15.0	17.5	
ASFPXI S170R	1.38	1.85	2.23	1.32	0.00	0.00	0.00	0.97
ASFPXI S172R	1.23	1.55	1.70	0.81	0.00	0.00	0.00	0.76
ASFPXI S178R	0.96	1.29	1.33	0.67	0.00	0.00	0.00	0.61
ASFPXI S181R	0.62	0.76	0.58	0.15	0.00	0.00	0.00	0.30
ASFPXI S183R	0.68	0.69	0.42	0.14	0.00	0.00	0.00	0.28
ASFPXI S164(b)E	0.72	0.75	0.52	0.11	0.00	0.00	0.00	0.30
ASFPXI S183E	0.89	1.02	0.88	0.24	0.00	0.00	0.00	0.43
ASFPXI WL17R	1.58	2.16	2.71	1.66	0.00	0.00	0.00	1.16
ASFPXI WL16R	1.04	1.20	1.17	0.52	0.00	0.00	0.00	0.56
ASFPI S3(2)S	1.25	3.05	5.97	0.00	0.00	0.00	0.00	1.47
ASFPI Dr6(8)S	1.22	2.48	3.35	0.00	0.00	0.00	0.00	1.01
ASFPIX S121(3)S	1.12	2.00	2.30	0.00	0.00	0.00	0.00	0.77
ASFPIX S131S	0.61	1.07	0.45	0.00	0.00	0.00	0.00	0.30
ASFPIX S121R	1.11	1.99	2.29	0.00	0.00	0.00	0.00	0.77
ASFPXI S155S	0.64	1.00	0.63	0.00	0.00	0.00	0.00	0.32
ASFPXI S176S	0.67	1.25	0.89	0.00	0.00	0.00	0.00	0.40
ASFPXI S178(1)S	0.84	1.77	2.27	0.00	0.00	0.00	0.00	0.70
ASFPXI S173S	0.49	0.63	0.16	0.00	0.00	0.00	0.00	0.18
ASFPXI S161(1)R	0.93	1.49	1.49	0.00	0.00	0.00	0.00	0.56
ASFPXI S161(2)R	1.11	1.96	2.24	0.00	0.00	0.00	0.00	0.76
ASFPXI S174(1)R	1.63	4.07	8.02	0.00	0.00	0.00	0.00	1.96
ASFPXI S174(2)R	1.20	2.45	3.32	0.00	0.00	0.00	0.00	1.00
ASFPXI S184R	1.09	1.88	2.09	0.00	0.00	0.00	0.00	0.72
ASFPXI S156E	1.11	2.09	2.53	0.00	0.00	0.00	0.00	0.82
ASFPXI S178E	0.65	0.91	0.66	0.00	0.00	0.00	0.00	0.32
ASFPXI S180E	0.60	1.04	0.40	0.00	0.00	0.00	0.00	0.29
ASFPXI WL16(1)R	0.83	1.54	1.60	0.00	0.00	0.00	0.00	0.57
ASFPXII S190R	0.99	1.72	1.86	0.00	0.00	0.00	0.00	0.65
ASFPXII Dr33R	1.24	2.98	5.57	0.00	0.00	0.00	0.00	1.40
Mean	1.14	1.68	2.01	0.99	0.38	0.34	0.21	
	NaCl (S)			Isolate (I)			S x I	
S.Em.±	0.06			0.17			0.46	
C.D.(0.01)	0.21			0.63			1.68	

Nine out of 12 isolates belonging to three functional groups (FNF, PSB and FP) were found to be on par with each other for cytokinin production. Of the four functional groups, least mean cytokinin production was recorded with associative N₂ fixers (ANF) and was in the range of 20.4 to 24.2 µg per l. Among nine efficient isolates, numerically higher amounts of cytokinin was recorded in ASFPI S4(1)S (38.2 µg/l) followed by ASFPI S6(1)S (37 µg/l), ASFNFIV S63(1)R (36.4 µg/l), ASPSBIX S125R (35 µg/l), ASFNFIV S45(1)R (34.7 µg/l), ASFPI S3(1)S (34.5 µg/l), ASPSBIX S122R (34.3 µg/l), ASFNFIV S75(1)R (32 µg/l) and ASPSBIX S124E (31.3 µg/l). The interaction effect was found to be non-significant.

4.3 Biochemical basis for salt tolerance

4.3.1 Exopolysaccharide (EPS) production

A total of 21 salt-tolerant isolates consisting of 3 ANF and 6 each of FNF, PSB and FP isolates were analyzed for exopolysaccharide (EPS) production in the presence of different concentrations of NaCl *in vitro*. The results clearly indicated that, increase in medium NaCl concentration resulted in significant increase in EPS production (Table 16). The amount of EPS produced by the salt-tolerant isolates under salt stress ranged from 29.9 to 100.4 and 32.1 to 113.3 µg per mg protein in the presence of 5 and 10 per cent NaCl respectively as compared to control which was in the range of 16.9 to 74.9 µg per mg protein.

The amount of EPS produced by the four functional groups viz., ANF, FNF, PSB and FP was in the range of 41.7 to 57.6 µg, 76.5 to 96.2 µg, 32.2 to 46.7 µg and 26.3 to 35.6 µg per mg protein. Among the isolates irrespective of NaCl concentrations, isolate ASFNFIV S45(1)R recorded the highest EPS production (96.2 µg/mg protein) followed by ASFNFIV Dr4(1) (92.2 µg/mg protein), ASFNFIV S75(1)R (89.1 µg/mg protein) and ASFNFIV S37R (89 µg/mg protein) all of which were on par with each other but significantly superior over other isolates. Among the NaCl concentrations, mean maximum EPS was recorded at 10 per cent NaCl (62 µg/mg protein) and was on par with 5 per cent NaCl (58.6 µg/mg protein) but both were significantly higher over control. There was a significant interaction between isolates and NaCl concentrations. The isolate ASFNFIV S45(1)R with 10 per cent NaCl concentration recorded significantly higher amounts of EPS (113.3 µg/mg protein) than most other treatment combinations.

4.3.2 ACC deaminase activity

Out of 57 salt-tolerant isolates positive for ACC deaminase activity, 15 ANF, 14 FNF, 19 PSB and 9 FP isolates were further subjected for ACC deaminase activity in the presence of 5 and 10% NaCl. The results obtained from this study are presented in Tables 17a to 17d.

4.3.2.1 Associative nitrogen fixers (ANF)

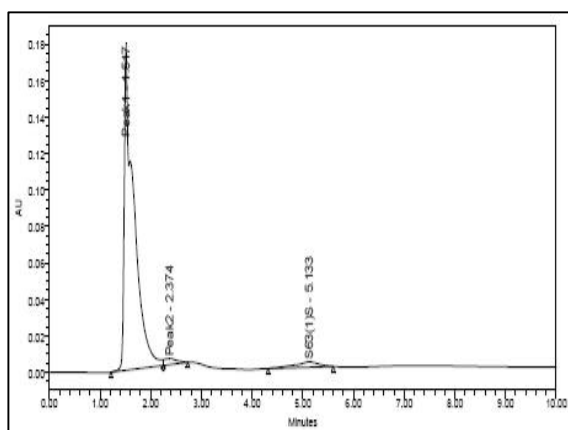
Among all the isolates, irrespective of NaCl concentrations, ASANFX S148E showed the highest ACC deaminase activity (178.2 nmol α ketobutyrate/g biomass/h) followed by ASANFXI S173E (152.6 nmol α ketobutyrate/g biomass/h) and ASANFX S145E (149.2 nmol α ketobutyrate/g biomass/h) all of which were significantly superior over rest of the isolates (Table 17a). While all 15 isolates showed the enzyme activity at 5% NaCl, only 3 were positive at 10% NaCl. Among the NaCl concentrations tested, maximum activity (137.2 nmol α ketobutyrate/g biomass/h) was recorded with control and minimum with 10 per cent NaCl treatment (6.4 nmol α ketobutyrate/g biomass/h). The interaction effect was found to be significant wherein the isolate ASANFX S148E without added NaCl recorded the maximum ACC deaminase activity of 360.6 nmol α ketobutyrate/g biomass/h.

4.3.2.2 Free-living nitrogen fixers (FNF)

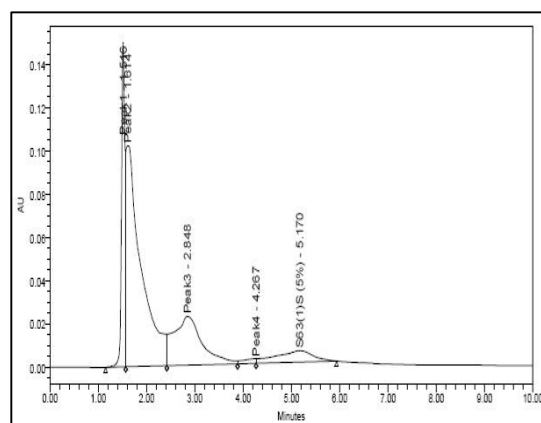
All 14 isolates were found to possess ACC deaminase activity. In general, the enzyme activity was adversely affected in the presence of NaCl. All 14 isolates showed enzyme activity at 5% NaCl whereas 13 isolates were positive at 10% NaCl (Table 17b). Among the FNF isolates tested, ASFNFIV S63(1)R showed mean maximum ACCD activity (222.1 nmol α ketobutyrate/g biomass/h) followed by ASFNFIV S75(1)R (208.4 nmol) and ASFNFIV S37R (202.9 nmol) all of which were on par with each other but significantly superior over remaining isolates. ACC deaminase activity of remaining isolates was in the range of 21.7 to 177.3 nmol α ketobutyrate/g biomass/h.

Table 15. Amount of cytokinin ($\mu\text{g/l}$) produced by salt tolerant AIM's at different salt concentrations

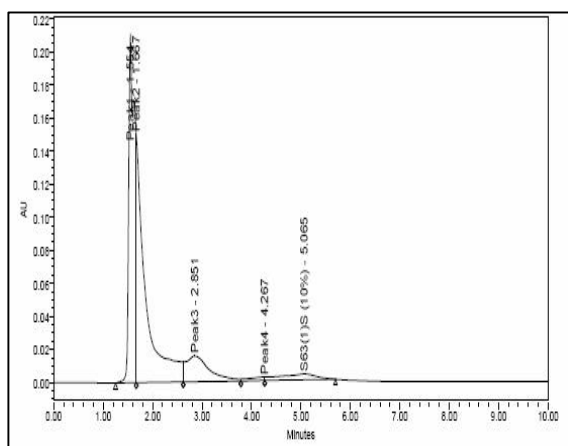
Isolate	NaCl concentrations (%)			Mean
	0	5	10	
ASANFX S145E	41.9	27.3	3.5	24.2
ASANFX S148E	40.9	23.9	2.8	22.5
ASANFXI S173E	39.3	20.7	1.3	20.4
ASFNFIV S63(1)R	49.8	39.1	20.4	36.4
ASFNFIV S75(1)R	44.2	34.2	17.6	32.0
ASFNFIV S45(1)R	46.7	38.7	18.9	34.7
ASPSBIX S122R	48.8	39.4	14.5	34.3
ASPSBIX S124E	44.3	36.7	12.8	31.3
ASPSBIX S125R	49.3	40.4	15.2	35.0
ASFPI S3(1)S	50.0	44.0	9.4	34.5
ASFPI S4(1)S	53.7	48.8	12.1	38.2
ASFPI S6(1)S	52.2	47.9	11.0	37.0
Mean	46.8	36.8	11.6	
	NaCl (S)	Isolate (I)		S x I
S.Em. $\pm$	1.0	1.9		3.4
C.D.(0.01)	3.7	7.5		NS



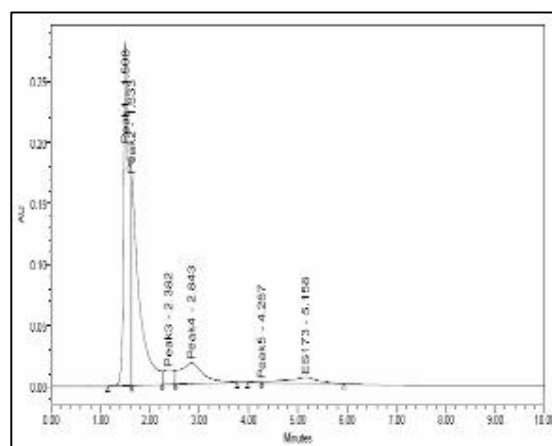
ASFNF S63(1)R (Control)



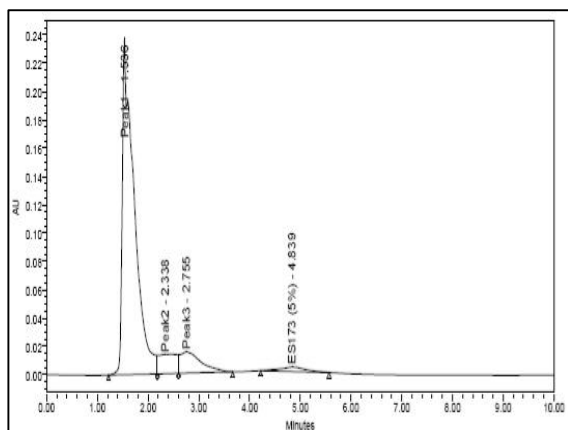
ASFNF S63(1)R (5% NaCl)



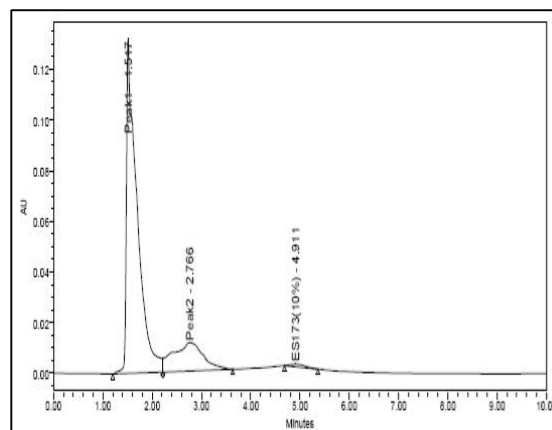
ASFNF S63(1)R (10% NaCl)



ASANF S173E (Control)

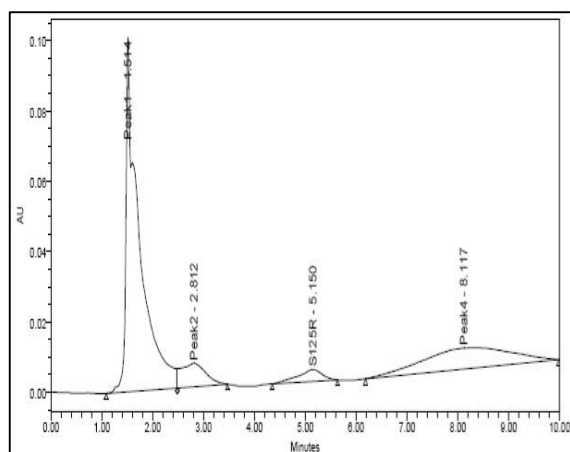


ASANF S173E (5% NaCl)

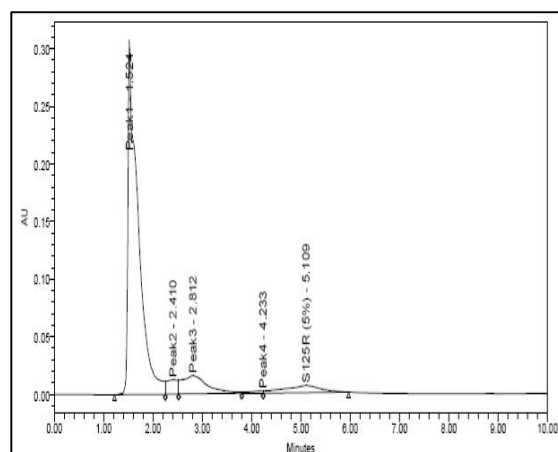


ASANF S173E (10% NaCl)

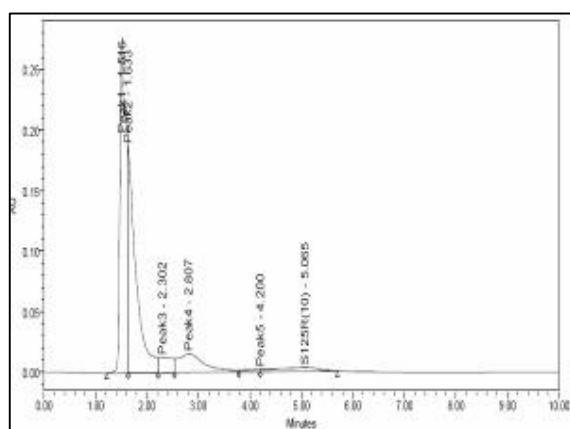
Fig. 8a. HPLC chromatograms of cytokinin produced by salt tolerant associative N_2 fixer (ASANFXI S173E) and free-living N_2 fixer (ASFNFIV S63(1)R) under salt stress



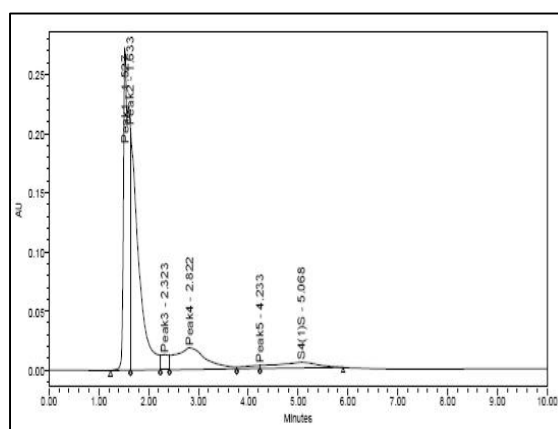
ASPSB S125R (Control)



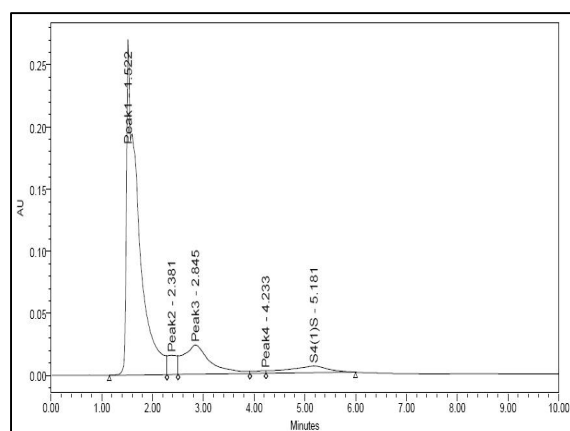
ASPSB S125R (5% NaCl)



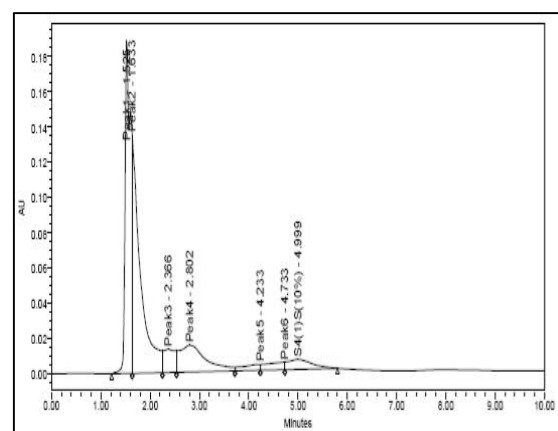
ASPSB S125R (5% NaCl)



ASFPV S4(1)S (Control)



ASFPV S4(1)S (5% NaCl)



ASFPV S4(1)S (10% NaCl)

Fig. 8b. HPLC chromatograms of cytokinin produced by salt tolerant P-solubilizing bacteria (ASPSBIX S125R) and fluorescent pseudomonad strain (ASFPI S4(1)S) under salt stress

Table 16. Amount of exo-polysaccharide ($\mu\text{g}/\text{mg}$ protein) produced by salt tolerant AIM's at different salt concentrations

Isolate	NaCl concentrations (%)			Mean
	0	5	10	
ASANFX S145E	33.9	49.3	53.2	45.5
ASANFX S148E	30.4	48.1	46.5	41.7
ASANFXI S173E	35.5	63.5	73.7	57.6
ASFNFIV S63(1)R	57.7	81.3	90.6	76.5
ASFNFIV S75(1)R	68.3	89.8	109.3	89.1
ASFNFIV S37R	70.1	87.9	109.1	89.0
ASFNFIV WL4(1)R	59.0	82.4	98.6	80.0
ASFNFI Dr4(1)	70.6	98.2	107.7	92.2
ASFNFIV S45(1)R	74.9	100.4	113.3	96.2
ASPSBIX S122R	26.5	44.0	45.2	38.6
ASPSBIX S124E	32.5	55.6	43.7	43.9
ASPSBIX S125R	39.0	62.4	51.4	50.9
ASPSBXI S156R	24.3	38.4	33.9	32.2
ASPSBXI S181R	29.9	51.2	32.9	38.0
ASPSBXI S189E	33.8	53.7	52.6	46.7
ASFPI S3(1)S	24.2	40.6	42.0	35.6
ASFPI S4(1)S	26.3	39.3	40.2	35.3
ASFPI S6(1)S	19.0	31.7	34.6	28.4
ASFPV Dr16E	26.2	39.6	37.9	34.6
ASFPV S94(2)E	24.0	43.2	53.6	40.3
ASFPV S100E	16.9	29.9	32.1	26.3
Mean	39.2	58.6	62.0	
	NaCl (S)	Isolate (I)		S x I
S.Em.±	1.0	2.5		4.4
C.D.(0.01)	3.6	9.6		16.6

Among the NaCl concentrations tested, mean maximum ACCD activity was recorded with control (207.6 nmol α ketobutyrate/g biomass/h) and minimum with 10 per cent NaCl treatment (46.6 nmol α ketobutyrate/g biomass/h). The interaction was also found to be significant wherein the isolate ASFNFIV S63(1)R without any added NaCl recorded maximum ACC deaminase activity (395.7 nmol α ketobutyrate/g biomass/h).

4.3.2.3 Phosphate solubilizing bacteria (PSB)

Effect of NaCl concentrations on the ACCD activity of 19 PSB isolates was assessed *in vitro* and the results obtained are presented in Table 17c. It was observed that, all the isolates showed ACCD activity at both 5 and 10% NaCl but the amount was reduced significantly with increase in NaCl concentration. The ACCD activity by the salt-tolerant PSB isolates under salt stress ranged from 20.4 to 145.7 and 16.1 to 118.5 nmol α ketobutyrate/ g biomass/ h in the presence of 5 and 10 per cent NaCl concentration respectively as compared to ACCD activity in the control treatment which was in the range of 40.2 to 454.8 nmol α ketobutyrate/ g biomass/ h.

Among the isolates tested, ASPSBIX S122R was found to be significantly superior over rest of the isolates and showed mean maximum ACCD activity of 239.7 nmol α ketobutyrate/ g biomass/ h. Among the NaCl concentrations tested, the highest ACCD activity was recorded with control (204.4 nmol α ketobutyrate/g biomass/h) and the lowest with 10 per cent NaCl (51.9 nmol α ketobutyrate/g biomass/h). The interaction between isolates and NaCl concentrations was also significant. The isolate ASPSBIX S122R without added NaCl recorded the highest ACC deaminase activity (454.8 nmol α ketobutyrate/ g biomass/ h).

4.3.2.4 Fluorescent pseudomonads (FP)

All the 9 test isolates were found to exhibit ACCD activity in the presence of 5 and 10% NaCl. Significant reduction in ACCD activity was observed with increase in NaCl concentration (Table 17d). The ACCD activity of salt-tolerant FP isolates under salt stress ranged from 51.7 to 133.5 and 28.8 to 77.3 nmol α ketobutyrate/ g biomass/ h in the presence of 5 and 10 per cent NaCl respectively as compared to ACCD activity in the control treatment (86.7 to 454.8 nmol α ketobutyrate/ g biomass/ h).

Among the isolates, ASFPI S4(1)S recorded significantly higher mean ACCD activity (221.9 nmol α ketobutyrate/ g biomass/ h) over all other isolates. It was followed by ASFPI S4(1)S (184.2 nmol α ketobutyrate/ g biomass/ h), ASFPV S100E (136.7 nmol α ketobutyrate/ g biomass/ h) and ASFPV 97R (120.2 nmol α ketobutyrate/ g biomass/ h) which were significantly superior over other isolates. Among the NaCl concentrations tested, treatment receiving 10 per cent NaCl showed the lowest ACCD activity (47.1 nmol α ketobutyrate/ g biomass/ h) whereas control treatment recorded significantly higher activity (234 nmol α ketobutyrate/ g biomass/ h). There was a significant interaction between isolates and NaCl concentrations for ACCD activity. Maximum activity of 454.8 nmol α ketobutyrate/ g biomass/ h was recorded by ASFPI S4(1)S without any added salts (control) while minimum of 28.8 nmol α ketobutyrate/ g biomass/ h by ASFPV Dr10E with 10 per cent NaCl.

4.3.3 Proline

Sixty seven salt-tolerant isolates consisting of 18 ANF, 14 FNF, 23 PSB and 12 FP isolates were analyzed for changes in proline content in response to increase in medium NaCl concentration and the results obtained are presented in Tables 18a to 18d.

4.3.3.1 Associative nitrogen fixers (ANF)

The amount of proline accumulated by 18 salt-tolerant ANF isolates under salt stress was assessed in M9 minimal medium supplemented with increasing NaCl concentrations viz., 5, 7.5 and 10 per cent as against the control medium (without any added salts). The data revealed that, the proline content of the isolates in general was increased with increase in NaCl concentration up to 7.5 per cent and decreased thereafter (Table 18a). While all the isolates showed proline production at 5 and 7.5% NaCl, only 3 isolates showed proline production at 10% NaCl. Among the isolates, ASANFXI S173E recorded the highest mean proline content (10.5 μ g per mg protein) which was significantly superior over remaining isolates except ASANFX S148E (10.3 μ g) with which it was on par.

Table 17a. ACC* deaminase activity (nmol α ketobutyrate/g biomass/h) of salt-tolerant associative N₂ fixers

Isolate	NaCl concentrations (%)			Mean
	0.05	5	10	
ASANFX S145E	315.3	111.8	20.4	149.2
ASANFX S148E	360.6	126.9	47.2	178.2
ASANFXI S173E	327.9	101.4	28.4	152.6
ASANFIX S125E	139.5	89.2	0.0	76.2
ASANFXI WL19E	138.2	85.6	0.0	74.6
ASANFX Dr31E	15.1	15.1	0.0	10.1
ASANFIX S132E	94.2	60.3	0.0	51.5
ASANFX S135E	56.5	20.1	0.0	25.5
ASANFX S138E	131.9	86.5	0.0	72.8
ASANFX S141E	154.5	93.7	0.0	82.7
ASANFX S149E	77.9	32.3	0.0	36.7
ASANFX S150E	51.5	18.1	0.0	23.2
ASANFX S154E	15.1	15.1	0.0	10.1
ASANFX WL15E	81.7	39.4	0.0	40.4
ASANFXI S170E	98.0	65.7	0.0	54.6
Mean	137.2	64.1	6.4	
	NaCl (S)	Isolate (I)		S x I
S.Em.±	1.6	4.0		7.0
C.D.(0.01)	6.2	15.2		26.3

* 1-Amino-Cyclopropane-1-Carboxylate Deaminase

Table 17b. ACC* deaminase activity (nmol α ketobutyrate/g biomass/h) of salt-tolerant free-living N₂ fixers

Isolate	NaCl concentrations (%)			Mean
	0.05	5	10	
ASFNFIV S63(1)R	395.7	176.8	93.7	222.1
ASFNFIV S75(1)R	368.1	168.0	89.2	208.4
ASFNFIV S37R	360.6	162.5	85.6	202.9
ASFNFIV WL4(1)R	283.9	118.5	60.3	154.2
ASFNFI Dr4(1)	332.9	133.4	65.7	177.3
ASFNFIV S45(1)R	261.3	84.1	53.1	132.8
ASFNFIV S36(1)R	149.5	53.1	41.3	81.3
ASFNFIV S40(1)R	140.7	41.3	31.3	71.1
ASFNFIV S43(2)R	85.4	29.6	20.6	45.2
ASFNFIV S60(1)S	49.0	16.1	0.0	21.7
ASFNFIV S61(1)S	149.5	46.3	31.3	75.7
ASFNFIV S75(2)R	56.5	23.0	16.1	31.9
ASFNFIV S75E	109.3	31.3	23.0	54.5
ASFNFIV WL5(2)R	164.6	60.6	41.3	88.8
Mean	207.6	81.8	46.6	
	NaCl (S)	Isolate (I)		S x I
S.Em.±	3.4	7.4		12.9
C.D.(0.01)	13.1	28.4		49.1

* 1-Amino-Cyclopropane-1-Carboxylate Deaminase

Table 17c. ACC* deaminase activity (nmol α ketobutyrate/g biomass/h) of salt-tolerant P-solubilizers

Isolate	NaCl concentrations (%)			Mean
	0.05	5	10	
ASPSBIX S122R	454.8	145.7	118.5	239.7
ASPSBIX S124E	275.1	111.8	85.6	157.5
ASPSBIX S125R	415.8	133.5	93.7	214.3
ASPSBXI S156R	234.9	93.7	53.1	127.2
ASPSBXI S181R	271.4	101.4	65.7	146.2
ASPSBXII S189E	302.8	101.4	77.3	160.5
ASPSBIX S129R	252.5	93.7	69.2	138.5
ASPSBX S135R	140.7	60.9	20.6	74.1
ASPSBX S139R	162.1	65.7	33.1	87.0
ASPSBX S150R	267.6	101.4	60.9	143.3
ASPSBX Dr29aR	56.5	20.4	16.1	31.0
ASPSBX S142(2)S	84.2	29.6	16.1	43.3
ASPSBX S144(1)S	157.0	65.7	53.1	91.9
ASPSBX S145(1)S	109.3	60.9	33.1	67.8
ASPSBX Dr29aS	140.7	60.9	41.3	81.0
ASPSBX Dr27S	40.2	31.3	20.6	30.7
ASPSBX S141E	144.5	53.1	33.1	76.9
ASPSBXI S172(2)S	120.6	69.2	41.3	77.0
ASPSBXII Dr33S	252.5	77.3	53.1	127.6
Mean	204.4	77.8	51.9	
	NaCl (S)	Isolate (I)		S x I
S.Em.±	2.2	6.2		10.7
C.D.(0.01)	8.3	23.1		40.0

* 1-Amino-Cyclopropane-1-Carboxylate Deaminase

Table 17d. ACC* deaminase activity (nmol α ketobutyrate/g biomass/h) of salt-tolerant fluorescent pseudomonads

Isolate	NaCl concentrations (%)			Mean
	0.05	5	10	
ASFPI S3(1)S	157.0	60.9	33.1	83.7
ASFPI S4(1)S	454.8	133.5	77.3	221.9
ASFPI S6(1)S	360.6	122.7	69.2	184.2
ASFPV Dr16E	199.8	69.2	40.1	103.0
ASFPV S100E	271.4	85.6	53.1	136.7
ASFPI S14(3)S	145.7	51.7	31.3	76.2
ASFPV Dr10E	86.7	52.9	28.8	56.1
ASFPV S97R	236.2	77.3	47.2	120.2
ASFPV S158(2)R	193.5	69.2	43.6	102.1
Mean	234.0	80.3	47.1	
	NaCl (S)	Isolate (I)		S x I
S.Em.±	3.4	6.8		11.8
C.D.(0.01)	13.1	26.2		45.4

* 1-Amino-Cyclopropane-1-Carboxylate Deaminase

The proline content of remaining isolates ranged from 2.3 to 7.9 µg per mg protein. Among the NaCl concentrations, the maximum mean proline content was recorded at 7.5% NaCl (13.4 µg) which was significantly superior over rest of the NaCl concentrations.

The interaction effect was found to be significant. Three isolate viz., ASANFXI S173E, ASANFX S148E and ASANFX S125E with 7.5 per cent NaCl recorded maximum proline content of 24.6, 23.2 and 22.3 µg per mg protein respectively and were significantly superior over other treatment combinations but were on par with each other.

4.3.3.2 Free-living nitrogen fixers (FNF)

All 14 isolates showed proline production in the presence of 7.5% NaCl, while only 6, 2 and one isolates showed proline production in the presence of 10, 12.5 and 15% NaCl respectively. The amount of proline produced by 14 salt-tolerant FNF isolates under salt stress ranged from 5.4 to 22.8, 0.8 to 51.7, 0.0 to 69.2 and 0.0 to 55.8 µg per mg protein in the presence of 5, 7.5, 10 and 12.5 per cent NaCl respectively as compared to control (without any added salts) which was in the range of 5.4 to 14.6 µg per mg protein. It was observed that, the amount proline produced by most of the isolates increased only up to 5 per cent NaCl and declined thereafter whereas two isolates ASFNFIV S63(1)R and ASFNFIV S75(1)R were found to produce increased amounts of proline with increase in salt concentration up to 10 per cent NaCl.

Among 14 FNF isolates, ASFNFIV S75(1)R recorded the highest amount of proline (35 µg/mg protein) and was significantly superior over all other isolates (Table 18b). Among the salt concentrations, 5 per cent NaCl treatment recorded maximum proline (15.7 µg/mg protein) while minimum was in 15 per cent NaCl treatment (0.2 µg/mg protein). The interaction effect between isolates and NaCl concentration was found to be significant wherein maximum proline content (69.2 µg/mg protein) was recorded by ASFNFIV S75(1)R isolate with 10 per cent NaCl.

4.3.3.3 Phosphate solubilizing bacteria (PSB)

Twenty three salt-tolerant PSB isolates were tested for their ability to produce proline in response to different concentrations of NaCl in M9 minimal medium. The data on amount of proline produced by the isolates are presented in Table 18c. The proline content, in general, was found to increase with increase in NaCl concentration up to 12.5 per cent and declined thereafter. However, two isolates ASPSBXI S156R and ASPSBIX S122R showed increased amount of proline even up to 15 per cent NaCl. Proline production was not detected in seven out of 23 isolates in the control medium whereas one isolate (ASPSBIX S122R) did not show proline accumulation even up to 7.5 per cent NaCl.

Irrespective of NaCl concentrations, ASPSBXI S156R among the isolates was found to produce significantly higher amount of proline (11.53 µg/mg protein) as compared to remaining isolates. The amount of proline produced by remaining isolates was in the range of 0.28 to 4.52 µg per mg protein. Among the salt concentrations, supplementation of 12.5 per cent NaCl to the growth medium resulted in maximum proline (4.52 µg/mg protein) while minimum of 0.36 µg per mg protein was recorded in control medium. The interaction effect was also found to be significant wherein maximum proline content was recorded by ASPSBXI S156R (38.63 µg/mg protein) with 15 per cent NaCl.

4.3.3.4 Fluorescent pseudomonads (FP)

Data pertaining to proline accumulation by twelve salt tolerant FP isolates are presented in Table 18d. The results of the study indicated that, an increase in NaCl concentration found to have significant influence on amount of proline accumulated by the isolates and was found to increase with increase in medium NaCl concentration up to 15 per cent and declined thereafter. All the isolates were found to produce proline in the presence of 12.5 per cent NaCl while, only 10 and 6 isolates produced proline at 15 and 17.5 per cent NaCl respectively. The amount of proline produced by FP isolates in response to salt stress ranged from 5.8 to 46.7 µg, 10.7 to 56.2 µg, 5.8 to 57.5 µg, 5.3 to 90.0 µg, 0.0 to 75.5 µg and 0.0 to 62.4 µg per mg protein at 5, 7.5, 10, 12.5, 15 and 17.5 per cent NaCl respectively as compared to 2.5 to 21.6 µg per mg protein in normal medium.

Table 18a. Amount of proline (µg/mg protein) accumulated by salt tolerant associative N₂ fixers at different salt concentrations

Isolate	NaCl concentrations (%)				Mean
	0.05	5	7.5	10	
ASANFX S145E	0.9	2.7	15.3	12.9	7.9
ASANFX S148E	1.6	3.7	23.2	12.5	10.3
ASANFXI S173E	1.9	5.4	24.6	10.1	10.5
ASANFIX S125E	2.0	2.9	22.3	0.0	6.8
ASANFXI WL19E	2.9	7.9	19.6	0.0	7.6
ASANFX Dr31E	1.5	3.0	20.2	0.0	6.2
ASANFIX S132E	0.5	2.4	9.0	0.0	3.0
ASANFX S135E	1.0	3.6	9.6	0.0	3.5
ASANFX S138E	0.4	2.9	8.0	0.0	2.8
ASANFX S141E	0.3	2.6	6.1	0.0	2.3
ASANFX S149E	0.6	3.4	8.8	0.0	3.2
ASANFX S150E	0.8	4.1	10.0	0.0	3.7
ASANFX S154E	0.3	2.4	7.4	0.0	2.5
ASANFX WL13E	0.7	4.1	12.1	0.0	4.2
ASANFX WL15E	1.3	5.5	13.8	0.0	5.2
ASANFXI S160E	1.2	5.3	13.4	0.0	5.0
ASANFXI S170E	0.3	2.8	11.5	0.0	3.6
ASANFXI WL17E	0.5	2.7	7.2	0.0	2.6
Mean	1.0	3.7	13.4	2.0	
	NaCl (S)		Isolate (I)		S x I
S.Em.±	0.2		0.3		0.6
C.D.(0.01)	0.6		1.2		2.4

Table 18b. Amount of proline (µg/mg protein) accumulated by salt tolerant free-living N₂ fixers at different salt concentrations

Isolate	NaCl concentrations (%)						Mean
	0.05	5	7.5	10	12.5	15.0	
ASFNFIV S63(1)R	4.3	5.4	36.4	40.3	20.1	3.2	18.3
ASFNFIV S75(1)R	14.6	18.5	51.7	69.2	55.8	0.0	35.0
ASFNFIV S37R	12.9	16.1	29.4	14.0	0.0	0.0	12.1
ASFNFIV WL4(1)R	7.0	14.3	32.9	17.7	0.0	0.0	12.0
ASFNFI Dr4(1)	9.6	21.7	7.0	3.9	0.0	0.0	7.0
ASFNFIV S45(1)R	5.4	18.5	15.6	3.4	0.0	0.0	7.2
ASFNFIV S36(1R)	5.4	13.3	3.7	0.0	0.0	0.0	3.7
ASFNFIV S40(1)R	6.6	17.8	6.3	0.0	0.0	0.0	5.1
ASFNFIV S43(2)R	5.6	17.8	0.8	0.0	0.0	0.0	4.0
ASFNFIV S60(1)S	12.5	22.8	0.8	0.0	0.0	0.0	6.0
ASFNFIV S61(1)S	13.5	5.8	3.6	0.0	0.0	0.0	3.8
ASFNFIV S75(2)R	14.0	15.9	2.7	0.0	0.0	0.0	5.4
ASFNFIV S75E	7.0	18.9	1.0	0.0	0.0	0.0	4.5
ASFNFIV WL5(2)R	8.0	12.6	0.8	0.0	0.0	0.0	3.6
Mean	9.0	15.7	13.8	10.6	5.4	0.2	
	NaCl (S)		Isolate (I)			S x I	
S.Em.±	0.4		0.6			1.4	
C.D.(0.01)	1.4		2.1			5.2	

Table 18c. Amount of proline (µg/mg protein) accumulated by salt tolerant phosphate solubilizers at different salt concentrations

Isolate	NaCl concentrations (%)						Mean
	0.05	5	7.5	10	12.5	15	
ASPSBIX S122R	0.00	0.00	0.00	0.93	7.42	18.75	4.52
ASPSBIX S124E	0.00	0.21	0.94	1.19	5.06	1.35	1.46
ASPSBIX S125R	0.00	0.22	0.32	0.49	0.27	0.43	0.29
ASPSBXI S156R	0.00	0.05	0.41	6.21	23.88	38.63	11.53
ASPSBXI S181R	1.35	1.70	2.31	2.46	3.66	2.24	2.29
ASPSBXII S189E	0.85	1.63	2.39	1.63	1.25	1.21	1.49
ASPSBIX S129R	0.16	1.66	2.55	3.29	4.79	0.00	2.08
ASPSBX S135R	0.04	0.13	0.45	2.49	9.34	0.00	2.08
ASPSBX S139R	0.14	0.77	1.61	2.99	8.19	0.00	2.28
ASPSBX S150R	0.00	0.08	0.41	2.15	6.30	0.00	1.49
ASPSBX Dr29aR	0.00	0.50	0.95	0.37	0.63	0.00	0.41
ASPSBX S142(2)S	0.29	1.00	2.60	1.53	3.10	0.00	1.42
ASPSBX S140S	0.15	0.31	0.65	2.04	9.38	0.00	2.09
ASPSBX S144(1)S	0.82	2.46	3.36	0.98	1.13	0.00	1.46
ASPSBX S145(1)S	0.73	2.03	3.10	1.70	2.96	0.00	1.75
ASPSBX Dr29aS	0.14	1.28	2.49	0.61	0.38	0.00	0.82
ASPSBX WL15(3)S	0.84	1.84	0.97	0.72	0.71	0.00	0.85
ASPSBX Dr27S	0.44	0.52	0.79	1.74	6.17	0.00	1.61
ASPSBX S141E	0.04	0.17	1.10	0.77	0.79	0.00	0.48
ASPSBXI S169(1)R	0.00	0.08	0.54	2.13	6.52	0.00	1.55
ASPSBXI S172(2)S	0.79	0.72	0.51	0.24	0.37	0.00	0.44
ASPSBXII Dr33S	0.85	2.14	2.91	1.43	1.33	0.00	1.44
ASPSBXII S190S	0.65	0.29	0.20	0.15	0.37	0.00	0.28
Mean	0.36	0.86	1.37	1.66	4.52	2.72	
	NaCl (S)		Isolate (I)			S x I	
S.Em.±	0.03		0.05			0.13	
C.D.(0.01)	0.10		0.20			0.48	

Table 18d. Amount of proline (µg/mg protein) accumulated by salt tolerant fluorescent pseudomonads at different salt concentrations

Isolate	NaCl concentrations (%)							Mean
	0.05	5	7.5	10	12.5	15	17.5	
ASFPI S3(1)S	7.0	16.1	24.5	37.6	57.7	54.6	62.4	37.1
ASFPI S4(1)S	21.6	46.7	56.2	57.5	90.0	75.5	29.4	53.8
ASFPI S6(1)S	1.5	8.1	13.1	5.8	5.3	5.1	4.4	6.2
ASFPV Dr16E	10.3	29.7	48.8	36.7	27.8	15.5	8.6	25.3
ASFPV S94(2)E	15.1	29.5	33.2	33.5	57.4	29.3	20.4	31.2
ASFPV S100E	7.6	15.4	15.6	24.1	23.3	12.6	9.1	15.4
ASFPI S9(1)S	7.0	14.3	16.0	25.5	28.4	33.3	0.0	17.8
ASFPI S14(3)S	4.3	9.2	16.1	21.9	26.8	61.9	0.0	20.0
ASFPV Dr10E	3.2	6.7	10.7	13.7	34.4	91.5	0.0	22.9
ASFPV S97R	6.4	23.3	18.8	15.8	16.3	38.6	0.0	17.0
ASFPV S112(a)R	2.5	8.3	16.0	7.8	11.9	0.0	0.0	6.7
ASFPV S158(2)R	3.3	5.8	13.2	8.3	12.6	0.0	0.0	6.2
Mean	7.5	17.8	23.5	24.0	32.7	34.8	11.2	
	NaCl (S)			Isolate (I)			S x I	
S.Em.±	0.7			0.9			2.5	
C.D.(0.01)	2.7			3.5			9.3	

Among the isolates, irrespective of NaCl concentration, the highest amount of proline was recorded by ASFPI S4(1)S (53.8 µg/mg protein) followed by ASFPI S3(1)S (37.1 µg/mg protein) and ASFPV S94(2)E (25.3 µg/mg protein) which differed significantly among themselves but all were significantly superior over rest of the isolates. Among NaCl concentrations tested, the maximum mean proline content (53.8 µg/mg protein) was recorded at 15 per cent NaCl which was significantly superior over all other NaCl concentrations except 12.5 per cent (32.7 µg) with which it was on par. The amount of proline produced was found to increase even up to 17.5 per cent NaCl by ASFPI S3(1)S isolate. The interaction effect was also found to be significant wherein maximum proline content (90.0 µg/mg protein) was recorded by the isolate ASFPI S4(1)S with 12.5 per cent NaCl which was significantly superior over most of other treatment combinations.

4.3.4 Glycine-betaine

The production of glycine-betaine (GBT) in 67 salt-tolerant AIMS at different NaCl concentrations was analyzed and the results are presented in Table 19a to 19d.

4.3.4.1 Associative nitrogen fixers (ANF)

Salt stress was found to have significant influence on the glycine-betaine (GBT) content of salt tolerant ANF isolates. In general, GBT content was found to be higher in the presence of NaCl as compared to control (Table 19a). All the isolates showed GBT production in the presence of 5 and 7.5% NaCl, while only 3 isolates showed GBT production in the presence of 10% NaCl. The amount of GBT produced by ANF isolates in response to NaCl stress ranged from 0.22 to 0.52, 0.43 to 1.22 and 0.00 to 1.85 µmol per mg protein in the presence of 5, 7.5 and 10 per cent NaCl respectively as compared to normal medium (0.08 to 0.27 µmol) (control).

Among the isolates, irrespective of NaCl concentrations, the highest GBT content was recorded (0.92 µmol/ mg protein) in ASANFXI S173E which was significantly superior over all other isolates (Table 19a). Isolates ASANFXI S145E (0.76 µmol/mg protein) and ASANFXI S173E (0.71 µmol/mg protein) also recorded significantly higher GBT over other strains but were on par among themselves. Among the NaCl concentrations (irrespective of isolates), the highest GBT production was recorded at 7.5% NaCl which was significantly superior over all other concentrations. The interaction effect was found to be significant wherein the isolate ASANFXI S173E at 10% NaCl recorded the highest GBT content (1.85 µmol) and was significantly superior over all other treatment combinations. Isolates ASANFXI S145E and ASANFXI S148E at 10 per cent NaCl also recorded significantly higher GBT content (1.52 and 1.38 µmol per mg protein respectively) than all other treatment combinations.

4.3.4.2 Free-living nitrogen fixers (FNF)

Wide variation in GBT content among the FNF isolates was observed at different NaCl concentrations (Table 19b). GBT content, in general was found to increase up to 7.5 per cent NaCl and declined thereafter. While the isolate ASFNFIVS63(1)R showed increased production of GBT up to 12.5% NaCl, isolate ASFNFIV S75(1)R produced increasing quantities of GBT up to 10% NaCl, whereas all other isolates recorded higher production of GBT up to 7.5% NaCl. Salt stress resulted in significant variation in GBT content of the isolates and was in the range of 0.31 to 0.56 µmol, 0.30 to 1.42 µmol, 0.00 to 1.61 µmol, 0.00 to 1.57 µmol and 0.00 to 0.84 µmol per mg protein at 5, 7.5, 10, 12.5 and 15 per cent NaCl respectively when compared to control broth (0.1 to 0.25 µmol per mg protein).

Among the isolates, irrespective of salt concentration, two isolates ASFNFIV S63(1)R and ASFNFIV S75(1)R were found to accumulate maximum GBT of 0.86 and 0.85 µmol per mg protein respectively and were found to be on par with each other but significantly superior over all other isolates. Irrespective of isolates, 7.5 per cent NaCl treatment recorded maximum GBT (0.84 µmol per mg protein) followed by 10 and 5 per cent NaCl levels. The isolate ASFNFIV S75(1)R with 10 per cent NaCl recorded the highest GBT content (1.61 µmol per mg protein) followed by ASANFIV S75(1)R and ASFNFIV S63(1)R with 12.5 per cent NaCl (1.57 and 1.52 µmol per mg protein respectively) and all of them were found to be on par with each other but significantly superior over other treatment combinations.

Table 19a. Amount of glycine betaine (µmol/mg protein) accumulated by salt tolerant associative N₂ fixers at different salt concentrations

Isolate	NaCl concentrations (%)				Mean
	0.05	5	7.5	10	
ASANFX S145E	0.17	0.45	0.89	1.52	0.76
ASANFX S148E	0.20	0.41	0.84	1.38	0.71
ASANFXI S173E	0.16	0.46	1.22	1.85	0.92
ASANFIX S125E	0.14	0.36	0.93	0.00	0.36
ASANFXI WL19E	0.21	0.47	0.98	0.00	0.42
ASANFX Dr31E	0.20	0.52	0.66	0.00	0.35
ASANFIX S132E	0.15	0.29	0.74	0.00	0.30
ASANFX S135E	0.22	0.41	0.82	0.00	0.36
ASANFX S138E	0.20	0.45	0.81	0.00	0.37
ASANFX S141E	0.20	0.31	0.51	0.00	0.26
ASANFX S149E	0.10	0.33	0.63	0.00	0.27
ASANFX S150E	0.15	0.34	0.70	0.00	0.30
ASANFX S154E	0.12	0.32	0.63	0.00	0.27
ASANFX WL13E	0.19	0.33	0.70	0.00	0.31
ASANFX WL15E	0.13	0.36	0.51	0.00	0.25
ASANFXI S160E	0.27	0.48	0.69	0.00	0.36
ASANFXI S170E	0.17	0.27	0.86	0.00	0.33
ASANFXI WL17E	0.08	0.22	0.43	0.00	0.18
Mean	0.17	0.38	0.75	0.26	
	NaCl (S)		Isolate (I)		S x I
S.Em.±	0.01		0.02		0.05
C.D.(0.01)	0.04		0.09		0.18

Table 19b. Amount of glycine betaine (µmol/mg protein) accumulated by salt tolerant free-living N₂ fixers at different salt concentrations

Isolate	NaCl concentrations (%)						Mean
	0.05	5	7.5	10	12.5	15	
ASFNFIV S63(1)R	0.14	0.35	1.07	1.22	1.52	0.84	0.86
ASFNFIV S75(1)R	0.16	0.45	1.31	1.61	1.57	0.00	0.85
ASFNFIV S37R	0.20	0.31	0.89	0.85	0.00	0.00	0.38
ASFNFIV WL4(1)R	0.13	0.35	0.82	0.70	0.00	0.00	0.33
ASFNFI Dr4(1)	0.13	0.41	1.02	0.64	0.00	0.00	0.37
ASFNFIV S45(1)R	0.25	0.54	1.42	1.32	0.00	0.00	0.59
ASFNFIV S36(1)R	0.15	0.43	0.57	0.00	0.00	0.00	0.19
ASFNFIV S40(1)R	0.22	0.44	0.62	0.00	0.00	0.00	0.21
ASFNFIV S43(2)R	0.14	0.46	0.70	0.00	0.00	0.00	0.22
ASFNFIV S60(1)S	0.11	0.52	0.79	0.00	0.00	0.00	0.24
ASFNFIV S61(1)S	0.11	0.41	0.69	0.00	0.00	0.00	0.20
ASFNFIV S75(2)R	0.19	0.37	0.30	0.00	0.00	0.00	0.14
ASFNFIV S75E	0.22	0.56	0.74	0.00	0.00	0.00	0.25
ASFNFIV WL5(2)R	0.10	0.43	0.82	0.00	0.00	0.00	0.23
Mean	0.16	0.43	0.84	0.45	0.22	0.06	
	NaCl (S)		Isolate (I)			S x I	
S.Em.±	0.03		0.04			0.10	
C.D.(0.01)	0.10		0.16			0.38	

Table 19c. Amount of glycine betaine (µmol/mg protein) accumulated by salt tolerant phosphate solubilizers at different salt concentrations

Isolate	NaCl concentrations (%)						Mean
	0.05	5	7.5	10	12.5	15.0	
ASPSBIX S122R	0.17	0.33	0.61	0.99	1.67	1.40	0.86
ASPSBIX S124E	0.14	0.25	0.55	0.79	1.39	1.26	0.73
ASPSBIX S125R	0.12	0.20	0.31	0.66	1.55	1.21	0.68
ASPSBXI S156R	0.12	0.25	0.41	0.99	1.79	1.49	0.84
ASPSBXI S181R	0.12	0.23	0.46	0.71	0.99	1.02	0.59
ASPSBXII S189E	0.16	0.30	0.45	0.79	1.49	1.21	0.73
ASPSBIX S129R	0.08	0.51	0.74	1.04	2.19	0.00	0.76
ASPSBX S135R	0.03	0.06	0.22	0.76	2.35	0.00	0.57
ASPSBX S139R	0.07	0.31	0.47	0.79	2.03	0.00	0.61
ASPSBX S150R	0.00	0.05	0.21	0.63	1.69	0.00	0.43
ASPSBX Dr29aR	0.00	0.28	0.42	0.18	0.42	0.00	0.22
ASPSBX S142(2)S	0.15	0.37	0.72	0.69	1.88	0.00	0.64
ASPSBX S140S	0.07	0.17	0.35	0.74	2.81	0.00	0.69
ASPSBX S144(1)S	0.33	0.62	0.81	0.59	0.48	0.00	0.47
ASPSBX S145(1)S	0.31	0.56	0.77	0.73	1.38	0.00	0.63
ASPSBX Dr29aS	0.07	0.44	0.65	0.32	0.25	0.00	0.29
ASPSBX WL15(3)S	0.34	0.49	0.38	0.41	0.35	0.00	0.33
ASPSBX Dr27S	0.23	0.28	0.40	0.71	2.31	0.00	0.66
ASPSBX S141E	0.03	0.08	0.42	0.44	0.39	0.00	0.23
ASPSBXI S169(1)R	0.00	0.05	0.30	0.71	2.15	0.00	0.54
ASPSBXI S172(2)S	0.32	0.31	0.31	0.13	0.24	0.00	0.22
ASPSBXII Dr33S	0.33	0.51	0.69	0.60	0.62	0.00	0.46
ASPSBXII S190S	0.30	0.16	0.10	0.11	0.22	0.00	0.15
Mean	0.15	0.30	0.47	0.63	1.33	0.33	
	NaCl (S)		Isolate (I)			S x I	
S.Em.±	0.02		0.03			0.07	
C.D.(0.01)	0.06		0.11			0.27	

Table 19d. Amount of glycine betaine (µmol/mg protein) accumulated by salt tolerant fluorescent pseudomonads at different salt concentrations

Isolate	NaCl concentrations (%)							Mean
	0.05	5	7.5	10	12.5	15	17.5	
ASFPI S3(1)S	0.06	0.15	0.34	0.79	1.21	1.02	0.80	0.62
ASFPI S4(1)S	0.13	0.30	0.60	0.62	1.28	0.59	0.45	0.57
ASFPI S6(1)S	0.06	0.14	0.30	0.43	0.97	0.66	0.41	0.42
ASFPV Dr16E	0.13	0.29	0.62	0.74	0.93	0.52	0.26	0.50
ASFPV S94(2)E	0.03	0.10	0.25	0.41	1.46	0.73	0.47	0.49
ASFPV S100E	0.02	0.07	0.17	0.35	0.71	0.29	0.25	0.27
ASFPI S9(1)S	0.04	0.12	0.31	0.49	0.93	0.34	0.00	0.32
ASFPI S14(3)S	0.04	0.11	0.27	0.49	0.89	0.48	0.00	0.33
ASFPV Dr10E	0.10	0.23	0.49	0.41	1.30	0.71	0.00	0.46
ASFPV S97R	0.09	0.22	0.51	0.51	0.81	0.68	0.00	0.40
ASFPV S112(a)R	0.08	0.17	0.37	0.34	0.81	0.00	0.00	0.25
ASFPV S158(2)R	0.03	0.09	0.26	0.36	0.74	0.00	0.00	0.21
Mean	0.07	0.17	0.37	0.50	1.00	0.50	0.22	
	NaCl (S)			Isolate (I)			S x I	
S.Em.±	0.02			0.02			0.06	
C.D.(0.01)	0.06			0.08			0.21	

4.3.4.3 Phosphate solubilizing bacteria (PSB)

The amount of GBT produced by salt-tolerant PSB isolates in response to changes in NaCl concentrations are presented in Table 19c. Salt-tolerant PSB isolates were found to produce higher amounts of GBT under NaCl stress which ranged from 0.05 to 0.62, 0.10 to 0.81, 0.11 to 1.04, 0.22 to 2.81 and 0.00 to 1.49 μmol per mg protein in the presence of 5, 7.5, 10, 12.5 and 15 per cent NaCl respectively as compared to normal medium (0.00 to 0.34 μmol per mg protein). In most of the isolates, GBT content was found to increase with increase in NaCl concentration up to 12.5 per cent NaCl and declined thereafter. All the isolates were found to produce GBT up to 12.5% NaCl but 3 and 17 isolates did not show GBT production in control and 15% NaCl respectively. Among the isolates, irrespective of NaCl concentration, isolate ASPSBIX S122R followed by ASPSBXI S156R recorded maximum GBT content (0.86 and 0.84 μmol per mg protein respectively) which were found to be on par with each other but significantly superior over most other isolates.

Of all the NaCl levels tested, addition of 12.5 per cent to the growth medium resulted in significantly higher accumulation of GBT (1.33 $\mu\text{mol}/\text{mg}$ protein) over all other concentrations of NaCl and control medium (0.15 $\mu\text{mol}/\text{mg}$ protein). The interaction effect was also found to be significant wherein isolate ASPSBX S140S with 12.5 per cent NaCl recorded significantly higher GBT (2.81 $\mu\text{mol}/\text{mg}$ protein) as compared to other treatment combinations (Table 19c).

4.3.4.4 Fluorescent pseudomonads (FP)

A total of 12 FP isolates were analyzed for GBT content in response to salt stress. It was observed that, increase in NaCl concentration up to 12.5 per cent resulted in increased biosynthesis of GBT and was decreased thereafter. The amount of GBT produced in response to NaCl stress ranged from 0.07 to 0.30, 0.25 to 0.62, 0.34 to 0.79, 0.74 to 1.46, 0.00 to 1.02 and 0.00 to 0.80 μmol per mg protein at 5, 7.5, 10, 12.5, 15 and 17.5 per cent NaCl respectively as compared to control medium (0.02 to 0.13 μmol per mg protein) (Table 19d).

Among the FP isolates, irrespective of NaCl concentration, ASFPI S3(1)S recorded maximum GBT (0.62 $\mu\text{mol}/\text{mg}$ protein) which was followed by ASFPI S4(1)S (0.57 $\mu\text{mol}/\text{mg}$ protein) and both were significantly superior over all other isolates except ASFPV Dr16E with which they were on par. Among the salt concentrations, 12.5 per cent NaCl recorded (1.0 $\mu\text{mol}/\text{mg}$ protein) significantly higher GBT over all other NaCl concentrations, and was least in control medium (0.07 $\mu\text{mol}/\text{mg}$ protein). Among all the treatment combinations, ASFPV S94(2)E with 12.5 per cent NaCl recorded significantly higher GBT (1.46 $\mu\text{mol}/\text{mg}$ protein) than most other treatment combinations.

4.3.5 Total sugars

Sixty seven salt-tolerant AIMs were examined for production of total sugars at different NaCl concentrations. All the isolates belonging to different functional groups were found to differ in their response to increased NaCl concentrations. Data obtained from the present study are presented in Tables 20a-d.

4.3.5.1 Associative nitrogen fixers (ANF)

Changes in the total sugar content of associative N_2 fixers in response to salt stress was recorded and presented in Table 20a. All the isolates showed increase in total sugar content with increase in the medium NaCl concentration up to 7.5 per cent and declined thereafter. All the isolates showed total sugar production in the presence of 5 and 7.5% NaCl while only 3 isolates showed total sugar production in the presence of 10% NaCl. However, two isolates ASANFX S148E and ASANFXI S173E were found to produce increased amount of total sugars even at 10 per cent NaCl. The amount of total sugar produced by the isolates ranged from 31.4 to 76.0, 32.3 to 87.3 and 0.0 to 63.5 μg per mg protein in the presence of 5, 7.5 and 10 per cent NaCl respectively as compared to 16.2 to 38.9 μg per mg protein in the normal medium. Irrespective of salt concentrations, three isolates ASANFXI S173E, ASANFX S148E and ASANFXI WL 19E showed significantly higher total sugar contents (52.8, 51.2 and 50.0 μg per mg protein respectively) over all other isolates but were on par with each other.

Among the salt concentrations tested, 7.5 per cent NaCl treatment recorded significantly higher mean total sugars (54.7 µg/mg protein) than all other NaCl concentrations including control. The interaction effect was found to be significant wherein the isolate ASANFXI WL19E with 7.5 per cent NaCl recorded significantly higher total sugar content (87.3 µg per mg protein) over all other treatment combinations except ASANFX Dr31E with 7.5 per cent NaCl (76.4 µg/mg protein) with which it was on par.

4.3.5.2 Free-living nitrogen fixers (FNF)

All the isolates were found to produce higher amounts of total sugars in the presence of NaCl than in control medium. In general, the total sugar content increased with increase in salt concentration up to 7.5 per cent NaCl only and declined thereafter. While, four out of 14 FNF isolates produced increased amounts of total sugars up to 10 per cent NaCl, isolate ASFNFIV S63(1)R produced increasing amounts of total sugars with increase in NaCl concentration up to 15% (Table 20b).

Among the different NaCl concentrations, irrespective of isolates, significantly higher total sugar content of 50.9 µg per mg protein was recorded with 7.5 per cent NaCl which was significantly higher than all other NaCl concentrations. Irrespective of NaCl concentrations, ASFNFIV S63(1)R among the isolates recorded the highest amount of total sugars (65 µg/mg protein) followed by ASFNFIV S75(1)R (55.7 µg/mg protein) both of which were significantly superior over all other isolates but were on par with each other. The interaction effect was also found to be significant. The highest total sugar content was recorded by ASFNFIV WL4(1)R at 15 per cent NaCl (116.1 µg/mg protein) which was however on par with ASFNFIV WL4(1)R (115.2 µg) and ASFNF S75(1)R (104.6 µg) both at 10% NaCl but significantly superior over all other treatment combinations.

4.3.5.3 Phosphate solubilizing bacteria (PSB)

Addition of NaCl to the growth medium in general resulted in increased production of total sugars by the salt tolerant PSB isolates (Table 20c). The amount of total sugars produced under salt stress ranged from 15.7 to 31.9, 17 to 34.5, 17 to 35.5, 26.9 to 109.6 and 0.0 to 137.4 µg per mg protein in the presence of 5, 7.5, 10, 12.5 and 15 per cent NaCl respectively as compared to normal medium wherein the total sugar produced was in the range of 10.7 to 19.5 µg per mg protein.

Among the isolates, irrespective of NaCl concentrations, significantly higher total sugar contents of 53.3, 53.1, 52.8, 52.3 and 50.7 µg per mg protein were produced by ASPSBXI S181R, ASPSBXI S156R, ASPSBIX S125R, ASPSBIX S122R and ASPSBIX S124E respectively over other isolates but were found to be on par with each other. Among the NaCl concentrations tested, maximum mean total sugar content was recorded (66.9 µg per mg protein) at 12.5 per cent NaCl which was significantly higher than all other NaCl concentrations. The interaction effect was also found to be significant wherein the highest sugar production was recorded in ASPSBXI S181R with 15 per cent NaCl (137.4 µg per mg protein) followed by ASPSBXI S156R at 15% NaCl (136.7 µg per mg protein) both of which were significantly superior over all other treatment combinations (Table 20c).

4.3.5.4 Fluorescent pseudomonads (FP)

All the isolates were found to produce higher amount of total sugars in the presence of NaCl as compared to control (Table 20d). However, two and six isolates failed to show accumulation of total sugar at 15 and 17.5% NaCl respectively. Under salt stress, the amount of total sugar production ranged from 8.4 to 19.2, 9.4 to 22.7, 10.0 to 22.8, 10.1 to 33.8, 0.0 to 33.6 and 0.0 to 26.0 µg per mg protein in the presence of 5, 7.5, 10, 12.5, 15 and 17.5 per cent NaCl respectively, whereas it was in the range of 6.8 to 12.8 µg per mg protein in the control medium. Irrespective of salt concentrations, significantly higher total sugar was recorded (22.6 µg/mg protein) by ASFPI S4(1)S followed by ASFPI S3(1)S (21.1 µg/mg protein) both of which were significantly superior over rest of the isolates. Among the NaCl concentrations, 15 per cent NaCl treatment recorded the maximum mean total sugar production (20.8 µg/mg protein) which was however on par with 12.5% NaCl but both were significantly superior over all other NaCl concentrations (Table 20d).

Table 20a. Changes in the total sugar ($\mu\text{g}/\text{mg}$ protein) content of salt tolerant associative N_2 fixers at different salt concentrations

Isolate	NaCl concentrations (%)				Mean
	0.05	5	7.5	10	
ASANFX S145E	32.9	39.9	51.0	49.7	43.4
ASANFX S148E	37.7	45.9	58.7	62.3	51.2
ASANFXI S173E	37.2	49.4	61.0	63.5	52.8
ASANFIX S125E	25.7	31.4	56.4	0.0	28.4
ASANFXI WL19E	36.6	76.0	87.3	0.0	50.0
ASANFX Dr31E	38.9	45.0	76.4	0.0	40.1
ASANFIX S132E	22.0	40.4	41.5	0.0	26.0
ASANFX S135E	19.6	38.5	38.8	0.0	24.2
ASANFX S138E	23.8	43.6	46.2	0.0	28.4
ASANFX S141E	16.2	31.8	32.3	0.0	20.1
ASANFX S149E	25.1	47.3	52.2	0.0	31.2
ASANFX S150E	27.1	49.0	54.2	0.0	32.6
ASANFX S154E	25.1	49.2	55.9	0.0	32.6
ASANFX WL13E	24.1	53.5	67.0	0.0	36.2
ASANFX WL15E	19.1	40.8	42.6	0.0	25.6
ASANFXI S160E	24.5	45.7	49.5	0.0	29.9
ASANFXI S170E	25.8	48.6	54.1	0.0	32.1
ASANFXI WL17E	25.8	51.4	60.0	0.0	34.3
Mean	27.1	46.0	54.7	9.8	
	NaCl (S)		Isolate (I)		S x I
S.Em. $\pm$	0.9		2.0		4.0
C.D.(0.01)	3.6		7.5		15.1

Table 20b. Changes in the total sugar ($\mu\text{g}/\text{mg}$ protein) content of salt tolerant free-living N_2 fixers at different salt concentrations

Isolate	NaCl concentrations (%)						Mean
	0.05	5	7.5	10	12.5	15.0	
ASFNFIV S63(1)R	21.9	27.4	66.2	67.8	90.7	116.1	65.0
ASFNFIV S75(1)R	24.0	29.7	87.2	104.6	88.4	0.0	55.7
ASFNFIV S37R	23.2	26.9	69.4	84.4	0.0	0.0	34.0
ASFNFIV WL4(1)R	27.5	32.5	64.1	115.2	0.0	0.0	39.9
ASFNFI Dr4(1)	27.9	38.3	74.4	67.9	0.0	0.0	34.8
ASFNFIV S45(1)R	24.7	35.0	73.7	64.4	0.0	0.0	33.0
ASFNFIV S36(1R)	26.1	37.9	36.5	0.0	0.0	0.0	16.8
ASFNFIV S40(1)R	18.9	33.5	34.7	0.0	0.0	0.0	14.5
ASFNFIV S43(2)R	24.6	28.2	32.9	0.0	0.0	0.0	14.3
ASFNFIV S60(1)S	28.7	35.8	40.9	0.0	0.0	0.0	17.6
ASFNFIV S61(1)S	26.1	37.3	41.1	0.0	0.0	0.0	17.4
ASFNFIV S75(2)R	17.9	22.3	23.3	0.0	0.0	0.0	10.6
ASFNFIV S75E	26.3	33.6	40.8	0.0	0.0	0.0	16.8
ASFNFIV WL5(2)R	20.2	26.7	27.9	0.0	0.0	0.0	12.5
Mean	24.1	31.8	50.9	36.0	12.8	8.3	
	NaCl (S)		Isolate (I)			S x I	
S.Em. $\pm$	1.3		1.9			4.8	
C.D.(0.01)	4.8		7.3			17.8	

Table 20c. Changes in the total sugar ($\mu\text{g}/\text{mg}$ protein) content of salt tolerant phosphate solubilizers at different salt concentrations

Isolate	NaCl concentrations (%)						Mean
	0.05	5	7.5	10	12.5	15.0	
ASPSBIX S122R	14.0	27.4	28.2	27.7	96.5	120.0	52.3
ASPSBIX S124E	17.3	26.9	27.5	26.2	109.6	96.6	50.7
ASPSBIX S125R	17.5	31.9	34.5	35.1	87.3	110.7	52.8
ASPSBIX S156R	13.3	25.0	27.7	28.3	87.3	136.7	53.1
ASPSBXI S181R	19.5	28.3	30.2	31.7	72.6	137.4	53.3
ASPSBXII S189E	15.3	26.8	28.2	27.7	61.0	98.1	42.9
ASPSBIX S129R	15.2	21.8	25.1	31.3	84.7	0.0	29.7
ASPSBX S135R	13.1	18.9	21.0	23.7	47.6	0.0	20.7
ASPSBX S139R	13.7	19.4	21.7	24.0	43.2	0.0	20.4
ASPSBX S150R	10.7	15.7	17.0	17.0	26.9	0.0	14.6
ASPSBX Dr29aR	12.2	18.5	20.8	24.3	60.5	0.0	22.7
ASPSBX S142(2)S	15.1	21.8	24.8	28.9	70.1	0.0	26.8
ASPSBX S140S	16.0	22.5	25.5	28.9	70.8	0.0	27.3
ASPSBX S144(1)S	18.3	25.0	29.5	35.5	82.9	0.0	31.9
ASPSBX S145(1)S	17.1	23.6	27.2	32.3	72.7	0.0	28.8
ASPSBX Dr29aS	13.4	18.8	20.8	22.0	52.1	0.0	21.2
ASPSBX WL15(3)S	17.0	23.1	26.2	30.1	65.3	0.0	26.9
ASPSBX Dr27S	17.0	23.6	26.7	31.6	73.3	0.0	28.7
ASPSBX S141E	12.7	18.4	20.5	22.2	47.9	0.0	20.3
ASPSBXI S169(1)R	10.7	15.8	17.2	17.1	36.2	0.0	16.2
ASPSBXI S172(2)S	16.7	22.8	25.5	28.5	68.5	0.0	27.0
ASPSBXII Dr33S	17.1	23.0	25.6	28.5	64.3	0.0	26.4
ASPSBXII S190S	15.6	21.5	24.4	27.8	57.9	0.0	24.5
Mean	15.2	22.6	25.0	27.4	66.9	30.4	
	NaCl (S)		Isolate (I)			S x I	
S.Em.±	0.8		1.6			4.0	
C.D.(0.01)	3.0		6.0			14.6	

Table 20d. Changes in the total sugar ($\mu\text{g}/\text{mg}$ protein) content of salt tolerant fluorescent pseudomonads at different salt concentrations

Isolate	NaCl concentrations (%)							Mean
	0.05	5	7.5	10	12.5	15	17.5	
ASFPI S3(1)S	11.4	15.5	17.2	18.8	31.5	27.4	26.0	21.1
ASFPI S4(1)S	11.9	17.7	21.6	22.8	28.6	33.6	22.3	22.6
ASFPI S6(1)S	8.4	10.5	11.5	15.5	19.2	18.5	16.3	14.3
ASFPV Dr16E	12.8	19.2	22.7	22.2	21.4	15.6	10.2	17.7
ASFPV S94(2)E	9.2	11.9	12.6	16.0	33.8	29.7	18.3	18.8
ASFPV S100E	6.8	8.4	9.4	15.7	17.9	18.3	11.4	12.5
ASFPI S9(1)S	8.7	11.3	12.0	15.0	14.1	10.1	0.0	10.2
ASFPI S14(3)S	8.7	11.0	11.5	15.9	18.9	33.3	0.0	14.2
ASFPV Dr10E	10.9	15.3	17.3	12.9	17.4	29.3	0.0	14.7
ASFPV S97R	11.5	13.1	14.1	14.4	16.5	33.9	0.0	14.8
ASFPV S112(a)R	9.4	12.4	13.4	10.0	10.1	0.0	0.0	7.9
ASFPV S158(2)R	8.7	11.3	12.0	13.1	13.8	0.0	0.0	8.4
Mean	9.9	13.1	14.6	16.0	20.3	20.8	8.7	
	NaCl (S)			Isolate (I)			S x I	
S.Em.±	0.5			0.6			1.6	
C.D.(0.01)	1.8			2.3			6.1	

Among all the treatment combinations, ASFPV 94(2)E with 12.5 per cent NaCl (33.8 µg/mg protein) followed by ASFPI S4(1)S with 15 per cent NaCl (33.6 µg/mg protein) produced significantly higher total sugar than all other treatment combinations.

4.3.6 Intracellular sodium (Na) and potassium (K) contents and their ratio

All 67 salt-tolerant AIMs consisting of 18 ANF, 14 FNF, 23 PSB and 12 FP isolates were examined for intracellular sodium and potassium contents in the presence of different NaCl concentrations and their intracellular Na to K ratio was calculated. The results obtained from this study are presented in Tables 21a to d.

4.3.6.1 Associative nitrogen fixers (ANF)

Among the ANF isolates, irrespective of NaCl concentrations, ASANFXI S173E recorded the highest mean intracellular Na and K contents (17.8 and 83.8 µg/ml respectively) which were significantly superior over all other isolates except ASANFX S148E with which it was on par (Table 21a). Among the salt concentrations, the highest mean intracellular Na and K contents were recorded at 7.5% NaCl (22.9 and 84.2 µg/ml respectively) which were significantly superior over rest of the NaCl concentrations examined. The interaction effect was also found to be significant. Among the treatment combinations, isolate ASANFXI S173E with 10% NaCl recorded the highest intracellular Na and K contents (30 and 113 µg/ml respectively) which was significantly superior over most other treatment combinations (Table 21a).

In general, the intracellular sodium to potassium (Na/K) ratio was found to increase with increase in NaCl concentrations up to 7.5 per cent and declined thereafter. All the isolates were found to exhibit the changes in the intracellular Na/K ratio up to 7.5% NaCl while, only 3 isolates showed it at 10% NaCl (Table 21a). The intracellular Na/K ratio under NaCl stress was ranged from 0.15 to 0.26, 0.23 to 0.37 and 0.00 to 0.27 in the presence of 5, 7.5 and 10 per cent NaCl respectively as compared to normal medium (0.11 to 0.19).

Among the isolates, irrespective of NaCl concentrations, ASANFX S145E recorded the highest mean Na/K ratio (0.22) which was significantly superior over most of the isolates but was on par with 11 ANF isolates whose Na/K ratio was in the range of 0.16 to 0.21. Among NaCl concentrations, significantly higher mean Na/K ratio was recorded at 7.5% NaCl (0.28) which was significantly superior over other salt concentrations examined. While the least Na/K ratio was recorded at 10% NaCl (0.04). The interaction effect was also found to be significant in which the isolate ASANFX S138E at 7.5% NaCl recorded the highest Na/K ratio (0.37) which was significantly superior over most other treatment combinations (Table 21a).

4.3.6.2 Free-living nitrogen fixers (FNF)

Significant differences in intracellular Na and K content of FNF isolates were observed. Isolates were found to have maximum amount of intracellular K than Na. Among the isolates, ASFNFIV S63(1)R recorded the highest mean intracellular Na and K contents (25 and 74 µg/ml respectively) and was significantly superior over all other isolates (Table 21b). Among the salt concentrations, the highest mean intracellular Na and K contents were recorded at 7.5% NaCl (27 and 75 µg/ml respectively) which were significantly higher than that recorded at rest of the NaCl concentrations. The interaction effect was also found to be significant. Among the treatment combinations, isolate ASFNFIV S63(1)R at 15% and 10% NaCl recorded the highest intracellular Na and K contents of 41 and 113 µg/ml respectively which were significantly superior over most other treatment combinations (Table 21b).

Significant differences in intracellular Na to K ratio of FNF isolates were observed. The intracellular Na/K ratio was recorded in all the isolates up to 7.5% NaCl while, only 6, 2 and one isolates showed Na/K ratio at 10, 12.5 and 15% NaCl respectively (Table 21b). The salt stress resulted in significant changes in the intracellular Na/K ratio and was ranged between 0.18 to 0.45, 0.22 to 0.48, 0.00 to 0.50 and 0.00 to 0.45 in the presence of 5, 7.5, 10, 12.5 and 15 per cent NaCl respectively when compared to control medium (0.09 to 0.34).

Among the isolates, the highest mean Na/K ratio (0.32) was recorded in ASFNFIV S63(1)R which was significantly superior over rest of the isolates except ASFNFIV WL4(1)R (0.24) and ASFNFIV S45(1)R (0.24) with which it was on par (Table 21b).

Table 21a: Changes in the intracellular sodium and potassium content and Na to K ratio of salt tolerant associative N₂ fixers at different salt concentrations

Isolate	Intracellular Na content (µg/ml)				Mean	Intracellular K content (µg/ml)				Mean	Na to K ratio				Mean
	NaCl concentrations (%)					NaCl concentrations (%)					NaCl concentrations (%)				
	0.05	5	7.5	10		0.05	5	7.5	10		0.05	5	7.5	10	
ASANFX S145E	7.0	10.0	17.0	25.0	14.8	41.0	59.0	70.0	93.0	65.8	0.17	0.17	0.25	0.27	0.22
ASANFX S148E	5.0	12.0	23.0	28.0	17.0	44.0	69.0	83.0	103.0	74.8	0.11	0.18	0.28	0.27	0.21
ASANFXI S173E	6.0	14.0	21.0	30.0	17.8	52.0	76.0	94.0	113.0	83.8	0.12	0.19	0.23	0.27	0.20
ASANFIX S125E	7.0	12.0	19.0	0.0	9.5	40.0	60.0	81.0	0.0	45.3	0.18	0.20	0.24	0.00	0.16
ASANFXI WL19E	10.0	19.0	24.0	0.0	13.3	62.0	75.0	89.0	0.0	56.5	0.16	0.26	0.27	0.00	0.17
ASANFX Dr31E	9.0	16.0	21.0	0.0	11.5	53.0	62.0	78.0	0.0	48.3	0.17	0.26	0.28	0.00	0.18
ASANFIX S132E	8.6	11.1	23.4	0.0	10.8	50.6	58.9	73.6	0.0	45.8	0.17	0.19	0.33	0.00	0.17
ASANFX S135E	7.5	9.3	22.6	0.0	9.9	48.9	57.9	70.6	0.0	44.4	0.15	0.17	0.33	0.00	0.16
ASANFX S138E	10.3	12.9	26.9	0.0	12.5	54.6	60.5	73.9	0.0	47.3	0.19	0.22	0.37	0.00	0.19
ASANFX S141E	7.2	11.9	24.8	0.0	11.0	52.3	62.5	94.0	0.0	52.2	0.14	0.19	0.27	0.00	0.15
ASANFX S149E	7.6	9.6	21.3	0.0	9.6	46.9	55.6	88.1	0.0	47.7	0.16	0.18	0.25	0.00	0.15
ASANFX S150E	7.9	10.1	21.3	0.0	9.8	49.3	58.9	82.6	0.0	47.7	0.16	0.18	0.26	0.00	0.15
ASANFX S154E	6.8	11.3	24.1	0.0	10.6	52.1	70.3	94.3	0.0	54.2	0.13	0.16	0.26	0.00	0.14
ASANFX WL13E	7.2	8.8	22.7	0.0	9.7	48.9	58.9	81.5	0.0	47.3	0.15	0.15	0.28	0.00	0.15
ASANFX WL15E	10.3	13.1	28.1	0.0	12.9	56.9	65.9	91.2	0.0	53.5	0.18	0.20	0.31	0.00	0.17
ASANFXI S160E	8.5	10.8	25.6	0.0	11.2	50.6	55.6	90.6	0.0	49.2	0.17	0.20	0.29	0.00	0.16
ASANFXI S170E	7.9	10.5	23.6	0.0	10.5	52.3	63.6	85.9	0.0	50.5	0.15	0.17	0.28	0.00	0.15
ASANFXI WL17E	5.3	8.8	21.9	0.0	9.0	44.6	55.6	94.2	0.0	48.6	0.12	0.16	0.24	0.00	0.13
Mean	7.7	11.7	22.9	4.6		50.0	62.5	84.2	17.2		0.15	0.19	0.28	0.04	
	NaCl (S)		Isolate (I)		S x I	NaCl (S)		Isolate (I)		S x I	NaCl (S)		Isolate (I)		S x I
S.Em.±	0.3		0.6		1.3	1.5		3.3		6.6	0.01		0.02		0.03
C.D. (0.01)	1.1		2.4		4.8	5.8		12.3		24.6	0.03		0.06		0.12

Table 21b: Changes in the intracellular sodium and potassium content and Na to K ratio of salt tolerant free-living N₂ fixers at different salt concentrations

Isolate	Intracellular Na content (µg/ml)						Mean	Intracellular K content (µg/ml)						Mean	N a to K ratio						Mean
	NaCl concentrations (%)							NaCl concentrations (%)							NaCl concentrations (%)						
	0.05	5	7.5	10	12.5	15		0.05	5	7.5	10	12.5	15		0.05	5	7.5	10	12.5	15	
ASFNFIV S63 (1)R	5	12	22	33	36	41	25	26	52	72	113	95	86	74	0.20	0.24	0.32	0.30	0.38	0.48	0.32
ASFNFIV S75 (1)R	7	10	19	30	33	0	17	38	57	89	99	73	0	59	0.19	0.18	0.22	0.31	0.45	0.00	0.23
ASFNFIV S37R	9	16	29	35	0	0	15	36	60	79	73	0	0	41	0.25	0.27	0.38	0.50	0.00	0.00	0.23
ASFNFIV WL4 (1)R	10	21	31	35	0	0	16	38	62	83	80	0	0	44	0.27	0.35	0.38	0.45	0.00	0.00	0.24
ASFNFI Dr4 (1)	6	21	29	33	0	0	15	41	75	71	71	0	0	43	0.15	0.28	0.42	0.48	0.00	0.00	0.22
ASFNFIV S45 (1)R	7	18	23	31	0	0	13	25	55	68	71	0	0	37	0.29	0.34	0.35	0.45	0.00	0.00	0.24
ASFNFIV S36 (1R)	5	19	27	0	0	0	9	20	50	62	0	0	0	22	0.27	0.39	0.45	0.00	0.00	0.00	0.18
ASFNFIV S40 (1)R	12	18	26	0	0	0	9	44	56	65	0	0	0	27	0.28	0.33	0.42	0.00	0.00	0.00	0.17
ASFNFIV S43 (2)R	11	22	30	0	0	0	11	41	69	82	0	0	0	32	0.27	0.32	0.37	0.00	0.00	0.00	0.16
ASFNFIV S60 (1)S	4	16	24	0	0	0	7	27	69	75	0	0	0	28	0.15	0.24	0.33	0.00	0.00	0.00	0.12
ASFNFIV S61 (1)S	9	31	37	0	0	0	13	33	70	79	0	0	0	30	0.28	0.45	0.48	0.00	0.00	0.00	0.20
ASFNFIV S75 (2)R	5	16	22	0	0	0	7	28	65	68	0	0	0	27	0.19	0.25	0.33	0.00	0.00	0.00	0.13
ASFNFIV S75E	3	21	30	0	0	0	9	36	77	81	0	0	0	32	0.09	0.28	0.38	0.00	0.00	0.00	0.12
ASFNFIV WL5 (2)R	13	28	33	0	0	0	12	39	65	74	0	0	0	30	0.34	0.44	0.46	0.00	0.00	0.00	0.21
Mean	8	19	27	14	5	3		34	63	75	36	12	6		0.23	0.31	0.38	0.18	0.06	0.03	
	NaCl (S)		Isolate (I)			S x I		NaCl (S)		Isolate (I)			S x I		NaCl (S)		Isolate (I)			S x I	
S.Em.±	1		1			2		2		2			6		0.01		0.02			0.06	
C.D. (0.01)	2		3			7		6		9			22		0.06		0.08			0.21	

Among the NaCl concentrations, the highest Na/K ratio was recorded at 7.5% NaCl (0.38) which was significantly superior over all other NaCl concentrations. The interaction effect was also found to be significant wherein the isolate ASFNFIV S37R at 10% NaCl recorded the highest intracellular Na/K ratio (0.50) which was significantly superior over most other treatment combinations (Table 21b).

4.3.6.3 Phosphate solubilizing bacteria (PSB)

It was also observed that, the intracellular Na and K contents of all the PSB isolates were found to increase with increase in NaCl concentration (Table 21c). Among the isolates, ASPSBXII S189E recorded the highest mean intracellular Na content (25.8 µg/ml) which was significantly superior over rest of the isolates except ASPSBIX S124E (24.0 µg), ASPSBIX S122R (23.4 µg) and ASPSBXI S181R (23.1 µg) with which it was on par. Similarly, the highest intracellular K content was recorded by ASPSBXII S189E which was significantly superior over most of the isolates but on par with five isolates viz., ASPSBXI S181R (61.0 µg), ASPSBIX S125R (60.1 µg), ASPSBIX S124E (59.3 µg), ASPSBIX S122R (59.2 µg) and ASPSBXI S156R (54.8 µg). Among the NaCl concentrations, treatment receiving 12.5% NaCl to the growth medium recorded the highest intracellular Na and K (28.2 and 74.8 µg/ml respectively) which were significantly superior over all other salt concentrations. No Na and K contents were observed in 17 out of 23 isolates at 15% NaCl.

The interaction effect was also found to be significant. Among the treatment combinations, isolates ASPSBIX S124E and ASPSBXI S156R at 15% NaCl recorded the highest intracellular Na (46.8 µg/ml) and K (113.4 µg/ml) respectively which were significantly superior over most other combinations.

All the isolates were found to exhibit changes in intracellular Na/K ratio up to 12.5% NaCl whereas only 6 isolates showed changes in Na/K ratio at 15% NaCl. The intracellular Na/K ratio of salt-tolerant PSB isolates under NaCl stress were in the range of 0.30 to 0.50, 0.30 to 0.48, 0.31 to 0.48, 0.31 to 0.49 and 0.00 to 0.43 in the presence of 5, 7.5, 10, 12.5 and 15 per cent NaCl respectively as compared to normal medium (0.20 to 0.35).

Among the isolates, ASPSBXI S181R recorded the highest Na/K ratio (0.41) which was significantly superior over most of the isolates but was on par with 12 salt-tolerant PSB isolates whose Na/K ratio was in the range of 0.31 to 0.41. Among the salt concentrations, the highest mean Na/K ratio was recorded at 5% NaCl (0.40) which was significantly superior over control (0.30) and 15% NaCl (0.10) but was on par with 12.5, 10 and 7.5% NaCl (0.38, 0.38 and 0.36 respectively). The interaction effect was found to be significant where in the isolates ASPSBXII Dr335 and ASPSBXII S190S both at 5% NaCl recorded the highest Na to K ratio (0.5 in both) but were on par with most other treatment combinations (Table 21c).

4.3.6.4 Fluorescent pseudomonads (FP)

A total of 12 FP isolates were analyzed for intracellular Na and K and the results obtained are presented in Table 21d. It was observed that, the intracellular Na and K content of all the isolates were found to increase with increase in salt concentrations. Among the FP isolates, the highest mean intracellular Na content (25.3 µg/ml) was recorded by ASFPI S6(1)S which was significantly superior over most of the isolates but on par with isolates ASFPV S100E (24.2 µg), ASFPI S3(1)S (23.5 µg), ASFPV S94(2)E (23.5 µg) and ASFPV Dr16E (22.6 µg). The isolate ASFPI S4(1)S recorded the highest mean intracellular K content (74.3 µg) and was significantly superior over all other isolates except ASFPI S3(1)S (74.0 µg), ASFPI S6(1)S (73.5 µg), ASFPV S94(2)E (73.4 µg), ASFPV S100E (70.5 µg) and ASFPV Dr16E (70.1 µg) with which it was on par.

Among the salt concentrations, growth medium supplemented with 15% NaCl recorded the highest mean intracellular Na content (22.6 µg/ml) which was significantly superior over most of the NaCl concentrations but was on par with that of mean intracellular Na content recorded at 12.5% (21.7 µg) and 17.5% NaCl (21.0 µg/ml). Similarly, the highest mean intracellular K content was recorded at 15% NaCl which was significantly superior over most of the salt concentrations but on par with the mean intracellular Na content recorded at 12.5% NaCl (74.5 µg/ml).

Table 21c: Changes in the intracellular sodium and potassium content and Na to K ratio of salt tolerant phosphate solubilizers at different salt concentrations

Isolate	Intracellular Na content (µg/ml)						Mean	Intracellular K content (µg/ml)						Mean	Na to K ratio						Mean
	NaCl concentrations (%)							NaCl concentrations (%)							NaCl concentrations (%)						
	0.05	5	7.5	10	12.5	15.0		0.05	5	7.5	10	12.5	15.0		0.05	5	7.5	10	12.5	15.0	
ASPSBIX S122R	6.8	14.7	18.1	22.6	33.7	44.7	23.4	27.3	41.3	48.9	54.9	76.9	106.1	59.2	0.25	0.36	0.38	0.42	0.45	0.42	0.38
ASPSBIX S124E	5.7	12.6	16.5	25.3	37.1	46.8	24.0	23.5	38.2	49.6	56.8	78.4	109.1	59.3	0.25	0.34	0.34	0.45	0.48	0.43	0.38
ASPSBIX S125R	8.7	16.5	17.9	18.9	28.9	37.3	21.4	27.6	42.7	46.3	55.1	75.9	112.9	60.1	0.32	0.39	0.39	0.35	0.39	0.33	0.36
ASPSBXI S156R	6.5	14.3	18.3	22.5	29.3	39.5	21.7	22.9	30.1	38.9	49.7	73.8	113.4	54.8	0.29	0.49	0.48	0.46	0.40	0.35	0.41
ASPSBXI S181R	5.2	11.9	14.9	26.3	36.1	44.2	23.1	26.4	40.1	51.3	58.1	77.9	112.3	61.0	0.20	0.30	0.30	0.46	0.47	0.39	0.35
ASPSBXII S189E	7.8	15.9	18.6	28.1	38.9	45.6	25.8	27.3	42.7	52.7	59.3	81.3	115.1	63.1	0.29	0.38	0.36	0.48	0.49	0.40	0.40
ASPSBIX S129R	8.8	15.0	17.6	20.9	28.7	0.0	15.2	25.6	36.1	47.1	55.3	75.3	0.0	39.9	0.35	0.42	0.38	0.39	0.39	0.00	0.32
ASPSBX S135R	7.5	12.6	17.1	18.7	23.3	0.0	13.2	24.1	30.3	46.5	54.1	70.1	0.0	37.5	0.32	0.43	0.37	0.35	0.34	0.00	0.30
ASPSBX S139R	6.9	10.9	14.7	16.9	22.1	0.0	11.9	23.2	29.1	42.9	52.1	70.2	0.0	36.3	0.30	0.39	0.35	0.33	0.32	0.00	0.28
ASPSBX S150R	5.3	9.3	12.9	15.1	20.9	0.0	10.6	21.5	28.3	41.2	50.5	68.5	0.0	35.0	0.25	0.34	0.32	0.31	0.31	0.00	0.26
ASPSBX Dr29aR	6.9	11.2	15.0	17.3	23.1	0.0	12.3	23.6	30.1	42.6	53.2	70.9	0.0	36.7	0.30	0.38	0.36	0.33	0.33	0.00	0.28
ASPSBX S142 (2)S	5.9	10.1	15.2	17.9	22.7	0.0	12.0	22.1	28.5	45.1	54.0	70.1	0.0	36.6	0.27	0.37	0.34	0.34	0.33	0.00	0.28
ASPSBX S140S	8.6	14.1	18.6	22.6	30.7	0.0	15.8	25.1	31.5	48.5	58.1	77.6	0.0	40.1	0.35	0.46	0.39	0.40	0.40	0.00	0.33
ASPSBX S144 (1)S	6.9	11.6	15.4	18.2	27.1	0.0	13.2	23.1	29.1	45.3	53.2	75.2	0.0	37.7	0.31	0.41	0.35	0.35	0.37	0.00	0.30
ASPSBX S145 (1)S	7.5	12.9	16.9	19.6	28.1	0.0	14.2	24.2	30.1	45.9	54.8	75.8	0.0	38.5	0.32	0.44	0.38	0.37	0.38	0.00	0.31
ASPSBX Dr29aS	5.8	8.6	12.4	15.7	23.2	0.0	11.0	22.6	28.1	40.6	51.1	71.3	0.0	35.6	0.26	0.32	0.31	0.32	0.33	0.00	0.26
ASPSBX WL15 (3)S	6.9	11.8	15.6	18.3	25.2	0.0	13.0	23.4	30.3	44.4	52.4	73.3	0.0	37.3	0.30	0.40	0.36	0.36	0.35	0.00	0.29
ASPSBX Dr27S	5.7	9.3	13.2	16.6	23.7	0.0	11.4	22.1	29.1	42.9	52.2	71.6	0.0	36.3	0.26	0.33	0.31	0.33	0.34	0.00	0.26
ASPSBX S141E	5.5	9.6	13.4	17.3	25.2	0.0	11.8	22.1	28.1	44.2	52.7	73.6	0.0	36.8	0.26	0.35	0.31	0.34	0.35	0.00	0.27
ASPSBXI S169 (1)R	6.8	11.3	15.8	19.3	27.1	0.0	13.4	23.1	29.4	46.2	54.5	75.6	0.0	38.1	0.30	0.40	0.35	0.36	0.37	0.00	0.30
ASPSBXI S172 (2)S	7.8	13.3	17.6	21.6	29.9	0.0	15.0	24.1	30.6	48.1	57.3	77.5	0.0	39.6	0.33	0.45	0.37	0.38	0.39	0.00	0.32
ASPSBXII Dr33S	8.7	15.2	18.9	22.9	31.3	0.0	16.2	25.2	31.3	48.8	58.0	78.9	0.0	40.4	0.35	0.50	0.39	0.40	0.40	0.00	0.34
ASPSBXII S190S	8.9	15.8	20.2	23.5	32.9	0.0	16.9	25.9	32.2	50.6	58.9	80.6	0.0	41.4	0.35	0.50	0.41	0.41	0.41	0.00	0.35
Mean	7.0	12.5	16.3	20.3	28.2	11.2		24.2	32.5	46.0	54.6	74.8	29.1		0.30	0.40	0.36	0.38	0.38	0.10	
	NaCl (S)		Isolate (I)			S x I		NaCl (S)		Isolate (I)			S x I		NaCl (S)		Isolate (I)			S x I	
S.Em.±	0.4		0.8			1.9		1.0		2.0			5.0		0.01		0.03			0.07	
C.D. (0.01)	1.4		2.8			6.9		3.8		7.5			18.4		0.05		0.10			0.25	

Table 21d: Changes in the intracellular sodium and potassium content and Na to K ratio of salt tolerant fluorescent pseudomonads at different salt concentrations

Isolate	Intracellular Na content (µg/ml)					Mean	Intracellular K content (µg/ml)					Mean	Na to K ratio					Mean
	NaCl concentrations (%)						NaCl concentrations (%)						NaCl concentrations (%)					
	0.05	10	12.5	15	17.5		0.05	10	12.5	15	17.5		0.05	10	12.5	15	17.5	
ASFPI S3 (1)S	6.8	16.4	20.1	28.4	45.9	23.5	27.9	54.6	74.8	98.4	114.2	74.0	0.25	0.31	0.27	0.29	0.41	0.31
ASFPI S4 (1)S	8.1	15.1	18.7	22.7	41.3	21.2	28.1	55.7	78.9	97.3	111.6	74.3	0.29	0.28	0.24	0.24	0.38	0.29
ASFPI S6 (1)S	5.6	18.1	24.1	32.1	46.7	25.3	26.7	56.9	75.8	99.6	108.7	73.5	0.22	0.32	0.32	0.33	0.44	0.33
ASFPV Dr16E	8.6	14.9	20.8	27.8	40.7	22.6	26.1	61.1	72.8	88.1	102.3	70.1	0.34	0.25	0.29	0.32	0.41	0.32
ASFPV S94 (2)E	7.8	13.3	23.8	30.7	41.9	23.5	28.1	51.7	76.4	101.8	109.1	73.4	0.28	0.26	0.32	0.31	0.39	0.31
ASFPV S100E	8.4	15.4	23.1	29.7	44.2	24.2	26.9	59.3	68.9	89.7	107.8	70.5	0.32	0.26	0.34	0.34	0.42	0.34
ASFPI S9 (1)S	8.2	16.5	22.7	26.7	0.0	14.8	25.8	57.6	77.1	98.3	0.0	51.8	0.33	0.29	0.30	0.28	0.00	0.24
ASFPI S14 (3)S	7.5	14.6	20.9	22.9	0.0	13.2	26.3	58.1	76.8	97.1	0.0	51.7	0.29	0.26	0.28	0.24	0.00	0.21
ASFPV Dr10E	5.9	12.9	20.6	22.9	0.0	12.5	23.4	54.3	72.4	96.8	0.0	49.4	0.26	0.24	0.29	0.24	0.00	0.21
ASFPV S97R	5.8	15.2	19.3	27.5	0.0	13.6	22.9	51.6	73.8	99.3	0.0	49.5	0.26	0.30	0.27	0.28	0.00	0.22
ASFPV S112 (a)R	5.1	13.6	19.3	0.0	0.0	7.6	26.1	52.8	76.1	0.0	0.0	31.0	0.20	0.26	0.26	0.00	0.00	0.14
ASFPV S158 (2)R	6.4	11.8	18.9	0.0	0.0	7.4	24.8	53.1	70.5	0.0	0.0	29.7	0.27	0.23	0.28	0.00	0.00	0.15
Mean	7.0	14.8	21.0	22.6	21.7		26.1	55.6	74.5	80.5	54.5		0.28	0.27	0.29	0.24	0.20	
	NaCl (S)		Isolate (I)		S x I		NaCl (S)		Isolate (I)		S x I		NaCl (S)		Isolate (I)		S x I	
S.Em.±	0.7		1.0		2.3		2.0		3.1		6.9		0.02		0.03		0.06	
C.D. (0.01)	2.5		3.9		8.8		7.4		11.5		25.8		0.06		0.10		0.22	

The interaction effect was also found to be significant. Among the treatment combinations, isolates ASFPI S6(1)S and ASFPI S3(1)S at 17.5% NaCl recorded the highest intracellular Na (46.7 $\mu\text{g/ml}$) and K (114.2 $\mu\text{g/ml}$) respectively which were significantly superior over most other combinations. No intracellular Na and K contents were recorded in two strains at 15% NaCl and in six strains at 17.5% NaCl.

Significant changes in the intracellular Na to K ratio were observed among the isolates and NaCl concentrations. The changes in intracellular Na and K were detected in all the 12 FP isolates upto 12.5% NaCl while, in only 10 and 6 isolates at 15 and 17.5% NaCl respectively (Table 21d).

Among the isolates, the highest mean intracellular Na/K ratio (0.34) was recorded by ASFPIV S100E which was significantly superior over few isolates but on par with 7 FP isolates whose Na/K ratio ranged from 0.24 to 0.34. Among the NaCl concentrations, the highest mean Na/K ratio was recorded at 12.5% NaCl (0.29) which was significantly superior over 17.5% NaCl (0.20) but on par with rest of the NaCl concentrations as well as control medium (Table 21d). The interaction effect was also found to be significant. Among the treatment combinations, the isolate ASFPI S6(1)S at 17.5% NaCl recorded the highest Na/K ratio (0.44) which was significantly superior over most other treatment combinations (Table 21d).

4.4 Plant growth promotional activity of potential salt-tolerant isolates

A total of nineteen salt tolerant isolates representing four groups of agriculturally important microorganisms viz., associative N_2 fixers (4), free-living N_2 fixers (5), P-solubilizing bacteria (4) and fungi (1) and fluorescent pseudomonads (5) were screened in environmental chamber for the plant growth promotional activity of wheat (cv. DWR 162) under different soil ECe (Electrical conductivity of saturation paste extract) levels (0.37, 2.32, 4.08, 5.38, 7.21 and 7.55 dS/m) against un-inoculated control.

4.4.1 Associative nitrogen fixers (ANF)

The data on seedling emergence, plant height and biomass content as influenced by inoculation of salt-tolerant ANF isolates are presented in Tables 22a to 22c.

Among all the isolates, wheat seeds inoculated with ASANFIV S61E recorded the highest mean seedling emergence (72.2%) followed by ASANFII S22E (71.1%) and ASANFIV S40E (70.0%) and least in UIC (56.7%). Among the isolates, inoculation of three ANF isolates (ASANFIV S61E and S78E and ASANFII S22E) recorded the highest seedling emergence (100%) up to soil ECe of 5.38 dS m^{-1} whereas the isolate ASANFIV S40E and un-inoculated control (UIC) recorded only 33.3 and 40 per cent respectively at 5.38 dS m^{-1} (Table 22a). While all inoculated strains recorded 13.3 to 20.0 per cent seedling emergence at 7.21 dS m^{-1} and 6.7 to 13.3% at 7.55 dS m^{-1} , no seedling emergence was noticed in UIC treatment at both ECe levels. Among the soil ECe levels, seedling emergence was not affected either in inoculated or UIC treatment with increase in soil ECe levels up to 4.08 dS/m (100%) but declined to 74.7, 13.3 and 8.0% at soil ECe of 5.38, 7.21 and 7.55 dS/m respectively (Table 22a).

All four isolates showed significant improvement in plant height (20.2 to 21.1 cm) over UIC (14.2 cm) but were on par each other (Table 22b). Among the soil ECe levels, irrespective of isolates, maximum plant height was recorded at 2.32 dS m^{-1} (28.7 cm) followed by 0.37 dS m^{-1} (26.4 cm) and 4.08 dS m^{-1} (25.8 cm) all of which were significantly superior over other higher ECe levels. The interaction effect was also found to be significant wherein isolate ASANFIV S78E at 2.32 dS/m recorded maximum plant height (31 cm) as compared to other treatment combinations (Table 22b).

The treatments also had a significant influence on the dry matter content of wheat plants (Table 22c). Among the treatments, irrespective of soil ECe levels, inoculation of salt-tolerant ANF isolates showed significant improvement in dry matter content of wheat (46.9 to 48.9 mg/plant) over UIC (32.6 mg/plant). Among the isolates, ASANFII S40E recorded the highest mean dry matter content and was on par with remaining isolates.

Table 22a. Seedling emergence (%) of wheat as influenced by inoculation of salt-tolerant associative N₂ fixing isolates under different soil ECe levels

Isolate	Soil ECe (dS/m)						Mean
	0.37	2.32	4.08	5.38	7.21	7.55	
ASANFIV S61E	100	100	100	100	20.0	13.3	72.2
ASANFIV S40E	100	100	100	100	13.3	6.7	70.0
ASANFII S22E	100	100	100	100	20.0	6.7	71.1
ASANFIV S78E	100	100	100	33.3	13.3	13.3	60.0
UIC	100	100	100	40.0	0.0	0.0	56.7
Mean	100	100	100	74.7	13.3	8.0	

Table 22b. Plant height (cm) of wheat as influenced by inoculation of salt-tolerant associative N₂ fixing isolates under different soil ECe levels

Isolate	Soil ECe (dS/m)						Mean
	0.37	2.32	4.08	5.38	7.21	7.55	
ASANFIV S61E	29.0	29.7	26.3	22.0	11.3	4.0	20.4
ASANFIV S40E	27.0	30.3	27.0	24.3	17.3	0.7	21.1
ASANFII S22E	28.7	27.0	27.3	24.3	13.0	0.7	20.2
ASANFIV S78E	25.7	31.0	26.0	22.7	16.3	4.3	21.0
UIC	21.7	25.3	22.3	15.7	0.0	0.0	14.2
Mean	26.4	28.7	25.8	21.8	11.6	1.9	
	ECe level (E)		Isolate (I)		E x I		
S _{Em} ±	0.5		0.5		1.2		
CD (0.01)	1.9		1.8		4.3		

Table 22c. Total dry matter (mg/plant) of wheat as influenced by inoculation of salt-tolerant associative N₂ fixing isolates under different soil ECe levels

Isolate	Soil ECe (dS/m)						Mean
	0.37	2.32	4.08	5.38	7.21	7.55	
ASANFIV S61E	56.9	57.7	61.6	44.5	39.2	21.7	46.9
ASANFIV S40E	54.1	58.7	63.3	52.1	47.3	17.7	48.9
ASANFII S22E	54.7	55.4	64.7	52.7	42.9	19.3	48.3
ASANFIV S78E	51.3	65.1	59.3	47.1	45.1	22.7	48.4
UIC	48.6	50.4	56.1	40.3	0.0	0.0	32.6
Mean	53.1	57.5	61.0	47.3	34.9	16.3	
	ECe level (E)		Isolate (I)		E x I		
S _{Em} ±	1.2		1.1		2.7		
CD (0.01)	4.6		4.2		10.3		

Among the soil ECe levels, maximum mean dry matter content (61.0 mg/plant) was recorded at soil ECe of 4.08 dS/m followed by 2.32 dS/m (57.5 mg/plant) both of which were on par with each other but significantly superior over rest of the soil ECe levels. The interaction effect was also found to be significant wherein isolate ASANFIV S78E at a soil ECe of 2.32 dS/m recorded the highest dry matter content (65.1 mg/plant) and was significantly superior over several other treatment combinations (Table 22c).

4.4.2 Free-living nitrogen fixers (FNF)

Inoculation of FNF isolates was found to have significant influence on seedling emergence, plant height and dry matter content of wheat (Tables 23a to 23c).

Wheat seeds inoculated with salt-tolerant FNF isolates recorded maximum mean seedling emergence (73.3 to 75.6%) as compared to UIC (56.7%). While, inoculation of salt-tolerant FNF isolates recorded 100% seedling emergence, UIC recorded only 40% seedling emergence at 5.38 dS/m (Table 23a). Seedling emergence at 7.21 dS m⁻¹ ranged from 13.3 to 53.3 per cent in inoculated treatments whereas no seeds germinated in UIC. Similarly, at 7.55 dS/m no seedlings were emerged in UIC and with isolate ASFNFIV S75(1)R. Among the soil ECe levels, the mean seedling emergence was unaffected upto 4.08 dS/m (100%) whereas it was decreased to 90.0, 26.7 and 11.1% at higher soil ECe levels of 5.38, 7.21 and 7.55 dS m⁻¹ respectively (Table 23a).

Irrespective of soil ECe levels, inoculation of two salt-tolerant FNF isolates viz., ASFNFIV S63(1)R and ASFNFIV S75(1)R showed significant improvement in plant height (20.0 and 18.5 cm respectively) over UIC (14.2 cm) but were on par with other (Table 23b). Among the soil ECe levels, maximum mean plant height (28.7 cm) was recorded at a soil ECe of 2.32 dS/m which was however on par with ECe levels of 0.37 and 4.08 dS/m but all three were significantly superior over all other higher ECe levels. The interaction effect was also significant. Among the treatment combinations, isolate ASFNFIV S75(1)R at a soil ECe of 2.32 dS/m recorded maximum plant height (30.7 cm) which was on par with all treatment combinations upto 4.08 ECe level but was significantly superior over most other treatment combinations (Table 23b).

The isolates also enhanced the dry matter content of wheat plants significantly over UIC (Table 23c). Among the isolates, irrespective of soil ECe levels, wheat plants inoculated with ASFNFIV S63(1)R recorded the highest mean dry matter content (54.0 mg/plant) which was significantly superior over remaining isolates except ASFNFIV WL4(1)R (42.6 mg/plant) with which it was on par. Similarly, dry matter content of wheat plants was found to increase with increase in soil ECe up to 4.08 dS/m and declined thereafter. Among the soil ECe levels tested, wheat plants grown at soil ECe of 4.08 dS/m recorded the highest mean dry matter content (64.9 mg/plant) which was significantly superior over all other ECe levels. The interaction effect was also found to be significant. Among the treatment combinations, ASFNFIV S63(1)R at a soil ECe of 4.08 dS/m recorded the highest dry matter content (72.1 mg/plant) and was significantly superior over most other treatment combinations (Table 23c).

4.4.3 P-solubilizers (PSB/ PSF)

Among the treatments, irrespective of soil ECe levels, maximum mean seedling emergence was recorded in wheat seeds inoculated with ASPSF WL9S (95.6%) followed by ASPSBIX S125R (83.3%) and least in UIC (56.7%). Wheat seeds inoculated with salt-tolerant P-solubilizing bacteria recorded 100 per cent seedling emergence upto 5.38 dS m⁻¹ but per cent seedling emergence ranged from 40 to 66.7% at 7.21 dS m⁻¹ and 6.7 to 33.3% at 7.55 dS m⁻¹. The lone fungal isolate ASPSF WL9S showed 100 per cent seedling emergence upto 7.21 dS m⁻¹ and 73.3% seedling emergence at ECe of 7.55 dS m⁻¹. Per cent seedling emergence noticed in UIC was only 40% at 5.38 dS m⁻¹ but at 7.21 and 7.55 dS m⁻¹ ECe levels no seeds germinated (Table 24a).

Similarly, inoculation of salt-tolerant P-solubilizers showed significant improvement in plant height of wheat over UIC (Table 24b). Among the treatments, irrespective of soil ECe levels, the fungal isolate ASPSF WL9S recorded the highest mean plant height (26.1 cm) which was significantly superior over all the bacterial isolates and UIC (14.2 cm). All four bacterial isolates also showed significant improvement in plant height (18.7 to 21.0 cm) over UIC (14.2cm) but were on par with each other.

Table 23a. Seedling emergence (%) of wheat as influenced by inoculation of salt-tolerant free-living N₂ fixing isolates under different soil ECe levels

Isolate	Soil ECe (dS/m)						Mean
	0.37	2.32	4.08	5.38	7.21	7.55	
ASFNFIV S63(1)R	100	100	100	100.0	26.7	20.0	74.5
ASFNFIV S75(1)R	100	100	100	100.0	53.3	0.0	75.6
ASFNFIV WL4(1)R	100	100	100	100.0	33.3	6.7	73.3
ASFNFIV S37R	100	100	100	100.0	13.3	20.0	72.2
ASFNFXII S178E	100	100	100	100.0	33.3	20.0	75.6
UIC	100	100	100	40.0	0.0	0.0	56.7
Mean	100	100	100	90.0	26.7	11.1	

Table 23b. Plant height (cm) of wheat as influenced by inoculation of salt-tolerant free-living N₂ fixing isolates under different soil ECe levels

Isolate	Soil ECe (dS/m)						Mean
	0.37	2.32	4.08	5.38	7.21	7.55	
ASFNFIV S63(1)R	27.3	30.3	27.3	19.7	9.7	5.7	20.0
ASFNFIV S75(1)R	26.7	30.7	27.0	21.0	5.7	0.0	18.5
ASFNFIV WL4(1)R	28.0	28.7	25.7	17.3	9.0	1.3	18.3
ASFNFIV S37R	28.3	29.0	22.3	15.3	1.7	0.0	16.1
ASFNFXII S178E	24.3	28.3	22.7	23.0	2.7	2.0	17.2
UIC	21.7	25.3	22.3	15.7	0.0	0.0	14.2
Mean	26.1	28.7	24.6	18.7	4.8	1.5	
	ECe level (E)		Isolate (I)		E x I		
SEm±	1.1		1.1		2.7		
CD (0.01)	4.2		4.2		10.2		

Table 23c. Total dry matter (mg/plant) of wheat as influenced by inoculation of salt-tolerant free-living N₂ fixing isolates under different soil ECe levels

Isolate	Soil ECe (dS/m)						Mean
	0.37	2.32	4.08	5.38	7.21	7.55	
ASFNFIV S63(1)R	53.3	64.3	72.1	48.6	42.8	42.8	54.0
ASFNFIV S75(1)R	52.3	65.6	69.1	56.4	32.9	0.0	46.1
ASFNFIV WL4(1)R	54.1	59.0	66.7	53.9	40.7	22.6	49.5
ASFNFIV S37R	55.7	61.9	64.9	47.6	13.7	0.0	40.6
ASFNFXII S178E	50.8	56.6	60.7	59.7	20.6	25.1	45.6
UIC	48.6	50.4	56.1	40.3	0.0	0.0	32.6
Mean	52.5	59.6	64.9	51.1	25.1	15.1	
	ECe level (E)		Isolate (I)		E x I		
SEm±	1.2		1.2		2.9		
CD (0.01)	4.5		4.5		10.9		

Table 24a. Seedling emergence (%) of wheat as influenced by inoculation of salt-tolerant phosphate solubilizing isolates under different soil ECe levels

Isolate	Soil ECe (dS/m)						Mean
	0.37	2.32	4.08	5.38	7.21	7.55	
ASPSBIX S122R	100	100	100	100.0	53.3	6.7	76.7
ASPSBIX S125R	100	100	100	100.0	66.7	33.3	83.3
ASPSBIX S124E	100	100	100	100.0	40.0	26.7	77.8
ASPSBXI WL19E	100	100	100	100.0	60.0	6.7	77.8
ASPSF WL9S	100	100	100	100.0	100.0	73.3	95.6
UIC	100	100	100	40.0	0.0	0.0	56.7
Mean	100	100	100	90.0	53.3	24.4	

Table 24b. Plant height (cm) of wheat as influenced by inoculation of salt-tolerant phosphate solubilizing isolates under different soil ECe levels

Isolate	Soil ECe (dS/m)						Mean
	0.37	2.32	4.08	5.38	7.21	7.55	
ASPSBIX S122R	27.3	29.3	24.7	24.0	9.3	3.0	19.6
ASPSBIX S125R	25.0	28.0	25.7	22.0	21.3	4.0	21.0
ASPSBIX S124E	24.7	27.3	23.3	21.7	14.0	4.0	19.2
ASPSBXI WL19E	24.7	24.7	26.0	24.7	11.3	1.0	18.7
ASPSF WL9S	29.3	32.7	28.0	26.7	22.7	17.3	26.1
UIC	21.7	25.3	22.3	15.7	0.0	0.0	14.2
Mean	25.4	27.9	25.0	22.4	13.1	4.9	
	ECe levels		Isolates		Interaction		
SEm±	0.6		0.6		1.5		
CD (0.01)	2.3		2.3		5.7		

Table 24c. Total dry matter (mg/plant) of wheat as influenced by inoculation of salt-tolerant phosphate solubilizing isolates under different soil ECe levels

Isolate	Soil ECe (dS/m)						Mean
	0.37	2.32	4.08	5.38	7.21	7.55	
ASPSBIX S122R	56.9	66.2	61.8	47.2	41.0	19.2	48.7
ASPSBIX S125R	54.2	60.2	63.9	45.0	56.8	21.4	50.3
ASPSBIX S124E	52.4	54.1	60.9	43.3	45.2	20.6	46.1
ASPSBXI WL19E	51.6	52.7	65.7	50.9	42.8	12.1	46.0
ASPSF WL9S	60.6	72.6	69.9	57.9	60.7	51.3	62.1
UIC	48.6	50.4	56.1	40.3	0.0	0.0	32.6
Mean	54.1	59.4	63.0	47.4	41.1	20.8	
	ECe level (E)		Isolate (I)		E x I		
SEm±	1.2		1.2		3.0		
CD (0.01)	4.5		4.5		11.1		

Among the soil ECe levels, maximum mean plant height was recorded at 2.32 dS/m (27.9 cm) which was significantly superior over rest of the soil ECe levels. The interaction effect was also found to be significant wherein isolate ASPSF WL9S at soil ECe of 2.32 dS/m recorded maximum plant height (32.7 cm) as compared to other treatment combinations (Table 24b).

Inoculation of salt-tolerant P-solubilizers resulted in enhancement of dry matter content of wheat (Table 24c). Among the isolates, inoculation of PSF WL9S recorded the highest mean dry matter content (62.1 mg/plant) which was significantly superior over remaining isolates and UIC (32.6 mg/plant). All the four bacterial isolates were found to have on par effect on dry matter content of wheat but were significantly superior over UIC. Of the four bacterial isolates, ASPSBIX S125R recorded higher mean dry matter content (50.3 mg/plant) as compared to other isolates (Table 24c).

Among the soil ECe levels, the highest mean dry matter content (63.0 mg/plant) was recorded at a soil ECe of 4.08 dS/m (63.0 mg/plant) followed by soil ECe of 2.32 dS/m (59.4 mg/plant) both of which were on par with each other but significantly superior over rest of the soil ECe levels tested (Table 24c). In general, dry matter content of wheat was found to increase with increase in soil ECe up to 4.08 dS/m (63.0 mg/plant) and decreased significantly thereafter. The interaction effect was also found to be significant where in isolate PSF WL9S with soil ECe of 2.32 recorded the highest dry matter content (72.6 mg/plant) as compared to rest of the treatment combinations (Table 24c).

4.4.4 Fluorescent pseudomonads

The data on seedling emergence, plant height and biomass content as influenced by inoculation of salt-tolerant FP isolates are presented in Tables 25a to 25c.

All five FP isolates showed marked increase in mean seedling emergence (66.7 to 84.5%) over UIC (56.7%). Among the isolates, inoculation of four isolates viz., ASFPV Dr12bR, ASFPV S4(1)S, ASFPV Dr10R and ASFPV S6(1)S recorded 100% seedling emergence up to soil ECe of 5.38 dS/m whereas the isolate ASFPV S100E and un-inoculated control (UIC) recorded only 53.3 and 40 per cent respectively at 5.38 dS/m (Table 25a). While all inoculated strains recorded 33.3–86.7 per cent seedling emergence at 7.21 dS/m and 13.3 to 33.3% at 7.55 dS/m, no seedling emergence was noticed in UIC treatment at both soil ECe levels. Among the soil ECe levels, the mean seedling emergence was not affected either in inoculated or UIC treatment with increase in soil ECe levels up to 4.08 dS/m (100%) but declined thereafter drastically (Table 25a).

All the isolates except ASFPV Dr12bR, showed significant improvement in mean plant height of wheat (18.5 to 20.7 cm) over UIC (14.2 cm) (Table 22b). Among the isolates, ASFPV Dr10R recorded the highest mean plant height (20.7 cm) which was however on par with isolates ASFPV S4(1)S (20.4 cm) and ASFPV S6(1)S (19.5 cm) but was significantly superior over the isolates ASFPV Dr12bR and ASFPV S100E. Among the soil ECe levels, irrespective of isolates, maximum mean plant height (25.3 cm) was recorded at 4.08 dS m⁻¹ followed by soil ECe of 2.32 dS/m (25.0 cm) and 0.37 dS/m (24.5 cm) all of which were on par with each other but significantly superior over other soil ECe levels. The interaction effect was also found to be significant. Wheat plants inoculated with isolate ASFPV Dr10R at soil ECe of 4.08 dS/m recorded the maximum plant height (28.7 cm) as compared to other treatment combinations (Table 25b).

The treatments also had a significant influence on the dry matter content of wheat (Table 25c). All five FP isolates showed significant improvement in mean dry matter content of wheat (40.9 to 53.1 mg/plant) over UIC (32.6 mg/plant). Among the isolates, ASFPV Dr10R recorded the highest mean dry matter content (53.1 mg/plant) which was on par with the isolates ASFPV S6(1)S (50.8 mg/plant) and ASFPV S4(1)S (50.3 mg/plant) but was significantly superior over the remaining two isolates. Among the soil ECe levels, the highest mean dry matter content (62.6 mg/plant) was recorded at soil ECe of 4.08 dS/m which was significantly superior over rest of the soil ECe levels. The mean dry matter content of wheat plants was found to increase with increase in soil ECe levels upto 4.08 dS/m but decreased thereafter significantly.

Table 25a. Seedling emergence (%) of wheat as influenced by inoculation of salt-tolerant fluorescent pseudomonads under different soil ECe levels

Isolate	Soil ECe (dS/m)						Mean
	0.37	2.32	4.08	5.38	7.21	7.55	
ASFPV Dr12bR	100	100	100	100.0	86.7	20.0	84.5
ASFPI S4(1)S	100	100	100	100.0	40.0	20.0	76.7
ASFPV Dr10R	100	100	100	100.0	40.0	33.3	78.9
ASFPI S6(1)S	100	100	100	100.0	66.7	20.0	81.1
ASFPV S100E	100	100	100	53.3	33.3	13.3	66.7
UIC	100	100	100	40.0	0.0	0.0	56.7
Mean	100	100	100	82.2	44.5	17.8	

Table 25b. Plant height (cm) of wheat as influenced by inoculation of salt-tolerant fluorescent pseudomonads under different soil ECe levels

Isolate	Soil ECe (dS/m)						Mean
	0.37	2.32	4.08	5.38	7.21	7.55	
ASFPV Dr12bR	22.7	25.7	23.2	16.3	2.3	1.3	15.3
ASFPI S4(1)S	27.3	26.0	26.7	24.3	14.0	4.0	20.4
ASFPV Dr10R	24.7	22.7	28.7	21.0	18.3	8.7	20.7
ASFPI S6(1)S	24.0	26.7	25.0	21.7	14.3	5.3	19.5
ASFPV S100E	26.7	23.3	25.7	18.0	15.0	2.3	18.5
UIC	21.7	25.3	22.3	15.7	0.0	0.0	14.2
Mean	24.5	25.0	25.3	19.5	10.7	3.6	
	ECe level (E)		Isolate (I)		E x I		
SEm±	0.5		0.5		1.2		
CD (0.01)	1.9		1.9		4.6		

Table 25c. Total dry matter (mg/plant) of wheat as influenced by inoculation of salt-tolerant fluorescent pseudomonads under different soil ECe levels

Isolate	Soil ECe (dS/m)						Mean
	0.37	2.32	4.08	5.38	7.21	7.55	
ASFPV Dr12bR	50.8	53.9	58.0	46.8	23.4	12.6	40.9
ASFPI S4(1)S	54.3	55.1	66.9	60.6	44.1	21.0	50.3
ASFPV Dr10R	52.5	52.6	69.7	50.6	52.3	40.8	53.1
ASFPI S6(1)S	51.6	56.1	61.6	56.6	46.1	32.6	50.8
ASFPV S100E	53.5	53.3	63.3	51.2	48.8	18.7	48.1
UIC	48.6	50.4	56.1	40.3	0.0	0.0	32.6
Mean	51.9	53.6	62.6	51.0	35.8	20.9	
	ECe level (E)		Isolate (I)		E x I		
SEm±	1.2		1.2		2.9		
CD (0.01)	4.5		4.5		10.9		

The interaction effect was also found to be significant. Among the treatment combinations, the highest dry matter content was recorded with isolate ASFPV Dr10R at 4.08 dS/m which was significantly superior over most other treatment combinations (Table 25c).

4.5 Field performance of potentially efficient salt-tolerant isolates in wheat under saline conditions

A field experiment was conducted to study the performance of efficient salt-tolerant isolates to promote the growth and yield of wheat crop at different soil salinity levels during Rabi 2010-11. The results obtained are presented below.

4.5.1 Growth parameters of wheat

4.5.1.1 Plant height

Observations on plant height of wheat as affected by inoculation of salt-tolerant agriculturally important microorganisms (AIMs) under graded soil ECe levels were recorded at 30, 60 and 90 days after sowing (DAS) and at harvest (Table 26 and Plate 3).

In general, the inoculation treatments significantly enhanced the plant height over uninoculated control at all stages of crop growth. Among the treatments, irrespective of salinity, the highest mean plant heights of 30.1, 79.9, 83.0 and 84.6 cm were recorded in wheat plants inoculated with microbial consortia [*Azospirillum irakense* S173E, *Enterobacter aerogenes* S63(1)R, *Enterobacter cloacae* S125R and *Pseudomonas* sp. S4(1)S] at 30, 60, 90 DAS and at harvest respectively and the consortia treatment was significantly superior over rest of the inoculation treatments at all stages of crop growth. Two other treatments viz., *Aspergillus niger* WL9S and *Enterobacter aerogenes* S63(1)R also recorded significant improvement in plant height (28.5 and 27.2 cm at 30 DAS, 77.4 and 75.2 cm at 60 DAS, 80.6 and 77.7 cm at 90 DAS and 82.4 and 79.2 cm at harvest respectively) over other inoculation treatments at all stages of crop growth (Table 26).

It was observed that, the mean plant height was found to increase with increase in soil ECe up to 4.9 dS m⁻¹ at 30 and 60 DAS and declined thereafter while it was decreased with every increase in soil ECe level at 90 DAS and at harvest (Table 26). Among all the ECe levels, maximum mean plant height of 32.4 and 80.8 cm was recorded with soil ECe of 4.9 dS m⁻¹ at 30 and 60 DAS respectively whereas the mean plant heights of 83.0 and 84.8 cm were recorded at 2.4 dS/m at 90 DAS and at harvest respectively as compared to higher soil ECe levels. The interaction effect was found to be non-significant (Table 26).

4.5.1.2 Chlorophyll

Leaf chlorophyll content of wheat plants was estimated by measuring leaf greenness using a portable SPAD (Soil Plant Analysis Development) chlorophyll meter and the SPAD values recorded at 30 and 60 DAS are presented in Table 27. The results of the study indicated that, inoculation of wheat plants with salt-tolerant AIMs resulted in significant increase in SPAD values over that of UIC plants. Among the inoculation treatments, consortia recorded significantly higher SPAD values (29.0 and 34.8 at 30 and 60 DAS respectively) over those of other inoculation treatments, except *Aspergillus niger* WL9S at 60 DAS, with which it was on par (Table 27).

SPAD readings taken at 30 and 60 DAS were found to increase with increase in soil ECe levels up to 4.9 dS/m and declined thereafter (Table 27). Among all the soil ECe levels, irrespective of inoculation, maximum mean SPAD values of 30.9 and 36.3 were recorded at soil ECe of 4.9 dS/m at 30 and 60 DAS respectively which was significantly superior over rest of the soil ECe levels. The interaction effect was found to be non-significant (Table 27).

4.5.1.3 Root length

Data on root length recorded at 30 and 60 DAS are presented in Table 28. Inoculation treatments showed significant improvement in root length. The mean root length was increased from 8.2 cm in UIC to 15.6 cm in consortia and 12.7 cm in UIC to 24.1 cm in consortia at 30 and 60 DAS respectively (Table 28). Among the treatments, consortia was significantly superior over remaining treatments.

Table 26. Plant height (cm) of wheat as influenced by salt tolerant AIMs at different soil salinity levels under field condition

Treatment	30 DAS						60 DAS					
	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	31.3	33.7	30.2	21.7	19.1	27.2	80.4	81.3	78.9	69.4	65.9	75.2
<i>Azospirillum irakense</i> S173E	29.1	31.1	26.3	18.8	16.1	24.3	78.2	79.4	74.8	65.5	60.9	71.8
<i>Enterobacter cloacae</i> S125R	30.7	33.1	28.1	20.3	17.7	26.0	78.7	79.9	76.9	66.9	62.7	73.0
<i>Pseudomonas</i> sp. S4(1)S	28.7	30.3	24.7	17.8	14.3	23.2	76.2	77.7	73.5	63.7	58.1	69.8
<i>Aspergillus niger</i> WL9S	32.1	34.3	31.1	23.1	20.5	28.2	81.5	82.9	79.3	70.9	67.9	76.5
Consortia*	33.2	36.3	33.1	25.1	22.6	30.1	84.4	86.9	83.7	73.7	70.7	79.9
UIC	26.3	27.9	22.3	16.1	12.4	21.0	74.9	76.8	69.7	59.7	54.8	67.2
Mean	30.2	32.4	28.0	20.4	17.5		79.2	80.7	76.7	67.1	63.0	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	0.4		0.6		1.5		0.8		1.1		3.2	
CD (0.05)	1.1		1.7		NS		2.3		3.1		NS	

Treatment	90 DAS						At harvest					
	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	84.8	83.5	80.2	71.8	68.4	77.7	85.5	85.1	82.1	73.3	70.0	79.2
<i>Azospirillum irakense</i> S173E	81.4	80.2	75.8	68.0	63.2	73.7	83.1	82.3	77.5	69.6	65.6	75.6
<i>Enterobacter cloacae</i> S125R	82.5	81.3	78.7	69.4	65.5	75.5	83.9	83.9	80.4	70.5	67.4	77.2
<i>Pseudomonas</i> sp. S4(1)S	80.2	79.0	74.4	66.2	60.9	72.1	82.3	81.4	76.0	68.0	62.5	74.0
<i>Aspergillus niger</i> WL9S	84.5	81.8	80.8	71.2	68.8	77.4	88.5	86.1	84.5	75.1	72.9	81.4
Consortia*	90.2	86.9	86.8	77.5	73.8	83.0	91.4	88.5	88.3	78.7	76.1	84.6
UIC	77.7	77.2	72.4	61.7	56.9	69.2	78.8	78.9	74.1	62.6	58.3	70.5
Mean	83.0	81.4	78.4	69.4	65.4		84.8	83.7	80.4	71.1	67.5	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	1.1		1.2		2.9		0.7		0.9		1.9	
CD (0.05)	2.9		3.4		NS		2.1		2.4		NS	

* *Enterobacter aerogenes* S63(1)R + *Azospirillum irakense* S173E + *Enterobacter cloacae* S125R + *Pseudomonas* sp. S4(1)S

SEm±: Standard error of means

CD: Critical difference

ECe: Electrical conductivity of the saturation paste extract



***Azospirillum irakense* S173E**



***Enterobacter aerogenes* S63 (1) R**



***Aspergillus niger* WL9S**



***Enterobacter cloacae* S125R**



Un inoculated control



Consortia *

Plate 3. Plant growth promotional activity of salt tolerant isolates at salinity level of 14.3 dS/m under field condition in wheat (45 DAS)

Aspergillus niger WL9S (13.7 cm) and *Enterobacter aerogenes* S63(1)R (12.9 cm) were on par with each other but were significantly superior over other strains and UIC (8.2 cm) at 30 DAS. Similar trend was observed at 60 DAS as well (Table 28).

It was observed that, mean root length of wheat was found to increase with increase in soil ECe upto 4.9 dS/m and decreased there after. Among the soil ECe levels, the highest mean root lengths of 14.9 and 23.2 cm were recorded with soil ECe of 4.9 dS m⁻¹ at 30 and 60 DAS respectively and were significantly superior over rest of the soil ECe levels at respective growth stages. The interaction was found to be non-significant (Table 28).

4.5.1.4 Dry matter production

Dry matter production in wheat crop was recorded at 30, 60, 90 DAS and at harvest and the results are presented in Table 29. In general, inoculation of salt-tolerant AIMS resulted in significant improvement in dry matter production over UIC at all the stages of crop growth and at all ECe levels. Inoculation of wheat plants with salt-tolerant AIMS was found to increase the mean dry matter content which ranged from 67.0 to 81.2 g, 119.7 to 147.1 g, 177.8 to 202.9 g and 190.0 to 217.5 g per m row length as against the dry matter content of 63.5, 108.8, 169.2 and 179.0 g per m row length recorded by UIC at 30, 60, 90 DAS and at harvest respectively. Increase in soil ECe level over and above 4.9 dS m⁻¹ resulted in reduction of mean dry matter content of wheat from 92.2 g to 47.0, 207.2 to 141.9, 235.8 to 160.1 and 254.1 to 170.2 g per m row length at 30, 60, 90 DAS and at harvest respectively (Table 29 and Fig. 9).

Among the inoculation treatments, wheat crop receiving consortia recorded the highest dry matter production (81.2, 147.1, 202.9 and 217.5 g per m row length at 30, 60, 90 DAS and at harvest, respectively) which was significantly superior over UIC and all other strains (Table 29). Two strains viz., *Aspergillus niger* WL9S and *Enterobacter aerogenes* S63(1)R also recorded significantly higher dry matter content (77.9 and 75.1 g, 134.5 and 130.4 g, 192.7 and 188.9 g and 204.8 and 201.1 g at 30, 60, 90 DAS and at harvest respectively) over *Enterobacter cloacae* S125R but were on par with each other at all stages of crop growth (Table 29).

Among the soil salinity levels, soil ECe of 4.9 dS/m recorded the highest mean dry matter content (92.2, 159.7, 214.2 and 232.0 g per m row length at 30, 60, 90 DAS and at harvest respectively) and was significantly superior over rest of the salinity levels (Table 29). It was also observed that, dry matter content was found to decrease significantly with increase in soil ECe from 4.9 to 14.3 dS/m. The interaction effect was found to be non-significant.

4.5.1.5 Number of tillers

All the salt-tolerant AIMS except *Pseudomonas* sp. S4(1)S showed significant improvement in tillering of wheat crop over uninoculated control (Table 30a). Among the inoculation treatments, the highest mean tiller counts of 440.0 and 531.6 per square meter (at 30 and 60 DAS respectively) were recorded in treatment receiving consortia which was significantly superior over all other inoculation treatments except *Aspergillus niger* WL9S (430.2) at 30 DAS and *A. niger* WL9S (517.9) and *Enterobacter aerogenes* S63(1)R (500.9) at 60 DAS with which it was on par.

Soil salinity levels above 4.9 dS/m significantly reduced the tillering of wheat crop. Among the soil salinity levels, mean tiller counts at 4.9 dS m⁻¹ were found to be highest and was significantly superior over all higher ECe levels but was on par with 2.4 dS/m at both stages crop growth (Table 30a).

4.5.2 Yield and yield attributing characters

4.5.2.1 Number of productive tillers

In general, inoculation of salt-tolerant AIMS was found to increase the productive tillers over uninoculated control. Similarly increase in soil ECe levels above 4.9 dS/m had a negative impact on number of productive tillers in wheat crop at both 90 DAS and at harvest (Table 30b and Fig. 10).

Table 27. Chlorophyll content (SPAD value) of wheat as influenced by salt tolerant AIMS at different soil salinity levels under field condition

Treatment	At 30 DAS						At 60 DAS					
	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	29.8	32.1	24.8	21.4	15.8	24.8	33.4	37.1	32.6	28.5	22.5	30.8
<i>Azospirillum irakense</i> S173E	26.9	29.3	21.6	18.9	13.7	22.1	31.1	34.2	29.7	25.4	20.2	28.1
<i>Enterobacter cloacae</i> S125R	27.5	30.7	23.1	19.8	14.5	23.1	32.6	36.3	31.1	26.7	20.8	29.5
<i>Pseudomonas</i> sp. S4(1)S	25.5	28.1	20.9	17.3	12.9	20.9	30.4	33.5	28.6	24.4	18.6	27.1
<i>Aspergillus niger</i> WL9S	32.5	33.6	26.7	22.7	16.6	26.4	36.1	40.1	34.5	29.2	23.7	32.7
Consortia*	34.7	35.8	29.8	25.3	19.2	29.0	39.7	41.3	36.2	31.3	25.6	34.8
UIC	24.3	26.7	19.2	15.8	11.8	19.6	28.3	31.4	26.1	22.7	16.1	24.9
Mean	28.7	30.9	23.7	20.2	14.9		33.1	36.3	31.3	26.9	21.1	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	0.6		0.7		1.5		0.9		1.0		2.3	
CD (0.05)	1.6		1.9		NS		2.5		2.9		NS	

Table 28. Root length (cm) of wheat as influenced by salt tolerant AIMS at different soil salinity levels under field condition

Treatment	30 DAS						60 DAS					
	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	15.1	16.2	14.9	10.0	8.5	12.9	22.6	25.2	22.4	16.6	13.9	20.1
<i>Azospirillum irakense</i> S173E	12.3	13.6	12.1	8.4	7.1	10.7	19.3	21.4	18.2	13.5	11.1	16.7
<i>Enterobacter cloacae</i> S125R	14.0	15.6	14.2	9.2	7.8	12.2	21.1	23.8	20.8	14.9	12.1	18.5
<i>Pseudomonas</i> sp. S4(1)S	11.9	12.8	10.2	7.5	6.3	9.7	18.7	20.0	15.7	12.0	10.0	15.3
<i>Aspergillus niger</i> WL9S	15.6	17.1	15.7	11.3	8.9	13.7	23.2	26.4	23.5	18.3	14.8	21.2
Consortia*	16.4	18.8	17.4	14.7	10.9	15.6	24.9	28.9	26.3	22.5	18.0	24.1
UIC	9.5	10.4	8.9	7.3	4.8	8.2	15.3	17.0	13.2	10.5	7.7	12.7
Mean	13.5	14.9	13.3	9.8	7.8		20.7	23.2	20.0	15.5	12.5	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	0.4		0.5		1.1		0.4		0.4		1.0	
CD (0.05)	1.1		1.4		NS		1.1		1.2		NS	

* *Enterobacter aerogenes* S63(1)R + *Azospirillum irakense* S173E + *Enterobacter cloacae* S125R + *Pseudomonas* sp. S4(1)S

SEm±: Standard error of means

CD: Critical difference

ECe: Electrical conductivity of the saturation paste extract

Table 29. Influence of salt tolerant AIMs at different soil salinity levels on dry matter production (g/m row length) of wheat under field condition

Treatment	30 DAS						60 DAS					
	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	84.6	93.6	82.0	65.8	49.7	75.1	142.2	161.6	142.2	115.4	90.7	130.4
<i>Azospirillum irakense</i> S173E	78.2	90.7	74.2	60.6	43.2	69.4	134.1	157.0	132.7	108.5	82.5	123.0
<i>Enterobacter cloacae</i> S125R	81.0	92.0	78.0	63.1	46.6	72.1	137.3	158.7	136.9	111.4	86.3	126.1
<i>Pseudomonas</i> sp. S4(1)S	75.6	89.0	70.1	58.7	41.8	67.0	130.6	154.4	127.7	105.7	80.2	119.7
<i>Aspergillus niger</i> WL9S	87.0	95.8	86.8	67.6	52.4	77.9	145.9	165.1	148.3	118.5	94.7	134.5
Consortia*	90.6	98.2	91.2	70.1	55.8	81.2	158.8	176.8	162.0	130.3	107.4	147.1
UIC	70.6	86.0	65.2	56.1	39.6	63.5	118.2	144.2	115.4	95.7	70.6	108.8
Mean	81.1	92.2	78.2	63.1	47.0		138.2	159.7	137.9	112.2	87.5	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	0.9		1.1		2.6		2.5		2.7		5.6	
CD (0.05)	2.8		3.1		NS		7.1		7.8		NS	

Treatment	90 DAS						At harvest					
	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	198.8	216.6	200.5	175.9	152.6	188.9	214.6	234.0	214.7	185.2	157.2	201.1
<i>Azospirillum irakense</i> S173E	189.4	210.7	189.7	167.7	143.1	180.1	206.5	229.4	205.2	178.3	149.0	193.7
<i>Enterobacter cloacae</i> S125R	193.2	213.0	194.5	171.2	147.5	183.9	210.6	232.0	210.3	182.1	153.7	197.7
<i>Pseudomonas</i> sp. S4(1)S	186.8	209.0	185.6	165.8	141.7	177.8	202.6	226.4	199.8	175.1	146.3	190.0
<i>Aspergillus niger</i> WL9S	202.2	219.8	206.3	178.7	156.3	192.7	217.9	237.1	220.4	187.9	160.8	204.8
Consortia*	212.8	229.2	217.7	188.2	166.7	202.9	230.9	248.9	234.2	199.8	173.6	217.5
UIC	176.8	201.0	175.7	158.2	134.5	169.2	190.3	216.1	187.6	165.2	135.8	179.0
Mean	194.3	214.2	195.7	172.2	148.9		210.5	232.0	210.3	181.9	153.8	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	2.2		2.6		7.1		1.8		2.4		5.7	
CD (0.05)	3.5		8.3		NS		5.2		6.6		NS	

* *Enterobacter aerogenes* S63(1)R + *Azospirillum irakense* S173E + *Enterobacter cloacae* S125R + *Pseudomonas* sp. S4(1)S

SEm±: Standard error of means

CD: Critical difference

ECe: Electrical conductivity of the saturation paste extract

Table 30a. Influence of salt tolerant AIMs on number of tillers (per sq.m) in wheat at different soil salinity levels under field condition

Treatment	30 DAS						60 DAS					
	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	458.2	475.1	448.1	372.4	300.4	410.8	591.1	566.6	516.4	467.1	363.1	500.9
<i>Azospirillum irakense</i> S173E	423.4	445.5	394.5	332.5	263.5	371.9	557.6	545.8	473.2	425.4	312.5	462.9
<i>Enterobacter cloacae</i> S125R	440.6	460.5	419.6	359.6	279.4	391.9	576.4	555.4	491.1	446.1	323.6	478.5
<i>Pseudomonas</i> sp. S4(1)S	407.8	422.6	369.7	311.0	227.4	347.7	437.3	516.6	460.7	411.2	293.9	423.9
<i>Aspergillus niger</i> WL9S	477.9	489.4	489.1	391.8	302.7	430.2	607.6	583.1	535.6	491.6	371.4	517.9
Consortia*	478.4	503.8	515.5	396.1	306.4	440.0	620.9	595.6	553.4	509.5	378.8	531.6
UIC	384.5	391.8	361.6	276.6	205.6	324.0	418.5	469.1	425.9	386.8	267.7	393.6
Mean	438.7	455.5	428.3	348.6	269.3		544.2	547.5	493.8	448.2	330.1	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	7.6		8.8		19.5		13.6		15.7		36.3	
CD (0.05)	20.4		24.6		NS		38.4		45.2		NS	

Table 30b. Influence of salt tolerant AIMs on number of productive tillers (per sq.m) in wheat at different soil salinity levels under field condition

Treatment	90 DAS						At harvest					
	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	446.4	444.9	389.5	315.6	223.6	364.0	448.3	446.9	391.4	318.3	225.8	366.1
<i>Azospirillum irakense</i> S173E	420.6	427.6	356.9	286.6	191.4	336.6	422.3	429.4	359.2	289.3	193.8	338.8
<i>Enterobacter cloacae</i> S125R	435.4	435.4	370.1	301.4	198.7	348.2	435.4	438.4	372.2	303.1	201.4	350.1
<i>Pseudomonas</i> sp. S4(1)S	329.9	404.7	347.5	277.8	179.4	307.9	331.8	406.8	349.6	281.5	181.1	310.2
<i>Aspergillus niger</i> WL9S	458.9	457.4	404.9	331.9	228.4	376.3	460.4	459.6	406.3	334.3	230.5	378.2
Consortia*	468.4	467.2	417.9	343.4	232.2	385.8	469.9	469.4	420.1	345.9	234.2	387.9
UIC	315.4	367.1	320.4	260.4	163.1	285.3	317.4	368.4	323.3	263.4	165.1	287.5
Mean	410.7	429.2	372.5	302.4	202.4		412.2	431.3	374.6	305.1	204.6	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	8.9		10.8		44		10.1		11.9		24.3	
CD (0.05)	25.7		28.9		NS		29.2		33.2		NS	

* *Enterobacter aerogenes* S63(1)R + *Azospirillum irakense* S173E + *Enterobacter cloacae* S125R + *Pseudomonas* sp. S4(1)S

SEm±: Standard error of means

CD: Critical difference

ECe: Electrical conductivity of the saturation paste extract

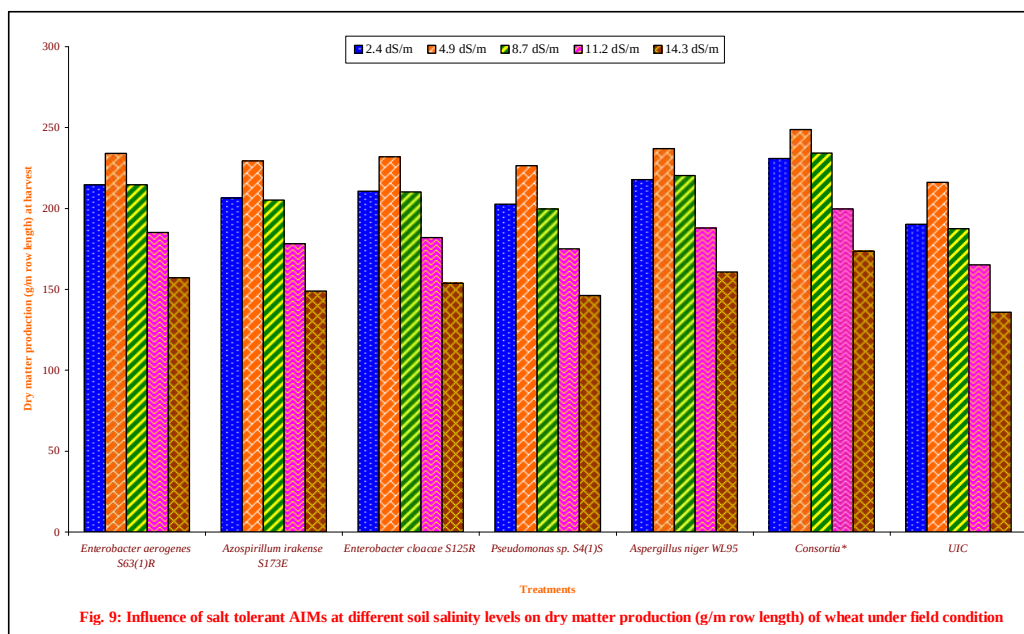


Fig. 9: Influence of salt tolerant AIMs at different soil salinity levels on dry matter production (g/m row length) of wheat under field condition

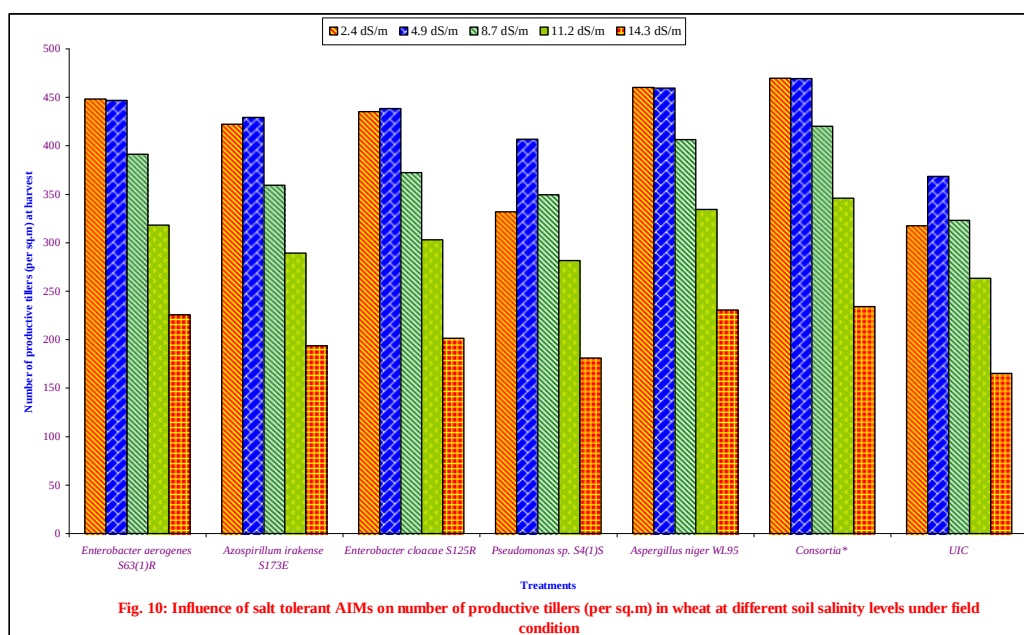


Fig. 10: Influence of salt tolerant AIMs on number of productive tillers (per sq.m) in wheat at different soil salinity levels under field condition

All inoculation treatments except *Pseudomonas* sp. S4(1)S showed significant improvement in mean number of productive tillers in wheat crop over UIC at 90 DAS and at harvest. Among the inoculation treatments, irrespective of soil ECe levels, treatment receiving consortia recorded the highest mean number of productive tillers per sq.m (385.8) followed by *Aspergillus niger* WL9S (376) and *Enterobacter aerogenes* S63(1)R (363) at 90 DAS which were on par with each other but significantly superior over other strains. Similar trend was observed at harvest (Table 30b).

Among the soil salinity levels, soil ECe of 4.9 dS m⁻¹ recorded maximum mean productive tillers (429.2 per sq.m) at 90 DAS which was however on par with ECe of 2.4 dS/m (410.7) but both were significantly superior over other soil ECe levels (Table 30b). An exactly similar trend was observed at harvest. The interaction effect was found to be non-significant (Table 30b).

4.5.2.2 Spike length

Among the treatments, maximum mean spike length was recorded in consortia (10.3 cm) which was significantly superior over UIC (8.3 cm) and all other inoculation treatments except *Aspergillus niger* WL9S (9.9 cm) with which it was on par (Table 31a). *Enterobacter aerogenes* S63(1)R (9.6 cm) and *Enterobacter cloacae* S125R (9.2 cm) showed significant increase in spike length over UIC but were statistically on par with each other (Table 31a).

Among the soil salinity levels, soil ECe levels of 4.9 and 2.4 dS/m recorded significantly higher mean spike length (10.0 and 9.8 cm respectively) over other ECe levels (8.4 to 9.3 cm) but were on par with each other. The interaction effect was found to be non-significant (Table 31a).

4.5.2.3 Number of grains per spike

All the inoculation treatments, except *Pseudomonas* sp. S4(1)S, recorded significantly higher mean number of grains per spike. Among the treatments, consortia recorded the highest mean number of grains per spike (59.6) which was significantly superior over other inoculation treatments (Table 31a). It was followed by *Aspergillus niger* WL9S (50.4) which also showed significantly higher number of grains per spike over other inoculation treatments but was on par with *Enterobacter aerogenes* S63(1)R (47.7).

Among the soil salinity levels, lower soil ECe levels (4.9 and 2.4 dS/m) recorded significantly higher mean number of grains per spike (52.7 and 50.7 respectively), over all other ECe levels but were on par with each other (Table 31a). The interaction effect was found to be non-significant (Table 31a).

4.5.2.4 Test weight

All inoculation treatments recorded significantly higher test weight over UIC. Among the treatments, the highest mean test weight (46.9 g) was recorded with consortia treatment which was significantly superior over other treatments. *Aspergillus niger* WL9S (45 g) and *Enterobacter aerogenes* S63(1)R (44.2 g) were on par with each other but were significantly superior over other isolates (Table 31a).

It was observed that, the mean test weights of wheat recorded at soil ECe levels 2.4 or 4.9 dS m⁻¹ were found to be on par but both were significantly superior over all other ECe levels (Table 31a). The mean test weight of wheat was decreased from 48 g to 42.3, 39.8 and 36.5 g with increase in soil ECe from 4.9 dS m⁻¹ to 8.7, 11.2 and 14.3 dS m⁻¹ respectively. Among the soil ECe levels, maximum mean test weight (48.0 g) was recorded at 4.9 dS/m followed by 2.4 dS/m (47.2 g). There was no significant interaction between salt-tolerant AIMS and soil ECe levels (Table 31a).

4.5.2.5 Grain yield

All salt-tolerant AIMS inoculated to wheat crop significantly increased the grain yield over UIC (Table 31b and Fig. 11). Among the treatments, wheat crop inoculated with consortia recorded the highest mean grain yield (39.5 q/ha) which was however on par with *Aspergillus niger* WL9S (37.80 q/ha) and *Enterobacter aerogenes* S63(1)R (37.2 q/ha) but was significantly superior over remaining salt-tolerant AIMS and that of UIC (25.9 q/ha). The grain yield of wheat as influenced by inoculation of remaining salt-tolerant AIMS was in the range of 35.0 to 36.5 q per ha and were on par with each other (Table 31b and Plate 4).

The grain yield of wheat was found to decrease with increase in soil ECe levels. It was reduced from 50.5 q per ha to 49.8, 33.6, 22.5 and 20.4 q per ha with increase in soil ECe from 2.4 dS m⁻¹ to 4.9, 8.7, 11.2 and 14.3 dS m⁻¹ respectively (Table 31b). Among the soil ECe levels, the highest mean grain yield was recorded with soil ECe level of 2.4 dS/m (50.5 q/ha) closely followed by 4.9 dS/m (49.8 q/ha) which were on par with each other but were significantly superior over all other higher soil ECe levels. The interaction effect was found to be non-significant (Table 31b and Fig. 11).

4.5.2.6 Straw yield

Inoculation of salt-tolerant AIMS also resulted in significant increase in straw yield of wheat (Table 31b and Fig. 11). All inoculation treatments enhanced the straw yield significantly over UIC.

Among the inoculation treatments, maximum mean straw yield (55.9 q/ha) was recorded by consortia followed by *Aspergillus niger* WL9S (54.2 q/ha) and *Enterobacter aerogenes* S63(1)R (53.7 q/ha) with which it was on par but was significantly superior over other strains. *Enterobacter cloacae* S125R (52.0 q/ha), *Azospirillum irakense* S173E (50.9 q/ha) and *Pseudomonas* sp. S4(1)S (50.5 q/ha) were on par with each other but were significantly superior over UIC (39.4 q/ha).

The straw yield of wheat was found to reduce significantly with increase in soil ECe from 4.9 to 14.3 dS m⁻¹. Among the soil ECe levels, 2.4 dS/m recorded the highest mean straw yield (76.9 q/ha) closely followed by ECe of 4.9 dS/m (76.09 q/ha) which were on par with each other but were significantly superior over all higher soil ECe levels. The interaction was found to be non-significant (Table 31b and Fig. 11).

4.5.2.7 Harvest index (HI)

All inoculation treatments, except *Pseudomonas* sp. S4(1)S, showed significant increase in HI of wheat over UIC (0.404). Among the treatments, maximum mean HI (0.421) was recorded with consortia and was significantly superior over all other isolates. *Aspergillus niger* WL9S (0.417) also recorded significantly higher HI over all other isolates except *Enterobacter aerogenes* S63(1)R (0.415) with which it was on par (Table 31b).

It was observed that, harvest index (HI) of wheat was found to increase significantly with increase in ECe level. Among the soil ECe levels, maximum HI (0.433) was recorded at highest soil ECe (14.3 dS/m) which was significantly superior over rest of the soil ECe levels. The interaction effect was found to be non-significant (Table 31b).

4.5.3 Nutrient uptake in wheat

In general, inoculation of salt-tolerant AIMS resulted in significant improvement in uptake of all three nutrients at all the soil ECe levels tested. While, increase in soil ECe levels over and above 4.9 dS/m was found to have negative impact on uptake of nutrients by wheat crop (Table 32). Inoculation of wheat plants with salt-tolerant AIMS was found to increase the mean total uptake of N, P and K in the range of 101.4 to 122.8 Kg, 14.6 to 24.3 Kg and 59.3 to 72.4 kg/ha as against the uptake of 75.1, 10.3 and 43.7 kg/ha in UIC respectively. The total uptake of N, P and K was found to increase with increase in soil ECe up to 4.9 dS/m and decreased thereafter. It was observed that, the mean N, P and K uptake decreased from 156.2 to 55.5 Kg, 29.4 to 7.4 Kg, 94.8 to 30.6 kg/ha with increase in soil ECe from 4.9 to 14.3 dS/m respectively (Table 32).

Among the treatments, wheat crop inoculated with consortia recorded the highest mean uptake of N (122.8 kg/ha) which was significantly superior over all other inoculation treatments except *Aspergillus niger* WL9S (114.0 Kg) and *Enterobacter aerogenes* S63(1)R (114.3 Kg) with which it was on par. Similarly consortia also recorded significantly higher P uptake (24.3 Kg) over all other strains. Potassium uptake was maximum in consortia treatment (72.4 Kg) and was on par with *Aspergillus niger* WL9S (67.5 Kg) and *Enterobacter aerogenes* S63(1)R (67.2 Kg) but was significantly superior over all other inoculation treatments.

Among the soil salinity levels, soil ECe of 4.9 dS/m recorded the highest uptake of N (156.2 kg/ha), P (29.4 kg/ha) and K (94.8 kg/ha) which were significantly superior over rest of the salinity levels (Table 32).

Table 31a. Influence of salt tolerant AIMs on yield attributes of wheat at different soil salinity levels under field condition

Treatment	Spike length (cm)						No of grains per spike						Test weight (g)					
	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	10.1	10.3	9.7	9.1	8.7	9.6	52.2	53.9	52.1	40.8	39.6	47.7	47.8	49.1	44.6	41.6	38.0	44.2
<i>Azospirillum irakense</i> S173E	9.1	9.5	8.9	8.6	8.2	8.9	46.0	45.8	45.0	33.0	32.3	40.4	46.6	48.0	40.4	38.3	36.2	41.9
<i>Enterobacter cloacae</i> S125R	9.6	9.7	9.2	8.9	8.5	9.2	48.1	51.2	48.2	37.2	36.2	44.2	47.2	48.3	42.1	40.1	37.1	43.0
<i>Pseudomonas</i> sp. S4(1)S	8.9	9.1	8.4	8.2	7.8	8.5	45.2	46.0	41.1	31.3	29.4	38.6	46.0	46.1	38.7	37.4	35.1	40.7
<i>Aspergillus niger</i> WL9S	10.7	11.0	9.9	9.1	8.9	9.9	54.3	58.1	55.3	44.0	40.5	50.4	48.2	50.3	45.9	42.1	38.4	45.0
Consortia*	11.0	11.3	10.3	9.5	9.3	10.3	65.3	73.3	61.5	52.1	45.7	59.6	49.3	52.1	47.8	45.3	40.2	46.9
UIC	8.9	8.9	8.5	7.7	7.3	8.3	43.8	40.7	35.2	28.1	25.2	34.6	45.1	42.3	36.7	33.9	30.2	37.6
Mean	9.8	10.0	9.3	8.7	8.4		50.7	52.7	48.3	38.1	35.6		47.2	48.0	42.3	39.8	36.5	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	0.2		0.2		0.5		1.2		1.4		3.2		0.5		0.6		1.2	
CD (0.05)	0.5		0.6		NS		3.4		4.0		NS		1.3		1.6		NS	

Table 31b. Influence of salt tolerant AIMs on grain and straw yield and harvest index of wheat at different soil salinity levels under field condition

Treatment	Grain yield (q/ha)						Straw yield (q/ha)						Harvest Index (HI)					
	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	51.8	52.1	36.0	24.0	22.0	37.2	78.8	79.6	49.3	32.2	28.6	53.7	0.397	0.395	0.422	0.427	0.436	0.415
<i>Azospirillum irakense</i> S173E	49.7	50.3	33.9	22.6	21.2	35.5	75.2	76.6	46.7	29.2	26.6	50.9	0.392	0.391	0.411	0.422	0.428	0.409
<i>Enterobacter cloacae</i> S125R	51.0	51.7	34.8	23.3	21.7	36.5	77.0	78.6	47.2	29.9	27.2	52.0	0.394	0.392	0.416	0.426	0.432	0.412
<i>Pseudomonas</i> sp. S4(1)S	49.2	49.9	33.2	22.2	20.4	35.0	75.1	76.6	46.1	28.9	25.6	50.5	0.390	0.389	0.410	0.420	0.428	0.407
<i>Aspergillus niger</i> WL9S	52.7	53.1	36.6	24.5	22.2	37.8	80.1	80.2	49.8	32.6	28.4	54.2	0.397	0.398	0.424	0.429	0.439	0.417
Consortia*	55.7	56.0	37.8	25.4	22.8	39.5	84.1	82.9	51.0	33.0	28.5	55.9	0.398	0.403	0.425	0.435	0.445	0.421
UIC	43.2	35.9	22.9	15.3	12.3	25.9	67.8	57.4	33.7	21.4	16.8	39.4	0.389	0.384	0.404	0.417	0.426	0.404
Mean	50.5	49.8	33.6	22.5	20.4		76.9	76.0	46.3	29.6	26.0		0.394	0.393	0.416	0.425	0.433	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	0.8		0.9		2.1		1.1		1.1		2.8		0.001		0.001		0.003	
CD (0.05)	2.2		2.6		NS		3.0		3.2		NS		0.003		0.003		0.09	

* *Enterobacter aerogenes* S63(1)R + *Azospirillum irakense* S173E + *Enterobacter cloacae* S125R + *Pseudomonas* sp. S4(1)S

SEm±: Standard error of means

CD: Critical difference

ECe: Electrical conductivity of the saturation paste extract

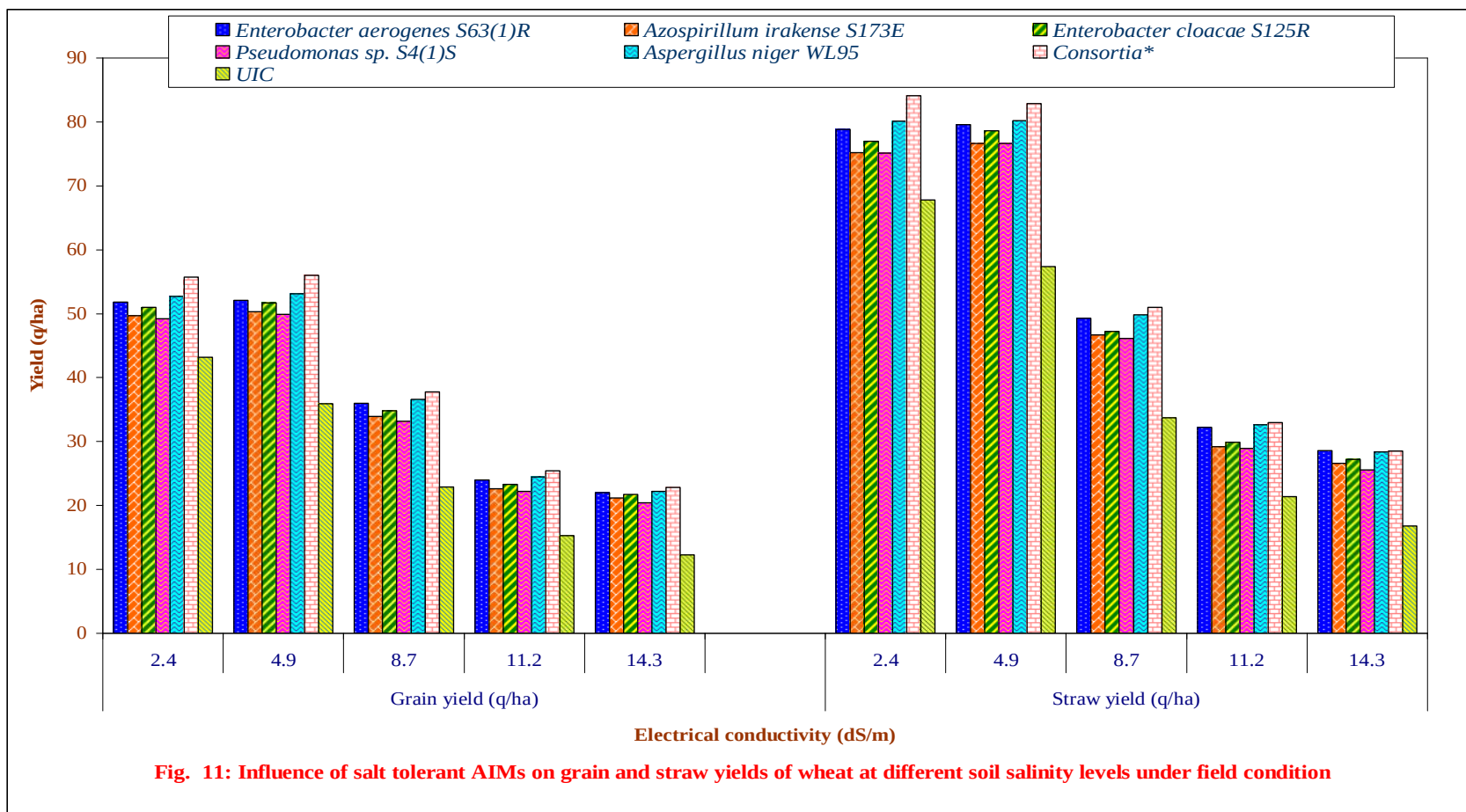


Fig. 11: Influence of salt tolerant AIMs on grain and straw yields of wheat at different soil salinity levels under field condition



***Enterobacter aerogenes* S63 (1) R**



***Azospirillum irakense* S173E**



***Enterobacter cloacae* S125R**



***Aspergillus niger* WL95**



Consortia *



Uninoculated control

Plate 4. Plant growth promotional activity of salt tolerant isolates at salinity level of 14.3 dS/m under field condition in wheat (at harvest)

It was also observed that, the total uptake was found to decrease gradually with increase in soil ECe from 4.9 to 14.3 dS/m and reaching the lowest total uptake of N (55.5 kg/ha), P (7.4 kg/ha) and K (30.6 kg/ha) at 14.3 dS/m. The interaction effect was found to be non-significant (Table 32).

4.5.4 Biochemical parameters

4.5.4.1 Activity of antioxidative enzymes (AOE)

Data on AOE activity in wheat plants as influenced by inoculation with salt-tolerant AIMs under salinity stress were recorded at 15, 21 and 30 days after sowing (DAS) and the results are presented in Tables 33a to 33c. The results of the study in general indicated that, catalase (CAT) and super-oxide dismutase (SOD) activities reached maximum at 21 DAS whereas peroxidase (POX) activity was maximum at 30 DAS. It was observed that, microbial inoculation resulted in significant reduction in AOE activity and vice versa with salinity.

4.5.4.1.1 Catalase activity (CAT)

Among the treatments, irrespective of soil ECe levels, significantly lower mean CAT activities (14.7, 20.4 and 17.4 U mg protein⁻¹ were recorded at 15, 21 and 30 DAS respectively) in consortia treated plants than all other treatments. The highest mean CAT activities of 38.5, 43.4 and 35.8 U mg protein⁻¹ were recorded in UIC plants at 15, 21 and 30 DAS respectively (Table 33a). Significant differences existed between the treatments at all stages of crop growth. Among the soil ECe levels, maximum mean CAT activities of 32.6, 38.3 and 30.6 U mg protein⁻¹ were recorded with a soil ECe of 14.3 dS m⁻¹ at 15, 21 and 30 DAS respectively, which were significantly superior over other soil ECe levels. While, the minimum mean CAT activity of 16.1, 21.2 and 19.2 U mg protein⁻¹ were recorded with a soil ECe of 2.4 dS m⁻¹ at 15, 21 and 30 DAS respectively. The interaction effect was found to be non-significant.

Though the inoculated plants showed lower CAT activity but the per cent increase in CAT activity with every increase in soil ECe over and above 2.4 dS/m were at maximum in inoculated than in UIC plants. CAT activity in consortia inoculated plants was increased by 32.6, 45.9, 78.5 and 100.7 per cent as compared to UIC plants wherein the CAT activity was increased by 27.8, 30.4, 31.3 and 57.6 per cent at soil ECe of 4.9, 8.7, 11.2 and 14.3 dS m⁻¹ respectively over and above 2.4 dS/m at 21 DAS. Similar trend was observed in plant samples drawn at 15 and 30 DAS (Fig. 12).

4.5.4.1.2 SOD activity

Among the treatments, the lowest mean SOD activity of 29.6 U/mg protein at 15 DAS, 41.7 U/mg protein at 21 DAS and 35.6 U/mg protein at 30 DAS were recorded in consortia treated plants and were significantly lower over remaining strains and UIC at the respective crop growth stages. The highest mean SOD activity of 70.9, 83.1 and 64.1 U mg protein⁻¹ were recorded in UIC plants at 15, 21 and 30 DAS respectively (Table 33b). Among all the soil ECe levels tested, maximum mean SOD activity of 72.6, 88.6 and 66.2 U mg protein⁻¹ were recorded at soil ECe of 14.3 dS/m and the minimum of 26.1, 32.6 and 30.9 U mg protein⁻¹ were recorded at soil ECe of 2.4 dS/m at 15, 21 and 30 DAS respectively.

The interaction effect was found to be significant at 15 DAS but non-significant at 21 and 30 DAS. Consortia with a soil ECe of 2.4 dS/m recorded the lowest SOD activity (16.0 U/mg protein) while UIC with soil ECe of 14.3 dS/m recorded the highest activity (106.6 U/mg protein) at 15 DAS (Table 33b).

Inoculated plants showed lower SOD activity as compared to uninoculated plants but the per cent increase in the activity with every increase in soil ECe over and above 2.4 dS/m were at maximum in inoculated plants (Fig. 13). The maximum per cent increase in SOD activity of 43.8, 79.5, 131.1 and 198.2 per cent was observed in consortia inoculated plants as against UIC plants which showed per cent increase in SOD activity by 40.9, 61.5, 75.9 and 130.0 per cent with increase in soil ECe from 2.4 dS m⁻¹ to 4.9, 8.7, 11.2 and 14.3 dS m⁻¹ respectively at 21 DAS. Similar trend was observed at 15 and 30 DAS (Fig. 13).

Table 32. Nutrient uptake (Kg/ha) in wheat plants as influenced by salt tolerant AIMs at different soil salinity levels under field condition

Treatment	Total uptake of nutrients (Kg/ha)																	
	Nitrogen						Phosphorous						Potassium					
	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	165.2	168.6	106.9	69.5	61.4	114.3	29.8	33.9	20.5	10.3	9.3	20.8	95.7	102.4	62.8	42.1	32.9	67.2
<i>Azospirillum irakense</i> S173E	151.3	153.6	96.9	63.6	57.4	104.5	26.3	26.6	15.6	8.2	7.1	16.8	89.0	92.3	57.8	37.4	31.7	61.6
<i>Enterobacter cloacae</i> S125R	157.4	160.2	100.0	64.9	58.6	108.2	26.9	29.9	16.7	8.4	7.7	17.9	92.0	97.3	59.7	38.2	32.2	63.9
<i>Pseudomonas</i> sp. S4(1)S	146.6	151.6	94.2	60.9	53.9	101.4	22.3	25.2	12.9	7.0	5.4	14.6	86.2	90.6	54.4	35.2	29.9	59.3
<i>Aspergillus niger</i> WL9S	164.5	168.0	107.2	69.9	60.3	114.0	30.3	33.1	20.8	9.9	8.8	20.6	97.3	102.9	62.1	41.1	34.1	67.5
Consortia*	179.9	181.2	113.5	74.2	65.2	122.8	36.1	38.7	23.2	12.5	11.1	24.3	104.3	111.9	65.6	44.0	36.3	72.4
UIC	127.8	110.0	64.9	41.1	32.0	75.1	18.5	18.3	8.4	3.8	2.5	10.3	74.7	65.8	37.2	23.4	17.2	43.7
Mean	156.1	156.2	97.6	63.4	55.5		27.2	29.4	16.9	8.6	7.4		91.3	94.8	57.1	37.3	30.6	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	2.5		2.9		6.5		0.5		0.6		1.3		1.5		1.8		4.1	
CD (0.05)	7.0		8.2		NS		1.3		1.6		NS		4.4		5.2		NS	

* *Enterobacter aerogenes* S63(1)R + *Azospirillum irakense* S173E + *Enterobacter cloacae* S125R + *Pseudomonas* sp. S4(1)S

SEm±: Standard error of means

CD: Critical difference

ECe: Electrical conductivity of the saturation paste extract

Table 33a. Catalase activity (U/mg protein) in wheat plants as influenced by salt tolerant AIMs at different soil salinity levels under field condition

Treatment	15 DAS						21 DAS						30 DAS					
	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	12.4	15.0	19.5	22.1	27.9	19.4	17.9	22.8	25.4	28.8	33.9	25.8	16.2	19.2	22.1	24.8	27.8	22.0
<i>Azospirillum irakense</i> S173E	17.1	20.9	23.4	26.7	35.0	24.6	22.8	28.9	28.9	33.8	41.1	31.1	20.3	23.4	25.0	28.3	32.8	25.9
<i>Enterobacter cloacae</i> S125R	14.3	18.0	21.1	24.4	30.6	21.7	19.6	26.3	26.7	31.3	36.6	28.1	18.0	21.6	23.3	26.6	29.4	23.8
<i>Pseudomonas</i> sp. S4(1)S	19.1	24.6	28.3	31.7	40.1	28.7	25.1	32.4	33.5	37.8	46.1	35.0	21.9	25.7	28.5	31.5	35.9	28.7
<i>Aspergillus niger</i> WL9S	11.1	13.5	16.2	19.6	24.7	17.0	15.8	21.2	22.0	26.3	30.6	23.2	14.7	17.6	19.1	22.6	25.1	19.8
Consortia*	9.4	11.2	13.9	17.4	21.7	14.7	13.5	17.9	19.7	24.1	27.1	20.4	12.8	15.1	16.5	20.6	21.9	17.4
UIC	29.0	36.7	39.2	39.1	48.5	38.5	33.5	42.8	43.7	44.0	52.8	43.4	30.6	33.8	36.8	36.4	41.4	35.8
Mean	16.1	20.0	23.1	25.9	32.6		21.2	27.5	28.6	32.3	38.3		19.2	22.4	24.5	27.3	30.6	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	0.6		0.7		1.5		0.9		1.0		2.2		0.7		0.8		1.8	
CD (0.05)	1.7		2.0		NS		2.4		2.6		NS		2.0		2.3		NS	

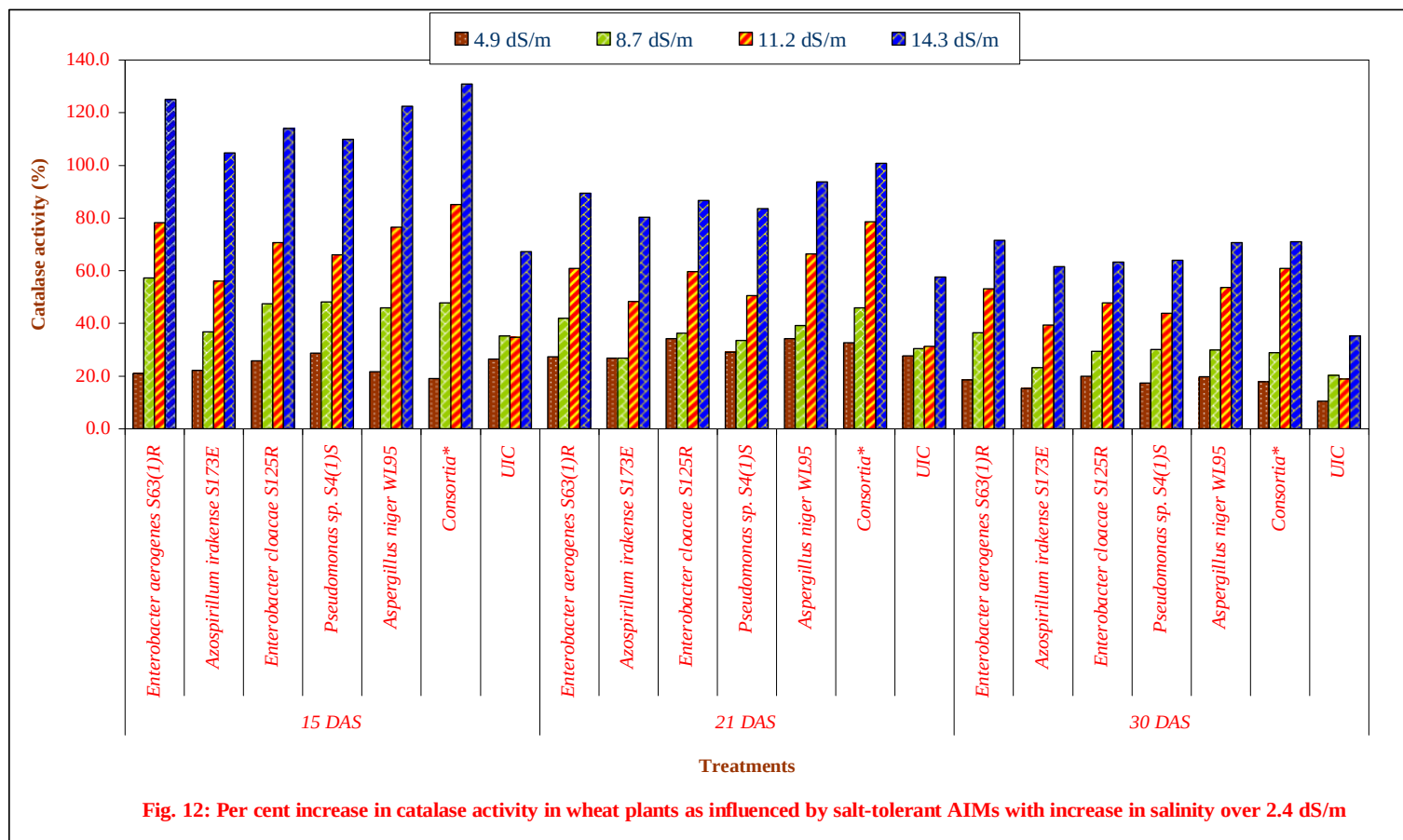


Fig. 12: Per cent increase in catalase activity in wheat plants as influenced by salt-tolerant AIMs with increase in salinity over 2.4 dS/m

4.5.4.1.3 Peroxidase activity (POX)

Among the treatments, consortia inoculated plants recorded the lowest POX activity (14.4, 18.6 and 21.9 U/mg protein at 15, 21 and 30 DAS respectively) which was significantly lower than that of other strains and UIC (Table 33c). The highest mean POX activity of 37.2, 39.4 and 41.5 U mg protein⁻¹ were recorded in UIC plants at 15, 21 and 30 DAS respectively (Table 33c). Similarly, among the soil ECe levels, maximum mean POX activity of 24.8, 28.3 and 38.4 U mg protein⁻¹ were recorded with a soil ECe of 14.3 dS m⁻¹ and the minimum of 20.4, 25.5 and 22.5 U mg protein⁻¹ with a soil ECe of 2.4 dS m⁻¹ at 15, 21 and 30 DAS respectively (Table 33c). The interaction effect was found to be non-significant.

However, the per cent increase in POX activity with increase in soil ECe levels was maximum in inoculated plants than in UIC (Fig. 14). POX activity in consortia inoculated plants was increased by 17.8, 35.0, 64.3 and 80.9 per cent as compared to UIC plants wherein the activity was increased by 10.1, 19.5, 35.5 and 49.4 per cent at soil ECe of 4.9, 8.7, 11.2 and 14.3 dS m⁻¹ over and above 2.4 dS/m respectively at 30 DAS (Fig. 14). Similar trend was recorded at remaining sampling stages (15 and 21 DAS).

4.5.4.2 Accumulation of osmo-protective compounds by wheat

Osmo-protective compounds such as proline, glycine-betaine like quaternary ammonium compounds (QACs), polyols, potassium, total sugar and protein were estimated in the wheat plants at 30 and 60 days of crop growth and the results are presented in Tables 34 to 38.

4.5.4.2.1 Proline

In general, proline content was found to increase significantly with increase in soil ECe up to 11.2 dS m⁻¹ and decreased thereafter whereas inoculated plants recorded significantly lower proline as compared to UIC at all the soil ECe levels examined (Table 34 and Fig. 15).

Significant differences existed between the inoculation treatments with respect to proline content at both 30 and 60 days of crop growth. Among the treatments, the lowest mean proline contents of 7.2 and 6.0 µmol per g fresh weight (FW) were recorded in consortia inoculated plants at 30 and 60 DAS respectively, which was significantly lower than the remaining treatments including UIC (Table 34). *Aspergillus niger* WL9S inoculated wheat plants also showed significantly lower proline content than other inoculation treatments at both the stages of crop growth. UIC plants recorded maximum proline content of 20.6 and 19.4 µmol per g FW at 30 and 60 DAS respectively (Table 34 and Fig. 15).

Among the soil ECe levels, maximum mean proline content of 16.8 and 15.7 µmol per g FW were recorded with a soil ECe of 11.2 dS m⁻¹ at 30 and 60 DAS respectively which were significantly higher over other soil ECe levels. Significantly lower proline content of 9.7 and 8.0 µmol per g FW were recorded at soil ECe of 2.4 dS/m at 30 and 60 DAS respectively. The interaction effect was found to be non-significant (Table 34).

4.5.4.2.2 Glycine-betaine like quaternary ammonium compounds (QACs)

The QACs content of wheat plants was found to increase with increase in soil ECe level up to 11.2 dS m⁻¹ and declined thereafter. Inoculation of salt-tolerant AIMS resulted in significant increase in QACs content of wheat plants (Table 35 and Fig. 16). In general, consortia inoculated wheat plants showed maximum QACs content and was least in UIC plants at both the stages of sampling and all the soil ECe levels.

Among the treatments, irrespective of soil ECe levels, the highest mean QACs content of 6.77 µmol per g FW at 30 DAS and 5.42 µmol per g FW at 60 DAS were recorded in consortia inoculated plants and was significantly superior over remaining treatments (Table 35). *Enterobacter aerogenes* S63(1)R also recorded significantly higher QACs in wheat plants as compared to other strains at both the stages of crop growth.

Among the soil ECe levels, maximum mean QACs content of 6.24 µmol per g FW was recorded with a soil ECe of 11.2 dS m⁻¹ at 30 DAS which was however on par with 14.3 dS/m (6.14 µmol) and both were significantly higher than that of other ECe levels.

Table 33b. Superoxide dismutase activity (U/mg protein) in wheat plants as influenced by salt tolerant AIMS at different soil salinity levels under field condition

Treatment	15 DAS						21 DAS						30 DAS					
	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	20.6	27.8	35.8	42.4	62.5	37.8	27.3	38.7	50.4	60.0	79.5	51.2	26.8	35.6	43.5	47.6	60.6	42.8
<i>Azospirillum irakense</i> S173E	27.6	36.5	40.9	50.1	77.9	46.6	34.8	48.9	56.6	69.9	94.5	61.0	32.9	41.8	48.3	54.0	70.1	49.4
<i>Enterobacter cloacae</i> S125R	23.2	32.6	37.7	46.0	67.6	41.4	30.1	44.5	52.6	64.6	85.2	55.4	29.2	38.8	45.6	50.8	64.4	45.8
<i>Pseudomonas</i> sp. S4(1)S	30.6	41.6	49.3	57.7	88.7	53.6	37.9	55.1	65.6	78.6	104.9	68.4	35.1	45.4	54.4	59.3	76.1	54.1
<i>Aspergillus niger</i> WL9S	18.5	25.6	30.8	38.2	55.8	33.8	24.9	36.5	43.8	55.4	72.8	46.7	24.7	32.9	39.0	44.5	56.2	39.5
Consortia*	16.0	21.8	27.2	34.3	48.8	29.6	21.9	31.5	39.3	50.6	65.3	41.7	22.2	28.9	35.3	41.0	50.8	35.6
UIC	46.3	59.7	66.6	75.5	106.6	70.9	51.4	72.4	83.0	90.4	118.2	83.1	45.2	56.3	66.3	67.5	85.0	64.1
Mean	26.1	35.1	41.2	49.2	72.6		32.6	46.8	55.9	67.1	88.6		30.9	40.0	47.5	52.1	66.2	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	0.9		1.1		2.4		1.1		1.3		2.9		1.2		1.4		3.2	
CD (0.05)	2.6		3.1		6.8		3.1		3.7		NS		3.4		3.8		NS	

* *Enterobacter aerogenes* S63(1)R + *Azospirillum irakense* S173E + *Enterobacter cloacae* S125R + *Pseudomonas* sp. S4(1)S

SEm±: Standard error of means

CD: Critical difference

ECe: Electrical conductivity of the saturation paste extract

Table 33c. Peroxidase activity (U/mg protein) in wheat plants as influenced by salt tolerant AIMS at different soil salinity levels under field condition

Treatment	15 DAS						21 DAS						30 DAS					
	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	15.9	17.0	19.2	20.8	21.1	18.8	21.2	21.8	25.0	23.0	25.0	23.2	19.3	22.8	26.3	30.5	35.3	26.8
<i>Azospirillum irakense</i> S173E	21.4	23.0	22.9	25.2	26.5	23.8	27.1	27.4	28.5	27.0	30.5	28.1	24.0	27.1	29.2	34.7	41.0	31.2
<i>Enterobacter cloacae</i> S125R	18.0	20.3	20.5	22.7	23.0	20.9	23.4	25.0	26.3	25.0	27.2	25.4	21.2	25.1	27.5	32.5	37.3	28.7
<i>Pseudomonas</i> sp. S4(1)S	23.9	26.5	27.7	28.9	30.4	27.5	29.7	30.5	33.0	30.2	33.6	31.4	25.8	29.7	33.4	38.2	44.8	34.4
<i>Aspergillus niger</i> WL9S	14.2	15.6	16.4	18.6	18.7	16.7	19.2	20.1	21.7	21.1	22.8	21.0	17.6	20.9	23.4	28.2	31.8	24.4
Consortia*	12.2	12.8	14.1	16.6	16.2	14.4	17.0	17.2	19.4	19.2	20.4	18.6	15.7	18.5	21.2	25.8	28.4	21.9
UIC	36.9	38.2	39.2	33.6	37.8	37.2	40.7	40.9	40.4	35.9	38.8	39.4	33.8	37.2	40.4	45.8	50.5	41.5
Mean	20.4	21.9	22.9	23.8	24.8		25.5	26.1	27.7	25.9	28.3		22.5	25.9	28.8	33.7	38.4	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	0.7		0.6		1.8		0.9		1.1		2.4		0.8		1.0		2.2	
CD (0.05)	1.9		2.3		NS		NS		3.0		NS		2.3		2.8		NS	

* *Enterobacter aerogenes* S63(1)R + *Azospirillum irakense* S173E + *Enterobacter cloacae* S125R + *Pseudomonas* sp. S4(1)S

SEm±: Standard error of means

CD: Critical difference

ECe: Electrical conductivity of the saturation paste extract

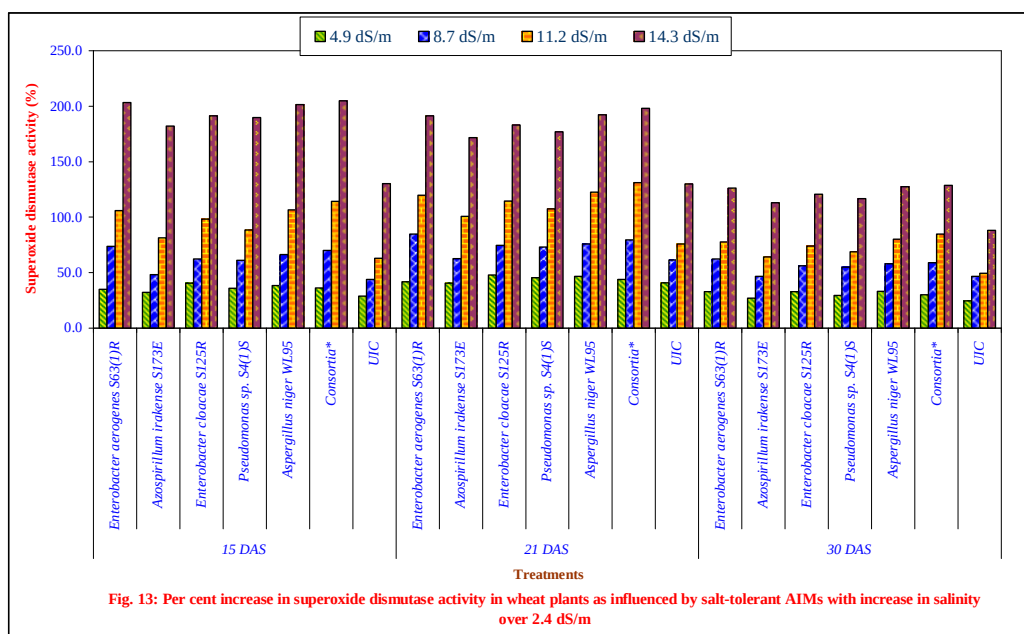


Fig. 13: Per cent increase in superoxide dismutase activity in wheat plants as influenced by salt-tolerant AIMs with increase in salinity over 2.4 dS/m

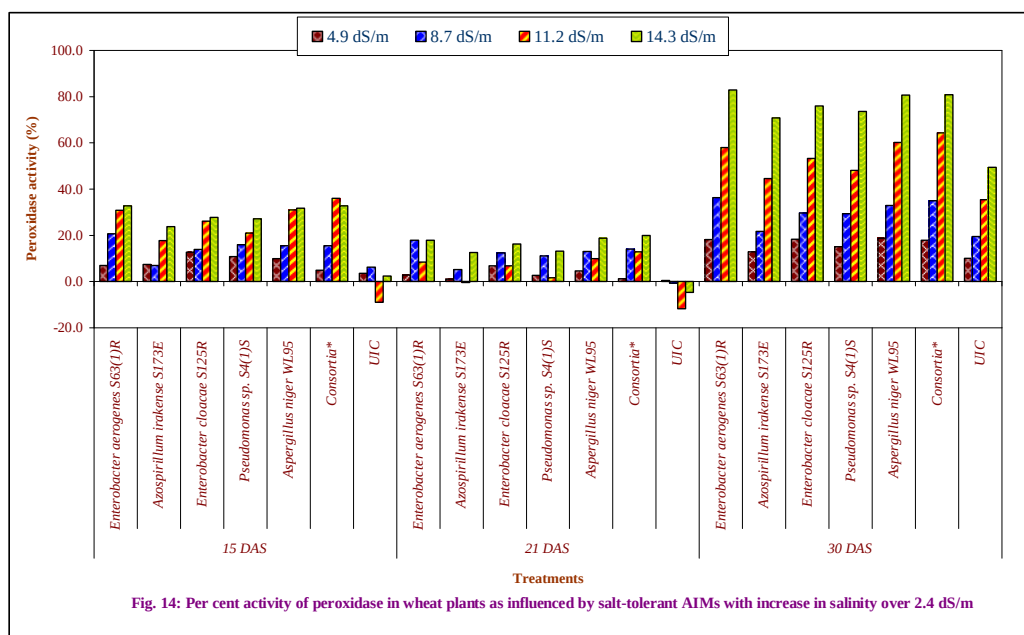


Fig. 14: Per cent activity of peroxidase in wheat plants as influenced by salt-tolerant AIMs with increase in salinity over 2.4 dS/m

Table 34. Influence of salt tolerant AIMs on proline ($\mu\text{mol/g FW}$) content of wheat at different soil salinity levels under field condition

Treatment	30 DAS						60 DAS					
	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	8.1	10.5	13.5	14.1	12.6	11.8	6.4	9.0	12.1	12.9	12.5	10.6
<i>Azospirillum irakense</i> S173E	10.7	14.9	18.3	18.1	16.3	15.7	9.0	13.4	16.9	16.9	16.2	14.5
<i>Enterobacter cloacae</i> S125R	9.4	12.6	16.7	16.8	14.9	14.1	7.7	11.1	15.3	15.6	14.8	12.9
<i>Pseudomonas</i> sp. S4(1)S	12.3	16.8	20.1	21.1	18.1	17.7	10.6	15.3	18.7	19.9	18.0	16.5
<i>Aspergillus niger</i> WL9S	7.6	9.3	11.9	12.3	9.1	10.0	5.9	7.8	10.5	11.1	9.0	8.9
Consortia*	5.8	6.4	8.2	8.7	6.8	7.2	4.1	4.9	6.8	7.5	6.7	6.0
UIC	14.1	18.9	22.4	26.8	20.7	20.6	12.4	17.4	21.0	25.6	20.6	19.4
Mean	9.7	12.8	15.9	16.8	14.1		8.0	11.2	14.5	15.7	14.0	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm $\pm$	0.3		0.3		0.8		0.2		0.3		0.6	
CD (0.05)	0.8		1.0		NS		0.7		0.8		NS	

Table 35. Influence of salt tolerant AIMs on glycine-betaine like QACs ($\mu\text{mol/g FW}$) content of wheat plants at different soil salinity levels under field condition

Treatment	30 DAS						60 DAS					
	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	3.42	4.87	5.73	7.87	8.22	6.02	2.39	3.69	4.51	6.37	6.41	4.67
<i>Azospirillum irakense</i> S173E	2.19	3.12	3.96	6.22	5.12	4.12	1.16	1.94	2.74	4.72	3.31	2.77
<i>Enterobacter cloacae</i> S125R	2.63	3.96	4.43	7.13	6.31	4.89	1.60	2.78	3.21	5.63	4.50	3.54
<i>Pseudomonas</i> sp. S4(1)S	1.87	2.68	3.11	5.03	4.33	3.40	0.84	1.50	1.89	3.53	2.52	2.06
<i>Aspergillus niger</i> WL9S	2.94	4.12	5.08	6.69	7.28	5.22	1.91	2.94	3.86	5.19	5.47	3.87
Consortia*	3.77	5.19	6.83	8.74	9.32	6.77	2.74	4.01	5.61	7.24	7.51	5.42
UIC	1.32	1.56	1.68	1.98	2.43	1.79	0.29	0.38	0.46	0.48	0.62	0.45
Mean	2.59	3.64	4.40	6.24	6.14		1.56	2.46	3.18	4.73	4.33	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm $\pm$	0.14		0.17		0.37		0.08		0.10		0.22	
CD (0.05)	0.40		0.47		NS		0.24		0.28		NS	

* *Enterobacter aerogenes* S63(1)R + *Azospirillum irakense* S173E + *Enterobacter cloacae* S125R + *Pseudomonas* sp. S4(1)S

SEm $\pm$: Standard error of means

CD: Critical difference

ECe: Electrical conductivity of the saturation paste extract

Table 36. Influence of salt tolerant AIMS on polyols ($\mu\text{g/g DW}$) content of wheat plants at different soil salinity levels under field condition

Treatment	30 DAS						60 DAS					
	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	611	737	932	1063	1082	885	273	342	456	482	517	414
<i>Azospirillum irakense</i> S173E	820	941	1086	1294	1322	1092	482	546	610	713	735	617
<i>Enterobacter cloacae</i> S125R	705	817	1010	1159	1223	983	367	422	534	578	618	504
<i>Pseudomonas</i> sp. S4(1)S	924	1061	1276	1426	1498	1237	586	666	800	845	893	758
<i>Aspergillus niger</i> WL9S	544	666	806	956	994	793	206	271	330	375	389	314
Consortia*	426	536	682	859	893	679	88	141	206	278	288	200
UIC	1069	1222	1437	1589	1682	1400	731	827	961	1008	1077	921
Mean	728	854	1033	1192	1242		390	459	557	611	645	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm $\pm$	23		27		61		12		15		33	
CD (0.05)	65		77		NS		35		41		NS	

Table 37. Total sugar (mg/g DW) content of wheat as influenced by salt tolerant AIMS at different soil salinity levels under field condition

Treatment	30 DAS						60 DAS					
	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	123.8	123.4	134.0	149.9	138.1	133.8	160.3	168.3	188.3	201.9	215.4	186.8
<i>Azospirillum irakense</i> S173E	103.0	108.0	123.5	134.6	122.7	118.4	152.6	161.8	176.7	191.6	206.9	177.9
<i>Enterobacter cloacae</i> S125R	113.7	117.1	129.6	144.1	133.9	127.7	154.1	164.2	178.9	196.3	212.6	181.2
<i>Pseudomonas</i> sp. S4(1)S	92.7	100.8	110.8	126.2	114.2	108.9	150.9	160.4	175.2	190.8	204.1	176.3
<i>Aspergillus niger</i> WL9S	130.4	133.9	148.4	157.3	149.9	144.0	165.2	171.6	190.7	204.6	223.1	191.0
Consortia*	142.1	148.4	159.3	167.2	161.9	155.8	168.3	178.3	195.8	211.3	232.6	197.3
UIC	79.2	87.9	98.0	116.9	108.3	98.1	142.6	152.3	163.6	173.9	191.3	164.7
Mean	112.1	117.1	129.1	142.3	132.7		156.3	165.3	181.3	195.8	212.3	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm $\pm$	3.1		3.7		8.3		5.0		5.9		13.3	
CD (0.05)	8.9		10.5		NS		14.2		16.7		NS	

* *Enterobacter aerogenes* S63(1)R + *Azospirillum irakense* S173E + *Enterobacter cloacae* S125R + *Pseudomonas* sp. S4(1)S

SEm $\pm$: Standard error of means

CD: Critical difference

ECe: Electrical conductivity of the saturation paste extract

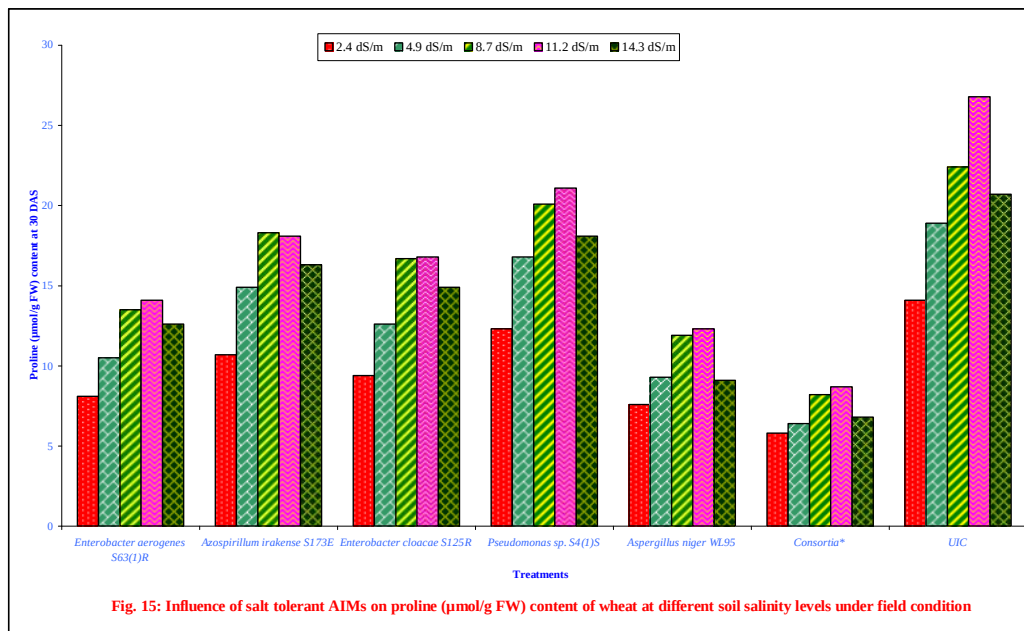


Fig. 15: Influence of salt tolerant AIMs on proline (µmol/g FW) content of wheat at different soil salinity levels under field condition

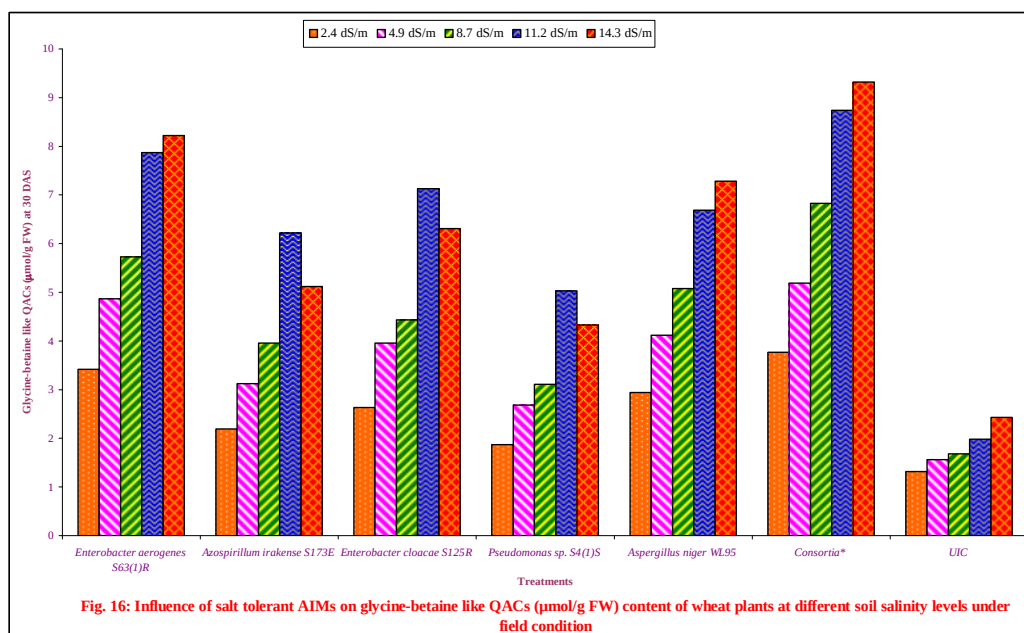


Fig. 16: Influence of salt tolerant AIMs on glycine-betaine like QACs (µmol/g FW) content of wheat plants at different soil salinity levels under field condition

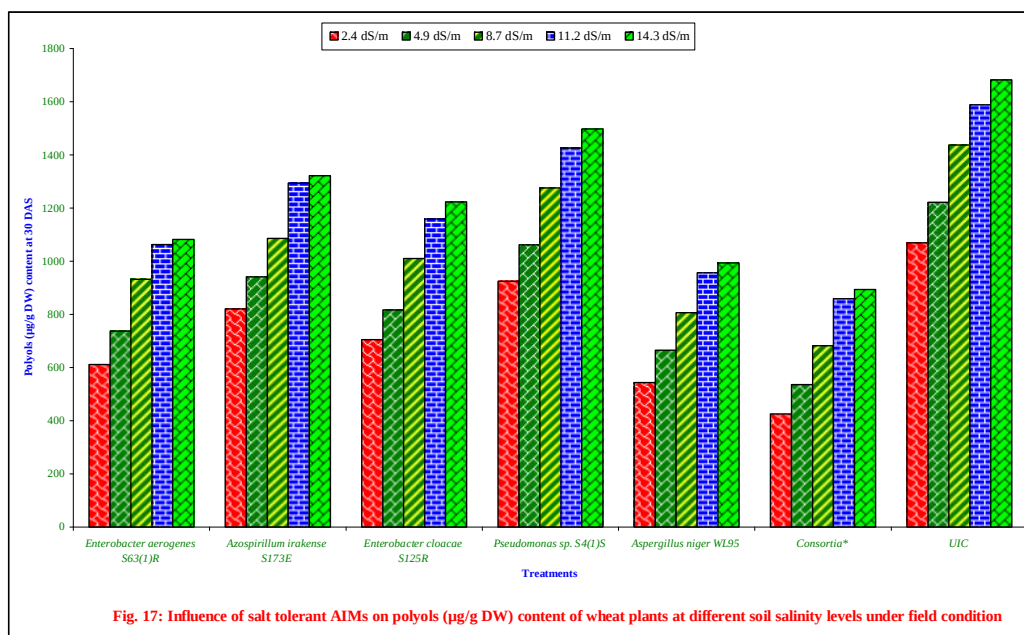


Fig. 17: Influence of salt tolerant AIMs on polyols (µg/g DW) content of wheat plants at different soil salinity levels under field condition

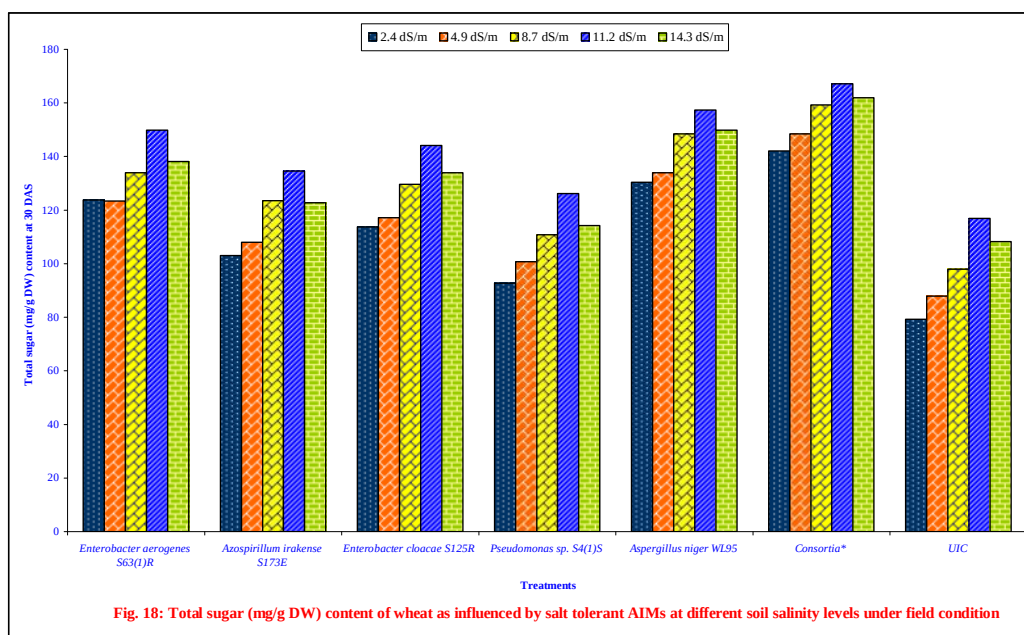


Fig. 18: Total sugar (mg/g DW) content of wheat as influenced by salt tolerant AIMs at different soil salinity levels under field condition

At 60 DAS, maximum QACs were recorded at ECe of 11.2 dS/m which was significantly higher than all other ECe levels. There was no significant interaction between microbial strains and soil ECe levels (Table 35 and Fig. 16).

4.5.4.2.3 Polyols

Inoculation of salt-tolerant AIMs was found to reduce the polyol contents in wheat plants. The inoculation effect was more pronounced with consortia than that of individual strains. However, there was significant increase in polyol contents in wheat plants with every increase in soil ECe level at both the stages of sampling. However, the polyols in wheat plants was reduced markedly from 30 DAS to 60 DAS (Table 36).

Among the treatments, UIC plants recorded significantly higher polyol content [1400 and 921 μg per g dry weight (DW) at 30 and 60 DAS respectively] as compared to inoculation treatments (Table 36). At 30 DAS, consortia inoculated plants showed least polyol contents (679 $\mu\text{g/g}$ DW) followed by *Aspergillus niger* WL9S (793 $\mu\text{g/g}$ DW) and *Enterobacter aerogenes* S63(1)R (885 $\mu\text{g/g}$ DW) which differed significantly among themselves as well as with other inoculation treatments. A similar trend was noticed at 60 days of crop growth (Table 36 and Fig. 17).

The mean polyol content in wheat plants, irrespective of inoculation, was found to increase with increase in soil ECe at both stages of crop growth. At 30 DAS, the highest polyol content (1242 $\mu\text{g/g}$ DW) was recorded at 14.3 dS/m which was however on par with ECe of 11.2 dS/m (1192 $\mu\text{g/g}$ DW) but both were significantly higher than that recorded at other ECe levels (Table 36). Same trend was observed at 60 DAS also. The interaction effect was found to be non-significant (Table 36 and Fig. 17).

4.5.4.2.4 Total sugar

Inoculation of salt-tolerant AIMs was found to increase the mean total sugar content in wheat plants which ranged from 108.9 to 155.8 and 176.3 to 197.3 mg/g DW as against the least total sugars content of 98.1 and 164.7 mg per g DW recorded in UIC at 30 and 60 DAS respectively (Table 37 and Fig. 18). The total sugar content of wheat plants was found to increase with increase in soil ECe level up to 11.2 dS m^{-1} (142.3 mg/g DW) at 30 DAS and decreased thereafter whereas it continued to increase with increase in soil ECe up to 14.3 dS/m (212.3 mg/g DW) at 60 DAS (Table 37).

Among the treatments, consortia recorded the highest sugar content of 155.8 mg/g DW in wheat plants at 30 DAS which was significantly superior over remaining treatments (Table 37). However, two isolates viz., *Aspergillus niger* WL9S and *Enterobacter aerogenes* S63(1)R also recorded significantly higher total sugar contents (144.0 and 133.8 mg/g DW respectively) than other inoculation treatments at 30 DAS but were on par with each other. At 60 DAS, the highest mean total sugar content was recorded in plants inoculated with consortia (197.3 mg) followed by *Aspergillus niger* WL9S (191.0 mg), *Enterobacter aerogenes* S63(1)R (186.8 mg) and *Enterobacter cloacae* (181.2 mg/g DW) which were on par with each other but were significantly superior over UIC (164.7 mg/g DW) (Table 37 and Fig. 18).

Among the soil ECe levels, the highest mean total sugar content (142.3 mg/g DW) was recorded with soil ECe 11.2 dS/m at 30 DAS which was on par with soil ECe of 14.3 dS/m (132.7 mg) but significantly superior over rest of the soil ECe levels (Table 37). At 60 DAS, the highest mean sugar content was recorded at 14.3 dS/m (212.3 mg) which was significantly superior over other soil ECe levels. The interaction effect was found to be non-significant (Table 37).

4.5.4.2.5 Potassium and sodium content and their ratio

Potassium and sodium contents in wheat plants as influenced by salt-tolerant AIMs at different soil salinity levels were determined at 30 and 60 days of crop growth and results are presented in Table 38. It was observed that, sodium content of wheat plants continued to increase with increase in soil ECe level upto 14.3 dS/m, while potassium content was increased only up to 8.7 dS/m and declined thereafter at both the stages crop growth. Maximum K to Na ratios were recorded at 4.9 dS/m which however declined thereafter with increase in soil ECe.

Table 38. Sodium and potassium contents ($\mu\text{g/g DW}$) and their ratio in wheat plants as influenced by salt tolerant AIMS at different soil salinity levels under field condition

Treatment	Potassium at 30 DAS						Sodium at 30 DAS						K to Na ratio at 30 DAS					
	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	9.1	26.8	31.3	29.9	28.4	25.1	4.0	6.9	10.1	12.6	15.5	9.8	2.3	3.9	3.1	2.4	1.8	2.7
<i>Azospirillum irakense</i> S173E	7.1	24.4	28.8	26.3	24.3	22.2	4.6	7.6	11.5	13.8	16.5	10.8	1.5	3.2	2.5	1.9	1.5	2.1
<i>Enterobacter cloacae</i> S125R	7.9	24.6	29.4	27.4	25.7	23.0	4.3	7.4	11.3	13.5	16.0	10.5	1.8	3.3	2.6	2.0	1.6	2.3
<i>Pseudomonas</i> sp. S4(1)S	7.8	23.4	26.8	25.6	23.4	21.4	4.8	7.7	11.5	14.1	16.8	11.0	1.6	3.0	2.3	1.8	1.4	2.0
<i>Aspergillus niger</i> WL9S	8.3	25.1	30.7	28.6	27.8	24.1	4.2	7.1	10.5	12.9	15.9	10.1	2.0	3.5	2.9	2.2	1.8	2.5
Consortia*	10.8	28.6	32.6	31.3	29.6	26.6	3.8	6.6	9.7	12.2	15.2	9.5	2.8	4.3	3.4	2.6	2.0	3.0
UIC	6.1	21.3	25.5	24.3	21.8	19.8	5.2	8.6	12.8	15.9	17.9	12.1	1.2	2.5	2.0	1.5	1.2	1.7
Mean	8.2	24.9	29.3	27.6	25.9	0.0	4.4	7.4	11.1	13.6	16.3	0.0	1.9	3.4	2.7	2.1	1.6	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	0.7		0.8		1.8		0.4		0.5		1.0		0.03		0.04		0.08	
CD (0.05)	1.9		2.2		NS		1.1		1.3		NS		0.09		0.11		0.24	
Treatment	Potassium at 60 DAS						Sodium at 60 DAS						K to Na ratio at 60 DAS					
	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean	Soil ECe (dS/m)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	15.2	49.3	63.5	63.7	61.6	50.7	5.2	9.3	16.3	21.2	28.1	16.0	2.9	5.3	3.9	3.0	2.2	3.5
<i>Azospirillum irakense</i> S173E	10.2	38.1	49.2	47.9	46.4	38.4	5.7	9.7	16.9	21.1	26.6	16.0	1.8	3.9	2.9	2.3	1.7	2.5
<i>Enterobacter cloacae</i> S125R	12.3	42.1	54.7	53.7	52.4	43.0	5.5	9.6	17.4	21.7	27.5	16.3	2.3	4.4	3.1	2.5	1.9	2.8
<i>Pseudomonas</i> sp. S4(1)S	11.8	38.6	48.2	48.1	46.6	38.7	6.0	10.0	17.5	22.6	28.6	16.9	2.0	3.9	2.8	2.1	1.6	2.5
<i>Aspergillus niger</i> WL9S	13.3	44.9	60.5	58.3	58.9	47.2	5.3	9.4	16.6	21.2	28.3	16.2	2.5	4.8	3.6	2.8	2.1	3.1
Consortia*	18.7	55.8	70.4	70.1	68.7	56.7	5.0	9.4	16.4	21.5	28.7	16.2	3.7	6.0	4.3	3.3	2.4	3.9
UIC	8.5	32.0	42.1	42.0	39.7	32.9	6.3	10.8	18.2	23.9	28.1	17.4	1.3	3.0	2.3	1.8	1.4	2.0
Mean	12.9	43.0	55.5	54.8	53.5		5.6	9.7	17.0	21.9	28.0		2.4	4.4	3.3	2.5	1.9	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	1.0		1.1		2.5		0.4		0.5		1.1		0.03		0.03		0.08	
CD (0.05)	2.7		3.2		NS		1.1		NS		NS		0.08		0.10		0.22	

* *Enterobacter aerogenes* S63(1)R + *Azospirillum irakense* S173E + *Enterobacter cloacae* S125R + *Pseudomonas* sp. S4(1)S

SEm $\pm$: Standard error of means

CD: Critical difference

ECe: Electrical conductivity of the saturation paste extract

Wheat plants inoculated with salt-tolerant AIMS recorded significantly higher potassium content and K to Na ratio but lower Na content as compared to UIC plants (Table 38).

4.5.4.2.5.1 Potassium

Among the treatments, irrespective of soil ECe levels, inoculation of microbial consortia recorded the highest mean K content of 26.6 μg per g dry weight (DW) at 30 DAS which was however on par with *Enterobacter aerogenes* S63(1)R (25.1 μg) but was significantly superior over other strains and UIC (19.8 μg). *Aspergillus niger* WL9S recorded significantly higher K content (24.1 $\mu\text{g/g}$ DW) than *Pseudomonas* sp S4(1)S (21.4 μg) but was on par with *Enterobacter cloacae* S125R (23.0 μg) and *Azospirillum irakense* S173E (22.2 μg). At 60 days of crop growth, the highest mean K content (56.7 $\mu\text{g/g}$ DW) was recorded by consortia treatment which was significantly superior over all other strains and UIC (Table 38). *Enterobacter aerogenes* S63(1)R also recorded significantly higher mean K content (50.7 $\mu\text{g/g}$ DW) than other strains and UIC (32.9 $\mu\text{g/g}$ DW) (Table 38).

Among the soil ECe levels, the maximum mean K content (29.3 $\mu\text{g/g}$ DW) was recorded at soil ECe of 8.7 dS/m and was significantly superior over other ECe levels except 11.2 dS/m (27.6 μg) with which it was on par at 30 DAS. At 60 DAS, the highest mean K content (55.5 μg) was again recorded with soil ECe of 8.7 dS/m which was however on par with soil ECe levels of 11.2 and 14.3 dS/m but significantly superior over other soil ECe. The interaction effect was found to be non-significant.

4.5.4.2.5.2 Sodium

It was observed that, UIC wheat plants had higher Na content than the inoculated plants (Table 38). The Na content of wheat plants was found to decrease from 5.2 to 3.8, 8.6 to 6.6, 12.8 to 9.7, 15.9 to 12.2 and 17.9 to 15.2 μg per g DW with inoculation of salt tolerant AIMS at soil ECe of 2.4, 4.9, 8.7, 11.2 and 14.3 dS m^{-1} respectively at 30 DAS. Among the treatments, consortia inoculated plants recorded the lowest mean Na content (9.5 $\mu\text{g/g}$ DW) which was on par with all other strains except *Pseudomonas* sp S4(1)S, but was significantly lower than UIC. However, at 60 DAS, the Na content in wheat plants as influenced by the microbial strains was found to be non-significant (Table 38).

Among the ECe levels, the highest mean Na contents of 16.3 and 28.0 μg per g DW were recorded at soil ECe of 14.3 dS m^{-1} at 30 and 60 DAS respectively, which were significantly higher than that recorded at other ECe levels. The least mean Na contents of 4.4 and 5.6 $\mu\text{g/g}$ DW were recorded with the soil ECe of 2.4 dS/m at 30 and 60 DAS respectively. The interaction effect was found to be non-significant (Table 38).

4.5.4.2.5.3 K to Na ratio

Significant differences in K to Na ratio in wheat plants were observed between the treatments and their combinations (Table 38). The K to Na ratio was found to increase due to inoculation but decreased with increase in soil ECe level above 4.9 dS m^{-1} . Among the treatments, consortia inoculated plants recorded the highest mean K to Na ratios of 3.0 and 3.9 at 30 and 60 DAS respectively which was significantly superior over other strains and UIC. *Enterobacter aerogenes* S63(1)R and *Aspergillus niger* WL9S also showed significantly higher K to Na ratio at both stages of sampling. The least Na to K ratios of 1.7 and 2.0 were recorded in UIC plants at 30 and 60 DAS respectively (Table 38).

Among the soil ECe levels, the highest K to Na ratio was recorded at soil ECe of 4.9 dS m^{-1} which decreased significantly with increase in soil ECe level (Table 38) but the K to Na ratio at all ECe levels was higher than that of 2.4 dS/m at both stages of crop growth. Among the treatment combinations, significantly higher K to Na ratios of 4.3 and 6.0 were recorded in consortia inoculated wheat plants at soil ECe of 4.9 dS m^{-1} at 30 and 60 DAS respectively, which were significantly superior over all other treatment combinations. Lower K to Na ratios of 1.2 and 1.3 were recorded in UIC plants at soil ECe of 2.4 dS m^{-1} at 30 and 60 DAS respectively (Table 38).

4.5.5 Microbiological parameters

4.5.5.1 Activity of soil enzymes

Data on activity of three soil enzymes viz., dehydrogenase, urease and phosphatase are presented in Tables 39a to 39c. In general, the activity of all 3 enzymes was highest at 45 days of crop growth. While increase in soil salinity from 2.4 dS m⁻¹ to 14.3 dS m⁻¹ adversely affected the activities of all the enzymes, inoculation of salt-tolerant AIMS showed significant improvement in enzyme activity.

4.5.5.1.1 Dehydrogenase

Among the inoculation treatments, irrespective of the soil salinity levels, consortia recorded the highest mean dehydrogenase activity of 65.6, 67.9, 64.3 and 55.8 µg TPF g soil⁻¹ day⁻¹ at 30, 45, 60 DAS and at harvest respectively and were significantly superior over all other inoculation treatments and UIC at the respective stages of sampling (Table 39a). The UIC treatment recorded the least mean dehydrogenase activity (30.7, 34.0, 28.4 and 25.2 µg TPF g soil⁻¹ day⁻¹ at 30, 45, 60 DAS and at harvest respectively). Among other inoculation treatments, *Aspergillus niger* WL9S recorded significantly higher mean dehydrogenase activity (53.0 µg TPF g soil⁻¹ day⁻¹) over all other strains except *Enterobacter aerogenes* S63(1)R (50.7 µg TPF g soil⁻¹ day⁻¹) with which it was on par at 30 DAS. Similar trends were observed at 60 and 90 DAS also. However, at harvest, *Aspergillus niger* WL9S recorded significantly higher dehydrogenase activity over all other strains (Table 39a).

Among the soil ECe levels, 2.4 dS/m recorded the highest mean dehydrogenase activity (67.3 µg TPF g soil⁻¹ day⁻¹) at 30 DAS which was significantly superior over all other soil ECe levels. Similar trend was noticed at 45 and 60 DAS as well as at harvest. The interaction effect was found to be non-significant (Table 39a).

Inoculation of salt-tolerant strains resulted in 25 to 78.8, 25 to 77.3, 37.5 to 103.1, 37.5 to 129.2 and 50 to 166.7 per cent increase in dehydrogenase activity over UIC at salinity levels of 2.4, 4.9, 8.7, 11.2 and 14.3 dS m⁻¹ respectively (Fig. 19).

4.5.5.1.2 Urease

All the inoculation treatments recorded significantly higher soil urease activity over UIC at all stages of crop growth. Among the inoculation treatments, consortia recorded the highest mean urease activity (99.1, 104.8, 91.4 and 86.7 µg NH₄⁺-N g soil⁻¹ day⁻¹ at 30, 45, 60 DAS and at harvest respectively) which was significantly superior over all other strains (Table 39b). UIC recorded the lowest mean urease activity (55, 63.4, 57 and 52 µg NH₄⁺-N g soil⁻¹ day⁻¹ at 30, 45, 60 DAS and at harvest respectively). Among other inoculation treatments, *Aspergillus niger* WL9S (86.2 µg NH₄⁺-N) recorded higher mean urease activity than other strains but was on par with *Enterobacter aerogenes* S63(1)R (83.6 µg NH₄⁺-N) and *Enterobacter cloacae* S125R (78.8 µg NH₄⁺-N) at 30 DAS. Exactly similar trends were observed at 45 DAS and at harvest. However, at 60 DAS, *Aspergillus niger* WL9S (82.5 µg NH₄⁺-N) was on par with *Enterobacter aerogenes* S63(1)R (79.4 µg NH₄⁺-N), but was significantly superior over rest of the strains (Table 39b).

Among the soil ECe levels, the lowest soil ECe (2.4 dS/m) recorded the highest mean urease activity (96.6 µg NH₄⁺-N g soil⁻¹ day⁻¹) at 30 DAS which was significantly superior over all other soil ECe levels. There was a significant reduction in soil urease activity with increase in soil ECe at 30 and 45 DAS. An almost similar trend was noticed at 60 DAS and at harvest except that the enzyme activity at ECe levels of 4.9 and 8.7 dS/m at 60 DAS and 2.4 and 4.9 dS/m at harvest were on par with each other. The interaction effect was found to be non-significant (Table 39b).

Inoculation of salt-tolerant AIMS resulted in 16.2 to 59.9, 17.8 to 54.6, 18.1 to 60.4, 20.3 to 77.5 and 20.8 to 88 per cent increase in urease activity over UIC at 2.4, 4.9, 8.7, 11.2 and 14.3 dS m⁻¹ respectively (Fig. 19).

4.5.5.1.3 Alkaline phosphatase

In general all the inoculation treatments recorded significant increase in soil alkaline phosphatase activity at all stages of crop growth except 30 and 60 DAS where in *Pseudomonas* sp. S4(1)S was on par with UIC.

Table 39a. Influence of salt-tolerant AIMs on dehydrogenase ($\mu\text{g TPF g soil}^{-1} \text{ day}^{-1}$) activity at different soil salinity levels under field condition

Treatment	30 DAS						45 DAS					
	Soil ECe (dS m ⁻¹)					Mean	Soil ECe (dS m ⁻¹)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	71.1	61.3	48.1	39.7	33.2	50.7	77.1	63.7	54.2	44.2	39.0	55.6
<i>Azospirillum irakense</i> S173E	61.9	54.8	44.2	34.1	27.1	44.4	68.2	59.1	45.8	36.0	30.2	47.9
<i>Enterobacter cloacae</i> S125R	68.3	58.9	45.7	38.3	29.0	48.0	75.0	61.2	49.1	41.0	35.1	52.3
<i>Pseudomonas</i> sp. S4(1)S	59.1	52.2	37.8	31.1	24.3	40.9	64.9	55.4	44.4	33.3	26.8	45.0
<i>Aspergillus niger</i> WL9S	74.8	62.0	51.2	42.2	35.0	53.0	79.7	67.2	56.1	46.1	40.1	57.8
Consortia*	88.2	73.3	66.4	55.8	44.1	65.6	93.2	77.9	65.2	54.7	48.3	67.9
UIC	48.0	40.2	29.1	21.2	14.8	30.7	52.1	43.8	31.7	24.1	18.1	34.0
Mean	67.3	57.5	46.1	37.5	29.6		72.9	61.2	49.5	39.9	33.9	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	1.7		2.0		4.5		1.8		2.2		4.9	
CD (0.05)	4.8		5.7		NS		5.2		6.1		NS	

Treatment	60 DAS						At harvest					
	Soil ECe (dS m ⁻¹)					Mean	Soil ECe (dS m ⁻¹)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	68.8	57.1	49.0	39.8	37.2	50.4	57.7	52.2	48.1	36.3	30.9	45.0
<i>Azospirillum irakense</i> S173E	61.1	48.0	44.3	33.3	30.1	43.4	52.7	45.2	41.4	29.7	24.8	38.8
<i>Enterobacter cloacae</i> S125R	66.2	51.9	47.1	37.0	33.3	47.1	55.8	48.3	45.2	33.1	27.1	41.9
<i>Pseudomonas</i> sp. S4(1)S	57.0	45.8	39.1	28.0	26.2	39.2	50.0	41.1	36.3	26.2	21.1	34.9
<i>Aspergillus niger</i> WL9S	72.1	59.4	50.2	42.4	40.0	52.8	61.2	55.5	50.1	37.9	33.3	47.6
Consortia*	83.1	73.3	62.1	55.0	48.2	64.3	70.1	63.5	57.5	48.2	39.5	55.8
UIC	44.8	37.3	27.0	18.7	14.2	28.4	41.1	32.7	25.2	14.9	12.1	25.2
Mean	64.7	53.3	45.5	36.3	32.7		55.5	48.4	43.4	32.3	27.0	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	1.0		1.2		2.6		0.7		0.9		2	
CD (0.05)	2.8		3.3		NS		2.1		2.5		NS	

* *Enterobacter aerogenes* S63(1)R + *Azospirillum irakense* S173E + *Enterobacter cloacae* S125R + *Pseudomonas* sp. S4(1)S

SEm $\pm$: Standard error of means

CD: Critical difference

ECe: Electrical conductivity of the saturation paste extract

Table 39b. Influence of salt-tolerant AIMs on urease ($\mu\text{g NH}_4^+ \text{-N g soil}^{-1} \text{ day}^{-1}$) activity at different soil salinity levels under field condition

Treatment	30 DAS						45 DAS					
	Soil ECe (dS m ⁻¹)					Mean	Soil ECe (dS m ⁻¹)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	102.8	93.3	84.4	76.0	61.4	83.6	107.3	99.1	90.8	75.2	66.5	87.8
<i>Azospirillum irakense</i> S173E	89.1	81.3	75.2	68.1	56.2	74.0	97.0	91.4	84.0	68.2	55.2	79.2
<i>Enterobacter cloacae</i> S125R	96.3	87.1	78.4	73.1	58.9	78.8	103.1	95.9	87.2	70.1	61.3	83.5
<i>Pseudomonas</i> sp. S4(1)S	86.2	75.0	71.5	62.4	49.1	68.8	93.4	86.4	78.2	65.0	52.4	75.1
<i>Aspergillus niger</i> WL9S	106.1	95.4	88.5	78.5	62.5	86.2	109.1	101.3	92.1	79.4	67.9	90.0
Consortia*	124.4	113.7	97.6	86.3	73.4	99.1	127.8	113.0	106.3	96.1	80.8	104.8
UIC	71.1	63.1	57.2	46.2	36.4	54.8	80.6	73.1	65.7	53.5	43.2	63.2
Mean	96.6	87.0	79.0	70.1	56.8		102.6	94.3	86.3	72.5	61.0	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	2.3		2.8		6.2		2.6		3.1		7.0	
CD (0.05)	6.6		7.8		NS		7.4		8.8		NS	

Treatment	60 DAS						At harvest					
	Soil ECe (dS m ⁻¹)					Mean	Soil ECe (dS m ⁻¹)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	97.0	88.8	86.2	69.1	55.7	79.4	87.9	87.2	77.1	67.4	56.1	75.1
<i>Azospirillum irakense</i> S173E	88.1	81.1	78.0	65.9	47.1	72.0	83.0	77.2	69.2	60.8	46.9	67.4
<i>Enterobacter cloacae</i> S125R	90.7	84.4	84.1	67.2	53.0	75.9	86.1	84.0	71.1	63.1	50.1	70.9
<i>Pseudomonas</i> sp. S4(1)S	84.2	75.1	73.4	61.0	46.2	68.0	78.7	73.2	63.8	55.2	42.2	62.6
<i>Aspergillus niger</i> WL9S	101.4	93.3	87.3	72.3	58.2	82.5	88.8	88.3	78.1	70.2	57.7	76.6
Consortia*	109.3	102.1	95.6	80.8	69.1	91.4	96.2	94.4	87.3	81.3	74.5	86.7
UIC	72.5	66.0	61.4	49.1	38.0	53.6	68.1	61.1	55.2	44.1	31.2	51.9
Mean	95.1	84.4	80.9	66.5	52.5		84.1	80.8	71.7	63.2	51.2	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	1.9		2.2		4.9		2.5		2.9		6.5	
CD (0.05)	5.2		6.2		NS		7.0		8.2		NS	

* *Enterobacter aerogenes* S63(1)R + *Azospirillum irakense* S173E + *Enterobacter cloacae* S125R + *Pseudomonas* sp. S4(1)S

SEm $\pm$: Standard error of means

CD: Critical difference

ECe: Electrical conductivity of the saturation paste extract

Table 39c. Influence of salt-tolerant AIMs on alkaline phosphatase ($\mu\text{g pNP g soil}^{-1} \text{h}^{-1}$) activity at different soil salinity levels under field condition

Treatment	30 DAS						45 DAS					
	Soil ECe (dS m ⁻¹)					Mean	Soil ECe (dS m ⁻¹)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	231.3	225.2	213.0	185.3	167.0	204.4	239.3	226.3	216.1	185.3	169.0	207.2
<i>Azospirillum irakense</i> S173E	213.4	204.2	188.3	167.2	145.1	183.6	223.1	209.0	195.0	166.1	149.0	188.4
<i>Enterobacter cloacae</i> S125R	226.2	213.4	202.0	171.5	150.5	192.7	227.1	213.4	203.0	172.0	156.0	194.3
<i>Pseudomonas</i> sp. S4(1)S	202.1	193.3	179.1	153.0	133.2	172.1	212.6	196.7	186.3	156.8	141.3	178.7
<i>Aspergillus niger</i> WL9S	236.5	226.0	217.4	188.2	171.1	207.8	242.2	228.1	219.8	187.9	170.0	209.6
Consortia*	252.7	243.2	236.2	203.8	189.6	225.1	259.2	245.6	236.7	206.3	186.5	226.9
UIC	188.1	174.0	162.0	140.0	119.3	156.7	195.9	185.4	171.1	146.1	124.0	164.5
Mean	221.5	211.3	199.7	172.7	153.7		228.5	214.9	204.0	174.4	156.5	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	4.6		5.8		13.8		5.5		6.7		15.5	
CD (0.05)	14.7		17.5		NS		16.6		20.3		NS	

Treatment	60 DAS						At harvest					
	Soil ECe (dS m ⁻¹)					Mean	Soil ECe (dS m ⁻¹)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	237.6	215.8	206.1	183.2	162.0	200.9	223.2	212.1	205.9	171.1	148.2	192.1
<i>Azospirillum irakense</i> S173E	217.1	195.9	186.0	158.2	142.0	179.8	199.1	191.3	182.2	158.2	136.5	173.5
<i>Enterobacter cloacae</i> S125R	226.5	201.3	190.0	166.7	149.2	186.7	206.3	196.4	190.1	165.8	139.4	179.6
<i>Pseudomonas</i> sp. S4(1)S	206.1	189.0	177.0	149.0	130.0	170.2	192.8	183.4	178.3	147.2	124.8	165.3
<i>Aspergillus niger</i> WL9S	241.1	219.2	205.9	187.1	165.8	203.8	226.4	213.5	210.2	174.2	151.6	195.2
Consortia*	255.8	231.5	223.3	202.0	181.4	218.8	241.7	229.7	224.5	191.7	172.5	212.0
UIC	188.7	176.3	164.3	138.1	117.2	156.9	180.7	170.2	159.4	133.8	113.3	151.5
Mean	224.7	204.1	193.2	169.2	149.7		210.0	199.5	192.9	163.1	140.9	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	4.6		5.7		13.6		2.7		3.7		8.8	
CD (0.05)	12.6		15.8		NS		9.6		11.6		NS	

* *Enterobacter aerogenes* S63(1)R + *Azospirillum irakense* S173E + *Enterobacter cloacae* S125R + *Pseudomonas* sp. S4(1)S

SEm $\pm$: Standard error of means

CD: Critical difference

ECe: Electrical conductivity of the saturation paste extract

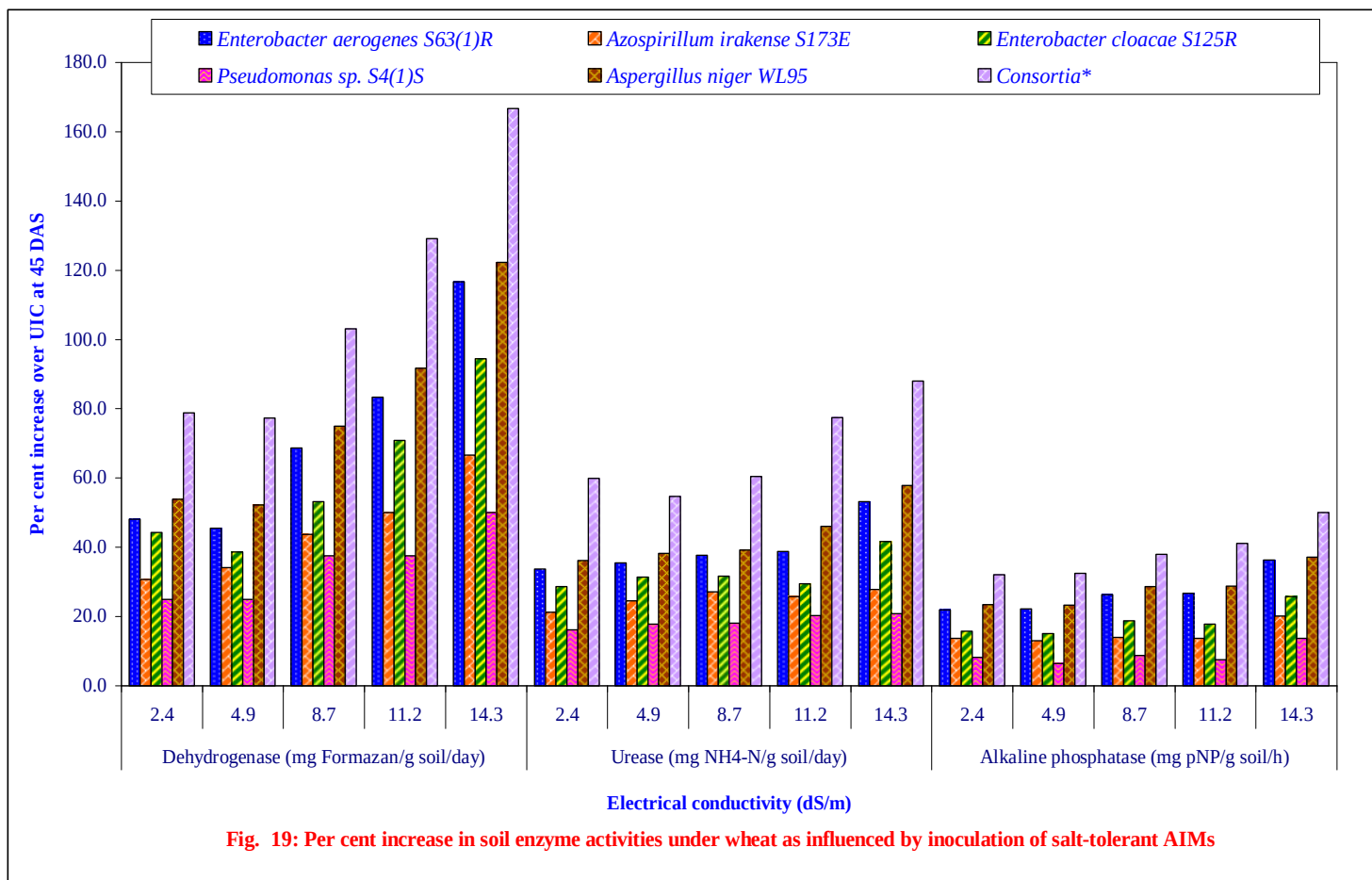


Fig. 19: Per cent increase in soil enzyme activities under wheat as influenced by inoculation of salt-tolerant AIMs

Among the inoculation treatments, consortia recorded the highest mean phosphatase activity ($225.1 \mu\text{g pNP g soil}^{-1} \text{ h}^{-1}$) at 30 DAS, which was however on par with *Aspergillus niger* WL9S ($207.8 \mu\text{g pNP}$) but was significantly superior over other strains and UIC (Table 39c). Similar trend was noticed at 60 DAS. At harvest, the highest mean phosphatase activity ($212.0 \mu\text{g pNP g soil}^{-1} \text{ h}^{-1}$) was recorded by consortia and was significantly superior over all the treatments. However, at 45 DAS consortia treatment was on par with *Aspergillus niger* WL9S ($209.6 \mu\text{g}$) and *Enterobacter aerogenes* S63(1)R ($207.2 \mu\text{g}$) but was significantly superior over other strains and UIC (Table 39c).

Among the soil ECe levels, the highest mean phosphatase activity ($221.5 \mu\text{g pNP g soil/h}$) was recorded with the lowest soil ECe (2.4 dS/m) which was on par with soil ECe of 4.9 dS/m ($211.3 \mu\text{g pNP/g soil/h}$) but significantly superior over other soil ECe levels at 30 DAS. Similarly, the mean enzyme activity at 4.9 ECe and 8.7 ECe was on par. An exactly similar trend was observed at 45 DAS. At 60 DAS and at harvest, ECe level of 2.4 dS/m recorded the highest mean phosphatase activity (224.7 and $210.0 \mu\text{g pNP/g soil/h}$ respectively) which was significantly superior over the enzyme activity at other soil ECe levels. The interaction effect was found to be non-significant (Table 39c).

Inoculation of salt-tolerant AIMs resulted in an increase in phosphatase activity over UIC by 8.2 to 32.1, 6.5 to 32.4, 8.8 to 38, 7.5 to 41.1 and 13.7 to 50 per cent at 2.4, 4.9, 8.7, 11.2 and 14.3 dS m^{-1} respectively (Fig. 19).

4.5.5.2 Microbial biomass carbon and nitrogen

The microbial biomass carbon and nitrogen as influenced by salt-tolerant AIMs at different soil ECe levels was estimated in soil under wheat crop at 30, 45, 60 DAS and at harvest. The results are presented here under.

4.5.5.2.1 Microbial biomass carbon (MBC)

Temporal variation in MBC was observed and results of the study indicated that, irrespective of treatments, increase in microbial biomass was observed from 30 to 60 DAS and decreased thereafter. Among the inoculation treatments, consortia recorded maximum mean MBC (336.1 mg) and was significantly superior over all other inoculation treatments except *Aspergillus niger* WL9S (316.4 mg) and *Enterobacter aerogenes* S63(1)R (304.6 mg) with which it was on par (Table 40a). The same trend was observed at 45 and 60 DAS. However, at harvest, consortia treatment showed mean MBC (277.8 mg) that was significantly higher than all other inoculation treatments except *Aspergillus niger* WL9S (257.4 mg) with which it was on par (Table 40a).

Irrespective of inoculation treatments, mean MBC was found to decrease with increase in soil salinity. At 30 DAS, maximum mean MBC was recorded at 2.4 dS/m (357.4 mg) which was however on par with 4.9 dS/m (336.4 mg) but was significantly superior over all other ECe levels (Table 40a). Almost similar trends were observed at 45 and 60 DAS. However, at harvest, maximum mean MBC recorded at 2.4 dS/m (306.0 mg) which was significantly superior over all other ECe levels. The interaction effect was found to be non-significant (Table 40a).

Per cent increase or decrease in MBC as influenced by inoculation of salt-tolerant AIMs at different soil ECe levels were calculated and depicted Fig. 20. Inoculation of salt-tolerant AIMs was found to increase the MBC content at all the soil ECe levels and at all crop growth stages. It was increased by 7.5 to 25.9, 7.4 to 25.6, 4.2 to 32.2, 6.3 to 34 and 11.5 to 39.1 per cent at soil ECe of 2.4, 4.9, 8.7, 11.2 and 14.3 dS m^{-1} respectively, over UIC at 60 DAS (Fig. 20).

4.5.5.2.2 Microbial biomass nitrogen (MBN)

Among the treatments, consortia recorded the highest mean MBN (60.8 and 67.6 mg/kg soil at 30 and 45 DAS respectively) which was significantly superior over rest of the treatments except *Aspergillus niger* WL9S (55.4 and 61.6 mg/kg soil at 30 and 60 DAS respectively) with which it was on par (Table 40b).

Table 40a. Microbial biomass C (mg kg soil⁻¹) content of soil under wheat as influenced by salt-tolerant AIMs at different soil salinity levels under field condition

Treatment	30 DAS						45 DAS					
	Soil ECe (dS m ⁻¹)					Mean	Soil ECe (dS m ⁻¹)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	374.3	342.8	295.2	261.7	249.0	304.6	396.7	377.3	325.9	301.3	277.9	335.8
<i>Azospirillum irakense</i> S173E	346.2	325.7	258.1	233.8	227.3	266.4	379.1	350.1	296.3	273.4	247.1	309.2
<i>Enterobacter cloacae</i> S125R	358.0	339.2	281.5	248.9	236.4	292.8	385.4	362.2	312.2	289.3	263.3	322.5
<i>Pseudomonas</i> sp. S4(1)S	334.1	317.3	249.4	229.2	213.1	268.6	362.1	335.8	285.1	257.9	238.8	295.9
<i>Aspergillus niger</i> WL9S	381.3	359.1	305.9	273.1	262.4	316.4	412.3	386.1	335.2	313.3	289.1	347.2
Consortia*	396.9	384.4	322.2	298.8	278.2	336.1	430.8	412.2	358.1	330.1	307.3	367.7
UIC	311.1	275.7	229.1	206.2	182.3	240.9	339.2	312.0	256.3	235.3	215.6	271.7
Mean	357.4	336.4	277.3	250.2	235.5		386.5	362.2	309.9	285.8	262.7	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	9.9		11.6		26.3		10.7		13.6		30.4	
CD (0.05)	26.7		32.1		NS		31.8		37.8		NS	

Treatment	60 DAS						At harvest					
	Soil ECe (dS m ⁻¹)					Mean	Soil ECe (dS m ⁻¹)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	432.3	408.3	342.1	326.7	307.0	363.3	318.8	295.2	239.0	216.3	176.0	249.1
<i>Azospirillum irakense</i> S173E	407.1	384.0	316.2	291.1	284.5	336.6	296.7	267.1	213.1	191.0	159.0	225.4
<i>Enterobacter cloacae</i> S125R	416.4	398.8	329.0	312.4	291.3	349.6	307.2	281.1	225.2	201.1	164.0	235.7
<i>Pseudomonas</i> sp. S4(1)S	399.2	378.3	301.1	285.1	271.4	327.0	283.3	259.1	202.4	186.4	145.3	215.3
<i>Aspergillus niger</i> WL9S	446.1	421.3	351.5	335.3	318.3	374.5	325.6	308.7	245.0	223.2	184.7	257.4
Consortia*	466.9	441.9	382.6	358.8	337.7	397.6	345.4	321.0	271.5	238.8	212.3	277.8
UIC	370.8	352.3	288.8	268.1	243.2	304.6	265.2	212.0	178.3	148.8	122.2	185.3
Mean	419.8	397.8	330.2	311.1	293.3		306.0	277.7	224.9	200.8	166.2	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	13.7		15.9		36.1		7.8		8.9		21.1	
CD (0.05)	37.6		44.7		NS		22.6		26.3		NS	

* *Enterobacter aerogenes* S63(1)R + *Azospirillum irakense* S173E + *Enterobacter cloacae* S125R + *Pseudomonas* sp. S4(1)S

SEm±: Standard error of means

CD: Critical difference

ECe: Electrical conductivity of the saturation paste extract

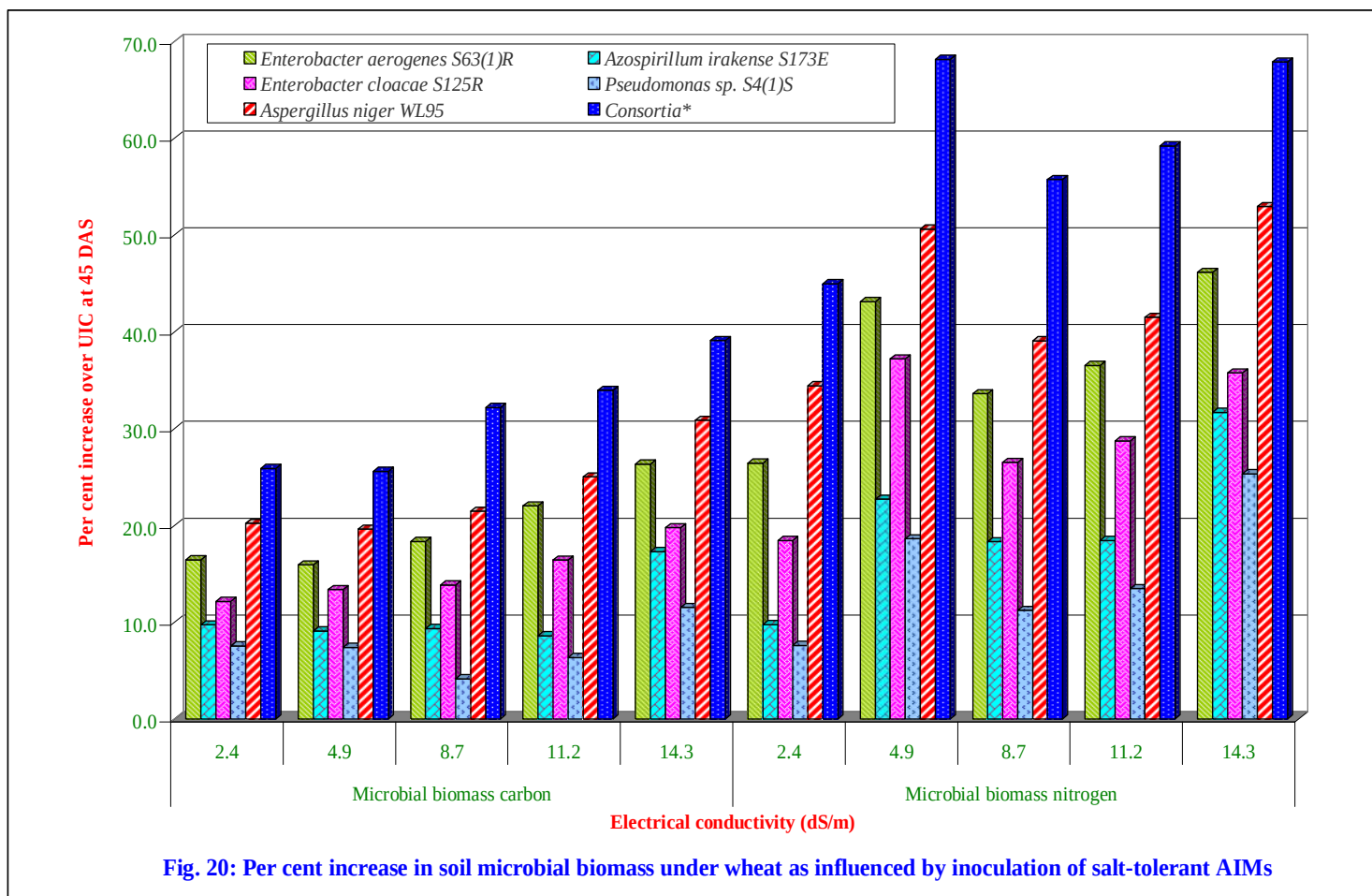


Fig. 20: Per cent increase in soil microbial biomass under wheat as influenced by inoculation of salt-tolerant AIMs

At 60 DAS, consortia again recorded the highest mean MBN content (74.9 mg/kg soil) which was significantly superior over most of the treatments but was on par with *Aspergillus niger* WL9S and *Enterobacter aerogenes* (68.0 and 64.6 mg/kg soil respectively). Similar trends were observed at harvest. Significantly low MBN contents were recorded in UIC at all the stages of crop growth (Table 40b).

In general, the MBN was found to decrease with increase in soil ECe levels. Among the soil salinity levels, the highest mean MBN contents (92.8, 103.4, 117.3 and 88.7 mg/kg soil at 30, 45, 60 DAS and at harvest respectively) were recorded at 2.4 dS/m which were significantly superior over rest of the soil ECe levels (Table 40b). The interaction effect was found to be non-significant (Table 40b).

The per cent increase or decrease in MBN as influenced by inoculation of salt-tolerant AIMS at different soil ECe levels were calculated and depicted in Fig.20. Inoculation of salt-tolerant AIMS was found to increase the MBN content at all the soil ECe levels and all crop growth stages. The inoculation of salt-tolerant AIMS was found to increase the soil MBN content by 7.6 to 45, 18.6 to 68.2, 11.2 to 55.7, 13.5 to 59.2 and 25.3 to 67.9 per cent at a soil ECe of 2.4, 4.9, 8.7, 11.2 and 14.3 dS m⁻¹ respectively at 60 DAS (Fig. 20).

4.6 Genes encoding tolerance/ resistance to salt concentration

A total of 21 salt-tolerant strains consisting of 6 each of free-living N₂ fixers, PSB and FP and 3 associative N₂ fixers, were examined for the presence *proBA*, *ectABC*, *otsAB* and *gbsTIBA* gene clusters encoding for biosynthesis of proline, ectoine, trehalose and glycine-betaine respectively, which were known to have role in salt-tolerance. A 16S rRNA gene was used as a positive control for all the strains under test (Table 41).

Genomic DNA of all the salt-tolerant AIMS as well as one salt-sensitive strain (ASPSBIV S61R) were isolated and examined for the presence of genes responsible for biosynthesis of osmoprotectants.

proBA

All the salt-tolerant AIMS including susceptible strain were found to exhibit the presence of *proBA* gene cluster. An expected amplicon size (1100 bp) was detected in all the strains which indicates the presence of gene responsible for proline biosynthesis (Plate 5).

ectABC

Out of 21 salt-tolerant AIMS examined, only 12 strains (6 FP, 4 PSB and 2 free-living N₂ fixers) were found positive for the presence of *ectABC* gene cluster while remaining salt-tolerant AIMS including susceptible strain did not show any amplification. An expected size of amplicon (750 bp) was detected in all the 12 positive isolates as evidenced by the gel images documented for the *ectABC* gene cluster (Table 41 and Plate 5).

otsAB

Salt-tolerant AIMS were also examined for the presence of gene responsible for trehalose biosynthesis. The products of the PCR amplification using gene specific degenerate primers were run on one per cent agarose gel and observed for the expected amplicon size (Plate 5). Gel images documented for *otsAB* gene cluster revealed that, only eighteen salt-tolerant AIMS showed the presence of expected amplicon (650 bp) but was not detected in three salt-tolerant AIMS viz., ASPSBXI S156R, ASPSBXI S189E and ASFPV Dr16E as well as in susceptible strain.

gbsTIBA

Gel images obtained from the PCR amplification of *gbsTIBA* revealed that, an expected amplicon size (750 bp) was detected in all the salt-tolerant AIMS but not in susceptible strain which indicated the presence of glycine-betaine biosynthetic gene in salt-tolerant AIMS (Table 41 and Plate 5).

Table 40b. Microbial biomass N (mg kg of soil⁻¹) content of soil under wheat as influenced by salt-tolerant AIMs at different soil salinity levels under field condition

Treatment	30 DAS						45 DAS					
	Soil ECe (dS m ⁻¹)					Mean	Soil ECe (dS m ⁻¹)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	98.4	66.0	41.0	30.5	25.9	52.4	110.3	73.9	45.9	35.4	29.3	59.0
<i>Azospirillum irakense</i> S173E	86.5	57.2	34.9	26.3	22.7	45.5	97.2	62.5	40.5	30.7	24.7	51.1
<i>Enterobacter cloacae</i> S125R	91.8	64.0	39.0	28.6	24.1	49.5	101.3	68.3	43.3	33.2	27.1	54.7
<i>Pseudomonas</i> sp. S4(1)S	83.5	53.7	33.2	24.9	21.1	43.3	92.8	58.9	38.5	28.7	23.9	48.6
<i>Aspergillus niger</i> WL9S	103.0	70.4	43.7	32.1	27.6	55.4	114.4	77.2	48.6	37.3	30.7	61.6
Consortia*	110.3	80.0	46.7	36.9	30.2	60.8	123.1	87.7	52.6	40.7	33.7	67.6
UIC	75.9	42.5	28.6	21.5	16.1	36.9	84.8	48.8	32.0	24.5	19.1	41.8
Mean	92.8	62.0	38.2	28.7	24.0		103.4	68.2	43.1	32.9	26.9	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	2.1		2.5		5.5		2.4		2.8		6.3	
CD (0.05)	5.9		7.0		NS		6.7		7.9		NS	

Treatment	60 DAS						At harvest					
	Soil ECe (dS m ⁻¹)					Mean	Soil ECe (dS m ⁻¹)					Mean
	2.4	4.9	8.7	11.2	14.3		2.4	4.9	8.7	11.2	14.3	
<i>Enterobacter aerogenes</i> S63(1)R	123.4	80.0	48.9	38.5	32.3	64.6	93.8	60.2	34.6	25.7	18.9	46.7
<i>Azospirillum irakense</i> S173E	107.1	68.6	43.3	33.4	29.1	56.3	82.5	49.4	30.0	22.0	16.4	40.1
<i>Enterobacter cloacae</i> S125R	115.6	76.7	46.3	36.3	30.0	61.0	87.7	55.1	32.1	23.6	17.3	43.2
<i>Pseudomonas</i> sp. S4(1)S	105.0	66.3	40.7	32.0	27.7	54.3	78.6	47.1	28.1	21.1	15.1	38.0
<i>Aspergillus niger</i> WL9S	131.2	84.2	50.9	39.9	33.8	68.0	98.8	64.4	36.0	27.2	20.1	49.3
Consortia*	141.5	94.0	57.0	44.9	37.1	74.9	107.8	71.3	41.1	30.3	23.8	54.9
UIC	97.6	55.9	36.6	28.2	22.1	48.1	71.6	34.2	22.8	15.9	11.3	31.2
Mean	117.3	75.1	46.2	36.2	30.3		88.7	54.5	32.1	23.7	17.6	
	Soil ECe (E)		Strain (S)		E x S		Soil ECe (E)		Strain (S)		E x S	
SEm±	3.1		3.6		8.1		2.4		2.9		6.5	
CD (0.05)	8.7		10.3		NS		6.9		8.2		NS	

* *Enterobacter aerogenes* S63(1)R + *Azospirillum irakense* S173E + *Enterobacter cloacae* S125R + *Pseudomonas* sp. S4(1)S

SEm±: Standard error of means

CD: Critical difference

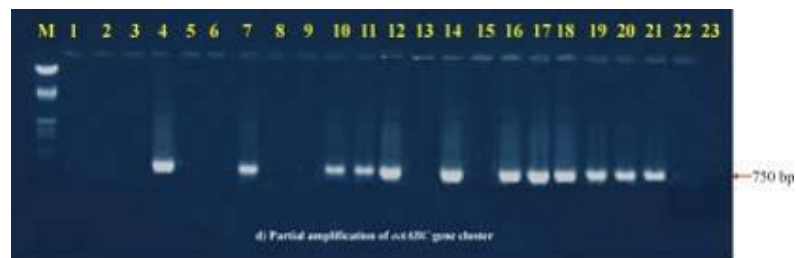
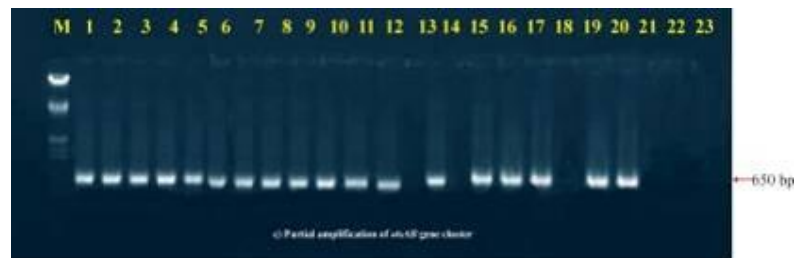
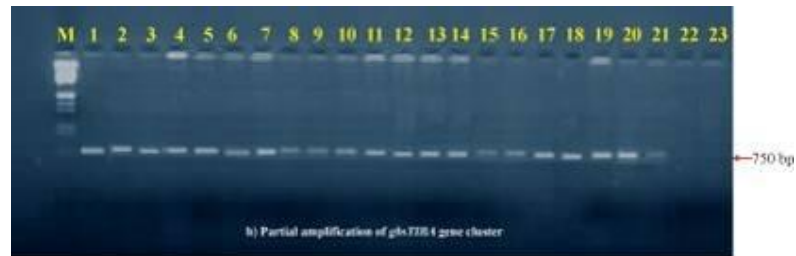
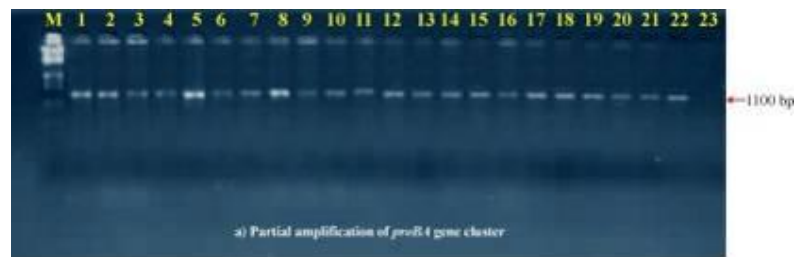
ECe: Electrical conductivity of the saturation paste extract

Table 41. Profile of gene cluster encoding salt-tolerance in salt-tolerant isolates

Isolate code	Identity	Salt tolerance (Max. % NaCl)	Presence or absence of gene cluster			
			<i>proBA</i>	<i>gbsTIBA</i>	<i>otsAB</i>	<i>ectABC</i>
ASANFX S145E	<i>Azospirillum irakense</i>	10	+	+	+	-
ASANFX S148E	<i>Azospirillum brasilense</i>	10	+	+	+	-
ASANFXI S173E	<i>Azospirillum irakense</i>	10	+	+	+	-
ASFNFIV S63(1)R	<i>Enterobacter aerogenes</i>	15	+	+	+	+
ASFNFIV S75(1)R	<i>Klebsiella pneumoniae</i>	12.5	+	+	+	-
ASFNFIV S37R	<i>Klebsiella oxytoca</i>	10	+	+	+	-
ASFNFIV WL4(1)R	<i>Enterobacter aerogenes</i>	10	+	+	+	+
ASFNFI Dr4(1)	<i>Klebsiella</i> sp.	10	+	+	+	-
ASFNFIV S45(1)R	<i>Klebsiella oxytoca</i>	10	+	+	+	-
ASPSBIX S122R	<i>Bacillus endophyticus</i>	15	+	+	+	+
ASPSBIX S124E	<i>Bacillus subtilis</i>	15	+	+	+	+
ASPSBIX S125R	<i>Enterobacter cloacae</i>	15	+	+	+	+
ASPSBXI S156R	<i>Staphylococcus</i> sp.	15	+	+	-	-
ASPSBXI S181R	<i>Bacillus subtilis</i>	15	+	+	+	+
ASPSBXI S189E	<i>Staphylococcus</i> sp.	15	+	+	-	-
ASFPI S3(1)S	<i>Pseudomonas putida</i>	17.5	+	+	+	+
ASFPI S4(1)S	<i>Pseudomonas</i> sp.	17.5	+	+	+	+
ASFPI S6(1)S	<i>Pseudomonas aeruginosa</i>	17.5	+	+	+	+
ASFPV Dr16E	<i>Pseudomonas chlororaphis</i> subsp. <i>aureofaciens</i>	17.5	+	+	-	+
ASFPV S94(2)E	<i>Pseudomonas putida</i>	17.5	+	+	+	+
ASFPV S100E	<i>Pseudomonas</i> sp.	17.5	+	+	+	+
ASPSBIV S61R	<i>Pseudomonas</i>	0	+	-	-	-

+: Present

-: Absent



M = Marker (1 kb+ ladder)	7) AATNIV 9147R	13) AATNIV 9148R	19) AATNIV 9149R
1) AATNIV 9147R	8) AATNIV 9148R	14) AATNIV 9149R	20) AATNIV 9150R
2) AATNIV 9148R	9) AATNIV 9149R	15) AATNIV 9150R	21) AATNIV 9151R
3) AATNIV 9149R	10) AATNIV 9150R	16) AATNIV 9151R	22) AATNIV 9152R
4) AATNIV 9150R	11) AATNIV 9151R	17) AATNIV 9152R	(salt sensitive)
5) AATNIV 9151R	12) AATNIV 9152R	18) AATNIV 9153R	23) NaCl (negative control)

Pate 5. PCR amplification of genes encoding for salt tolerance in the salt- tolerant AIMS of the present study

5. DISCUSSION

Plants face various biotic and abiotic stresses in adverse environmental conditions. Abiotic stress is a complex process against which signals the cells to adapt themselves at the molecular, biochemical and physiological levels. Among abiotic stresses, salinity causes osmotic stress and reduction in plant growth and crop productivity in irrigated areas of arid and semiarid regions (Cicek and Cakirlar, 2002).

Salt stress affects many aspects of plant metabolism and, as a result, the growth and yield are reduced. Excess salt in the soil solution may adversely affect plant growth through either osmotic inhibition of water uptake by roots or specific ion effects. Specific ion effects may cause direct toxicity or alternatively the insolubility or competitive absorption of ions may affect the plant's nutritional balance. Salinity was shown to increase the uptake of Na^+ or decrease the uptake of Ca^{2+} and K^+ (Yildirim *et al.*, 2006). Accumulation of excess Na^+ may cause metabolic disturbances in processes where low Na^+ and high K^+ or Ca^{2+} are required for optimum function. Uptake and accumulation of Cl^- may disrupt photosynthetic function through the inhibition of nitrate reductase activity (Xu *et al.*, 2000).

In addition to the use of traditional breeding and transgenic plants, the utilization of plant growth-promoting rhizobacteria (PGPR) is useful in strategies to facilitate plant growth in saline soils. PGPR are beneficial soil microorganisms which are known to facilitate plant growth both directly and indirectly.

Direct stimulation may include providing plants with fixed nitrogen, phytohormones, iron chelators and soluble phosphate, while indirect stimulation of plant growth includes preventing phytopathogens (biocontrol). Harsh conditions in the field affect the expression of many microbial traits (Patel *et al.*, 2012). Hence, application of PGPR strains that are tolerant to such harsh abiotic stresses is necessary.

In this context, microbial strains isolated from the stress ecosystems comprising saline, arid and waterlogged soils of the Karnataka state (Anonymous, 2008) were analyzed for their possible role in alleviation of salt stress.

A total of 195 strains tolerating high salt concentration (5 to 17.5% NaCl) were found to perform multiple functions in the presence of different salt concentrations. Thus, attempts were made to explore the dual properties *viz.*, agriculturally important functions and salt tolerance of these isolates to analyze their potential to improve the plant growth and development of wheat in saline soil.

5.1 Identification of salt-tolerant AIMS

Human beings appear to have an innate need to name things. Naming helps us to understand our world and to communicate with others about it. In science, accurate and standardized names are essential. All chemists must mean the same thing when they talk about an element or a compound; physicists must agree on terms when they discuss matter or energy; and biologists must agree on the names of organisms, be they tigers or bacteria. Since the days of Koch and Pasteur, bacteria were mainly classified according to morphological, physiological and biochemical criteria.

These properties are usually counted equally and bacteria are grouped together as to how many of these features they have in common. Faced with the great number and diversity of organisms, biologists use the characteristics of different organisms to describe specific forms of life and to identify new ones. The grouping of related organisms together is the basis of classification. The most obvious reasons for classification are (1) to establish the criteria for identifying organisms (2) to arrange related organisms into groups and (3) to provide important information on how organisms evolved.

Taxonomy is the science of classification. It provides an orderly basis for the naming of organisms and for placing organisms into a category, or taxon. This classical approach is also known as numerical taxonomy and the most widely used treatise in bacterial classification Bergey's Manual of Determinative Bacteriology depends heavily on numerical taxonomy (<http://www.wiley.com/college/black>). On these lines attempts were made in the present study to identify the salt-tolerant agriculturally important microorganisms.

All the isolates exhibited wide variation in colony size, shape, physiological and biochemical characteristics. Based on the above observations, the preliminary identity comprising 16 genera was assigned to all the 195 isolates and they classified into four bacterial groups of the Bergey's manual of determinative bacteriology (BMDB), namely, Group 4 (Gram negative, aerobic/ microaerophilic rods and cocci) and group 5 (facultative anaerobic gram negative rods) of Category I and group 17 (gram positive cocci) and 18 (endospore forming gram positive rods and cocci) of category II. Majority of the isolates belonged to group 4 (Gram negative, aerobic/ microaerophilic rods and cocci) of BMDB comprising of *Pseudomonas*, *Azotobacter* and *Azospirillum* genera. The results indicated that these groups predominates the rhizosphere and plant surfaces.

One hundred fourty were identified upto species level (23 as *Pseudomonas aeruginosa*, 18 as *Azotobacter chroococcum*, 16 as *Azospirillum irakense*, 15 as *Pseudomonas fluorescens*, 12 as *Azospirillum brasilense*, 11 each as *Pseudomonas aureofaciens* and *Azotobacter vinelandii*; 10 as *Azotobacter paspali*, eight as *Azospirillum halopraeferens*, seven as *Pseudomonas pseudomallei*; four as *Azotobacter nigricans*, three as *Pseudomonas mendocina* and one each as *P. putida* and *P. vesicularis*, while, 55 were tentatively identified only upto genus level (17 as *Bacillus*, 14 as *Pseudomonas*, five as *Staphylococcus*, four as *Aminobacter*, three as *Escherichia*, two each as *Glucanobacter*, *Acetobacter* and *Enterobacter* and one each as *Alcaligenes*, *Flavimonas*, *Rhizomonas*, *Rhizobacter*, *Xanthobacter* and *Xanthomonas*).

The above observations were also evidenced by evolutionary distances calculated for each functional group. All the isolates have been categorized into four functional groups to analyze their evolutionary relationship. The group specific phylogenetic tree for associative N₂ fixers (ANF), free-living N₂ fixers (FNF), P-solubilizing bacteria (PSB) and fluorescent pseudomonads (FP) constructed using 61, 55, 43 and 36 strains respectively confirmed their diversity.

For examples, wide variation in the evolutionary distances among the associative nitrogen fixers was noticed as evidenced by a minimum distance of 0.18 and maximum of 0.36. Similarly, all the ANF isolates exhibited variation with a minimum evolutionary distance of 0.11 and maximum of 0.44. Like wise, PSB isolates also recorded wide variation. The maximum variation was observed with an evolutionary distance of 0.38 and minimum with 0.08. While FP isolates showed lot of variation as evidenced by evolutionary distances calculated in which maximum distance of 0.48 and minimum of 0.12 were recorded.

Several scientists characterized the rhizobacterial strains using the numerical taxonomy. In one of the study, six phosphate solubilizing bacteria (PSB) were isolated from paddy fields and identified the taxonomic delineation employing morphological, biochemical, 16S rRNA gene sequences and phylogenetic affiliations and results suggested that they belonged to members of *Enterobacter* and *Exiguobacterium* genera (Kumar *et al.*, 2010).

More recently, however, it has become apparent that many of these original criteria of numerical taxonomy have little bearing at all in defining a particular taxonomic group; this includes important properties such as cell shape, photosynthesis and to a lesser extent gram staining. Due to these shortcomings and the paucity in diversity within alternative techniques, scientists were for a long time interested in the development of more useful classification tools. Recent developments in molecular genetics and gene cloning provided exactly these tools giving rise to the new era of molecular taxonomy where a wealth of data can readily be obtained from any microorganism. Ideally one could just sequence the entire genome of every organism and compare them with each other. This, however, is not a practical option at this time. It takes a lot of hard work and time to sequence just one genome. Several faster and easier techniques for estimating genetic homology are available. Similarities in DNA can be studied directly by determining the base composition of the DNA, by sequencing the bases in portions of DNA or RNA and by using DNA hybridization.

Currently, several molecular based methodologies are being used to identify microorganisms. One such method is sequencing of the 16S rRNA gene. The genomes of all bacterial organisms contain this highly conserved gene. Variability within this region is unique and specific to each species (Woese *et al.*, 1985). Microbial identity of salt-tolerant AIMs was recorded through 16S rRNA gene sequencing followed by basic local search alignment tool (BLAST) analysis.

Analysis of the 16S rRNA sequences of 91 representative bacterial isolates belonging to four functional groups (18 each of ANF and FNF; 28 PSB and 27 FP) and ITS sequence of a fungal strain WL9S revealed that, the sequence of each strain was found to have a homology of >99 per cent with that of the reported sequences in the NCBI database.

All the isolates represented any of the six family viz., Rhodospirillaceae and Rhizobiaceae of the Class α -Proteobacteria, Pseudomonadaceae and Enterobacteriaceae of Class γ -Proteobacteria and, Bacillaceae and Staphylococcaceae of Phylum Firmicutes (Class-Bacilli). Of the 91 isolates, 69 were grouped in Phyla Proteobacteria while 22 in Firmicutes. Sensitivity and specificity of 16S rRNA gene sequencing has been proved in the present study wherein fourteen salt-tolerant AIMs were identified only upto genus level indicating their uniqueness as species level.

Majority of the isolates identified using 16S rRNA sequencing represented the bacterial class γ -Proteobacteria, followed by Bacilli and α -Proteobacteria. For example, of the 61 salt-tolerant strains representing the class γ -Proteobacteria, 12 strains were identified as *Pseudomonas nitroreducens*, 16 as *Pseudomonas aeruginosa*, 7 as *Pseudomonas* sp., six each as *Azotobacter* (3 as *A. vinelandii*, 2 as *A. chroococcum* and one as *A. salinestris*) and *Enterobacter* (five as *E. aerogenes* and one as *E. oyzae*), four as *Klebsiella* (*K. oxytoca* and one each as *K. pneumoniae* and *Klebsiella* sp.), three each as *Pseudomonas putida* and *P. chlororaphis* sub sp. *aureofasciens*, two as *Enterobacter cloacae* and one each as *Pseudomonas monteilli* and *P. fluorescens*. In case of Firmicutes, 17 strains were identified as *Bacillus* (9 as *B. endophyticus*, 5 as *B. megaterium* and 2 as *B. subtilis*) and five as *Staphylococcus* sp. Out of the eight salt-tolerant strains representing α -Proteobacteria, six were identified as *Azospirillum* (3 as *A. irakense* and 1 each as *A. brasilense*, *A. halopraeferens* and *Azospirillum* sp.) and 2 as *Agrobacterium* (*A. tumefaciens*).

Sequencing the 16S rRNA gene has proven to be a very sensitive and specific method, although care must be taken to evaluate the accuracy of the sequence used to determine identification. Molecular phylogeny increasingly supports our understanding to the organism relationships and provides the basis for the conventional identification techniques (Singh *et al.*, 2007). The sequence analysis of the genes encoding rRNA, usually 16S rRNA, has been well-established as a standard procedure for the identification of bacteria at the levels of species, genera and family (Gurtler and Mayall, 2001). Recently, the strain YCWA18 which can survive at high concentrations of NaCl (up to 20%) and pH range of 4.0-10.0 was isolated and identified as *Kushneria* sp. according to its 16S rRNA gene sequence (Zhu *et al.*, 2011).

In the present study, salt-tolerant isolates were identified using conventional and molecular methods. It was observed that, conventional microbiological methods adequately identified salt-tolerant fluorescent pseudomonads and P-solubilizers. While, identity of N_2 fixers was found to vary between the conventional and molecular method employed. Out of 91 salt-tolerant AIMs, preliminary identity of 22 isolates was cent per cent conformity with their identity using 16S rRNA sequencing up to species level whereas, preliminary identity of 45 isolates was exactly similar only up to genera level to that of their identity using 16S rRNA sequencing. Significant differences were noticed between the two identification methods wherein identity of 24 isolates did not match with each of the method adopted. The 16S rRNA gene sequencing method was found to be substantially more accurate for bacterial identification when compared to conventional methods (Morgan *et al.*, 2009). 16S rRNA sequence analysis was particularly useful in identification of salt-tolerant N_2 fixers since diverse groups of bacteria were found to possess the property of N_2 fixation. Similar findings on comparative study were also reported by Morgan *et al.* (2009).

5.2 Functional characterization of salt-tolerant AIMs under salt-stress

Stress can be detrimental for sensitive microorganisms and decrease the activity of surviving cells, due to the metabolic load imposed by the need for stress tolerance mechanisms. Soil salinity is a stress factor relating to microbial selection process and can reduce bacterial diversity and control microbial abundance, composition and functions (Ibekwe *et al.*, 2010).

The analysis of functional variability indicated that, all the strains were found to produce phytohormones but differed in other functions like dinitrogen fixation, phosphate solubilization and biocontrol of plant pathogens. Out of 195 isolates, 79 exhibited dinitrogen fixation, 78 showed phosphate solubilization and only 18 isolates were found to have biocontrol activity against one or the other plant pathogens tested.

Biological nitrogen fixation is an energy demanding process and is subjected to elaborate controls in all the diazotrophs. It is catalyzed by the nitrogenase complex, which consists of dinitrogenase reductase (also known as Fe protein, encoded by *nifH*) and dinitrogenase (also known as Mo-Fe protein, encoded by *nifDK*). The former transfers electrons from low potential electron donors to the dinitrogenase, which then reduces dinitrogen to ammonia. Both free-living and associative diazotrophs are equipped with an effective system of control of nitrogen fixation in response to environmental conditions (Tripathi and Klingmueller, 1992), particularly at the level of synthesis and activity of nitrogenase (Tripathi *et al.*, 2002).

The present study revealed the existence of an effective nitrogen fixation system in salt-tolerant N₂ fixing isolates. However, large differences in the quantity of N₂ fixation were noticed among the N₂ fixing isolates. Of the 79 isolates, 43 were free-living N₂ fixers and 36 associative N₂ fixers. Variation in the type of N₂ fixation was later confirmed with their molecular identity in eighteen each of the high salt-tolerant free-living and associative N₂ fixing isolates using 16S rRNA sequencing. Free-living N₂ fixing strains tolerating higher NaCl concentration represented the genera *Azotobacter*, *Enterobacter*, *Klebsiella* and *Agrobacterium*. Similarly, out of 18 high salt-tolerant associative N₂ fixers, only six were identified as *Azospirillum* while twelve as *Pseudomonas nitroreducens*.

The presence of associative nitrogen fixation in twelve *Pseudomonas nitroreducens* strains, when most other *Pseudomonas* species lack this property, raises the question of their origin. The nitrogen fixation process is believed to have played a crucial role in early cellular evolution, in particular when the geochemical reserves of fixed nitrogen in the biosphere became depleted. Because nitrogen fixation is maintained in bacteria and archaea, it is often hypothesized that *nif* genes may originate from the last common ancestor, even though other scenarios are proposed (Fani *et al.*, 2000 and Raymond *et al.*, 2004). It can be presumed that *nif* genes were lost in most lineages of bacteria and archaea and that lateral gene transfer played a role in recent acquisition of *nif* genes in some lineages (Kechris *et al.*, 2006). The rareness of nitrogen fixation properties in true *Pseudomonas* suggests that *nif* genes were lost during evolution in most (if not all) *Pseudomonas* species. Similar results were reported from China by You and Zhou (1989) wherein they observed the property of microaerophilic N₂ fixation in two strains of *Pseudomonas stutzeri* namely CMT9A and A15 isolated from the roots of sorghum and rice respectively.

Wide variation in N₂ fixing ability among the isolates and between NaCl concentrations was noticed. The amount of N₂ fixation in general decreased with increase in NaCl concentration. Irrespective of isolates, the mean amount of N₂ fixation by associative N₂ fixers was reduced by nearly 18.8 per cent with increase in NaCl concentration up to 7.5 per cent. However, three isolates viz., ASANFX S145E, S148E and S173E retained their property with increase in medium NaCl concentration up to 10 per cent and the rate of N₂ fixation was reduced by 24.0, 31.2 and 36.8 per cent respectively as compared to N₂ fixation under normal semi-solid medium. The N₂ fixing ability of free-living N₂ fixers was also decreased by 16.1, 58.8 and 78.9 per cent at NaCl concentrations of 5.0, 7.5 and 10 per cent respectively over the amount of N₂ fixed in normal medium.

It was clearly observed that, the medium NaCl concentration in the medium affected the functional properties quantitatively. It can be attributed to the fact that, nitrogenase synthesis is inhibited by a variety of salts. Also increase in NaCl concentration results in decrease in the cell population and initiation of osmoadaptive mechanisms thereby decreasing the quantity of N₂ fixed. It is also evidenced by the research findings of Deits and Howard (1990) on nitrogenase catalysis in *Azotobacter vinelandii*. According to them, salinity has at least three levels of effects: a decrease in the apparent binding constant for nucleotides; the abolition of enzyme turnover; and the inhibition of Mg-ATP dependent iron chelation. The large effect of NaCl on the rate of Mg-ATP dependent iron chelation unequivocally establishes one site of salt interaction as the Fe-protein.

They have further emphasized that Fe:S cluster reactivity is a sensitive probe of the state of the Fe-protein and of the nitrogenase complex under conditions that do not require high protein concentrations or cryogenic temperatures. Conversely, only minimal changes induced by NaCl have been detected in the Fe:S cluster when the protein was studied by spectral methods.

It is also established that microorganisms exposed to salinity stress deviate cellular energy towards osmoregulation, especially Na⁺ efflux and hence inhibits nitrogen fixation and synthesis of nitrogenase by the isolates (Barua *et al.*, 2011). Similar study conducted by Dhanraj *et al.* (2012) noticed the loss of nitrogenase activity in dinitrogen fixing bacterial strain *Azotobacter chroococcum* N-21. The isolate though tolerated 16 per cent NaCl but could not fix elemental nitrogen beyond 4 per cent NaCl.

Another important function of agriculturally important microorganisms is mineral phosphate solubilization. Phosphate-solubilizing microorganisms are known to improve solubilization of fixed soil phosphorus and applied insoluble phosphates, thereby improving crop growth and yield. Phosphate solubilizing rhizobacteria are also confronted with various environmental stresses in natural habitats like soil. The ability to withstand the adverse environmental conditions such as high salinity, high/low pH and high temperature is significant not only for rhizobacterial survival in agricultural soils but also to involve in nutrient recycling..

In the present investigation, 81 salt-tolerant AIMs (78 bacterial and 3 fungal isolates) were found to exhibit P-solubilization. In general, the amount of Pi released from TCP was found to increase with increase in NaCl concentrations up to 7.5 per cent and declined thereafter. While, the isolate ASPSBIX S122R showed increase in per cent Pi release till 12.5 per cent NaCl but decreased thereafter.

The above results were similar to other findings in which P-solubilization though recorded at various NaCl concentrations viz., one (Kim *et al.*, 1997), five (Johri *et al.*, 1999 and Srinivasan *et al.*, 2012) and ten per cent (Nautiyal, 1999) but in general found to decrease with the increase in sodium chloride concentration (Zhu *et al.*, 2011). This might be due to two stresses at the same time which may harm cell growth and proliferation resulting in less efficiency of solubilization or it is possible that accelerated chloride levels may chelate or neutralize proton ions or acid produced in the media (Gaiind and Gaur, 1999). The researchers also mentioned that, third possible reason could be accumulation of specific solutes (Gaiind and Gaur, 1999).

One of the possible effects of salinity on plant growth inhibition is increased susceptibility of the plant towards phytopathogens (Werner and Finkelstein, 1995). In the present study, eighteen salt-tolerant fluorescent pseudomonad isolates exhibited inhibitory activity against one or the other plant pathogen tested. Out of these 18 antagonistic isolates, 6 were inhibitory to *Alternaria carthami*, 17 to *Fusarium oxysporum* f. sp. *carthami*, 11 to *Sclerotium rolfsii*, 5 to *Rhizoctonia bataticola* and 11 to *Pyricularia oryzae*. There was significant variation in the antagonistic activity of isolates. While, five isolates [ASFPV S94(2)E, ASFPIX S134(3)S, ASFPXI S158(1)R, ASFPXI S174(1)R and ASFPXI S187S] showed inhibition potential against all the five pathogens tested, two isolates were inhibitory to three pathogens each and 8 inhibited two pathogens each.

Suppression of plant pathogens by fluorescent pseudomonads could be due to existence of various biocontrol mechanisms in these strains. Mechanisms include direct antagonism, production of several metabolites with antimicrobial activity toward fungi and cell wall degrading enzymes (Haas and Keel, 2003). Antibiotics produced by *Pseudomonas chlororaphis* is reported to inhibit both mycelial growth and conidial germination of *Helminthosporium solani* (Martinez *et al.*, 2006). Several pathogenic fungi such as *F. solani*, *Gaeumannomyces graminis* pv. *tritici*, *Pythium ultimum*, *Alternaria alternate* and *Botrytis cinerea* were inhibited by *P. chlororaphis* TSAU 13 suggesting that antibiosis is a contributor to the antagonistic effect of these bacteria through the diffusion of antifungal compounds into the medium (Egamberdieva, 2012). Similar results were also reported by Validov *et al.* (2009) wherein *Pseudomonas putida* strain PCL1760 showed significant biological control of tomato foot and root rot, a disease caused by *Fusarium oxysporum* f. sp. *radicis lycopersici* (Forl). Thus the isolates herein can be very good source for development of biocontrol aspects especially in saline areas.

Most of the AIMs improve plant growth through N₂ fixation/ P-solubilization/ anti-phytopathogenic activity and/ or production of plant hormones. PGPR can alter root architecture and promote plant development with the production of different phytohormones like indole 3-acetic acid (IAA), gibberellic acid (GA) and cytokinins (Kloepper *et al.*, 2007). Several PGPRs as well as some pathogenic, symbiotic and free living rhizobacterial species are reported to produce IAA and GA in the rhizospheric soil and thereby play a significant role in increasing the root surface area and number of root tips in many plants (Han *et al.*, 2005). Reports describing factors that alter the level of phytohormone biosynthesis and/or the expression of biosynthetic genes in bacteria are numerous (Kloepper *et al.*, 2007). However, it is not yet possible to integrate these factors into a comprehensive regulatory scheme of phytohormone biosynthesis in bacteria. A first class of factors influencing IAA biosynthesis in diverse bacteria is related to environmental stress, including acidic pH, osmotic and matrix stress and carbon limitation (Spaepen *et al.*, 2007).

Several authors have addressed the importance of phytohormones and their role in plant growth promotion under different extreme stress conditions like salinity, alkalinity, sodicity, drought, flooding etc (Sachdev *et al.*, 2009; Upadhyay *et al.*, 2009 and Sadeghi *et al.*, 2012). In the present study, addition of NaCl had a positive impact on IAA and GA production whereas it had negative impact on cytokinin. The mean amount of IAA produced by salt-tolerant ANF, FP and PSB isolates was increased by 11.6, 82.9 and 22.8 per cent with increase in NaCl concentration up to 5, 7.5 and 10 per cent respectively and declined thereafter. Whereas the mean amount of IAA produced by salt-tolerant FNF isolates was found to decrease with increase in NaCl concentration.

Similarly, the mean amount of GA produced by the ANF and FNF isolates was decreased with increase in NaCl concentration. Whereas, the GA producing ability of FP and PSB isolates was increased by 76.3 and 41.9 per cent with increase in NaCl concentration up to 7.5 and 10 per cent and declined thereafter. However, salinity adversely affected the cytokinin producing ability of the salt-tolerant AIMs. The amount of cytokinin produced by the salt-tolerant AIMs was reduced by 21.4 and 75.2 per cent in the presence of 5 and 10 per cent NaCl respectively as compared to that of control medium. Increased production of IAA and GA by the salt-tolerant AIMs might be due to use of indole and related compounds as one of the strategy by the bacterial cells to overcome the salt stress. While decline in IAA and GA production at 15 and 17.5 per cent NaCl can be attributed to decline in population of the bacteria and initiation of other osmoadaptation mechanisms.

Similar observations were made by Sachdev *et al.* (2009) who isolated *Klebsiella* strains and demonstrated IAA production *in vitro*. *Klebsiella* strains were able to grow and produce IAA at varied NaCl concentrations (0-5% w/v). One strain K8 showed maximum IAA production of 21.9 µg per ml at 0.5 per cent NaCl and minimum of 11.2 µg per ml in absence of salt in the medium. Similarly, Sadeghi *et al.* (2012) studied the effect of graded concentration of NaCl (0, 100, 200, 300, mM NaCl) on the IAA biosynthesis by *Streptomyces* and reported gradual increase in IAA production with increase in salt concentration. The amount of IAA production was increased from 2.4 to 4.7 µg per ml with addition of 300 mM NaCl.

5.3 Biochemical basis for salt tolerance in AIMs

Abiotic stress tolerance in bacteria has been studied to provide a biological understanding of the adaptation and survival of living microorganisms in extreme environments. Bacteria adapt to the fluctuations in soil salinity and osmolarity by either modulating the composition of the cell envelope (Lopez *et al.*, 2000) or accumulating osmolytes such as potassium ions, glycine betaine, proline, proline betaine, trehalose, ectoines, *etc.* The adaptation of PGPRs to osmotic stress is of great significance, because soil salinity inhibits many of the vital plant growth promoting activities of bacteria, such as nitrogen fixation, mineral phosphate solubilization and phytohormone production (Miller and Woods, 1996).

Possible strategies of salt-tolerant AIMs to counteract the ill effects of salt stress include accumulation of compatible solutes, sugar and exopolysaccharide (EPS) production, adjustment of intracellular Na to K ratio. In the present study, all the three osmoadaptive mechanisms were in operation to overcome the effect of salt stress in the growth medium.

In general, increase in medium salt concentration resulted in enhanced accumulation of compatible solutes, EPS production and total sugars and increased intracellular Na/K ratio by the salt-tolerant AIMs. Isolates exhibited differential response within each of the NaCl concentration tested. They were found to vary in the amount and type of osmoprotectants accumulated. It could be due to variation in the mechanisms adapted by isolates under test.

In the first strategy of osmoadaptation, salt-tolerant AIMs were examined for two compatible solutes such as proline and glycine-betaine. Isolates recorded significantly lower proline in control medium (without any added salts) but increased its accumulation significantly with increase in salt concentration. Proline content of salt-tolerant fluorescent pseudomonads was increased by 137.3, 213.3, 220.0, 336.1, 364.3 and 49.3 per cent over control medium in the presence of 5, 7.5, 10, 12.5, 15 and 17.5 per cent NaCl respectively. Similar increasing trends were observed in other groups as well. Results of the study demonstrated that, salt-tolerant AIMs might have used proline accumulation as one of the main strategy to counteract the higher salt stress at a NaCl concentration over and above 10 per cent besides accumulating proline at a slower rate at lower salt concentrations up to 10 per cent NaCl. Isolates also exhibited the accumulation of glycine-betaine under salt stress. The glycine betaine content of salt-tolerant FP was increased by 142.8, 428.6, 614.3, 1328.5, 614.3 and 214.3 per cent over control medium in the presence of 5, 7.5, 10, 12.5, 15 and 17.5 per cent NaCl respectively. Similar increasing trends were observed in other groups as well. In this case, salt-tolerant AIMs exhibited sudden increase in glycine-betaine accumulation and used as a major strategy to counter balance the effect of salt concentration between 10 to 15 per cent NaCl.

The second strategy used by salt-tolerant AIMs was enhanced synthesis of total sugars and EPS. Both the compounds were found to have significant role in osmoadaptation by the rhizobacteria since their accumulation was found to increase with increase in salt concentration. In the present investigation, EPS production was not affected by increasing levels of NaCl concentration in the medium, rather it was increased under salt stress by 49.5 and 58.2 per cent in the presence of 5 and 10 per cent NaCl respectively over control medium. EPS are high molecular weight carbohydrate polymers that make up a substantial component of the extracellular polymers surrounding most microbial cells in the saline environment (Chowdhury *et al.*, 2007). They constitute a large fraction of the reduced carbon reservoir in the adverse ecosystems and enhance the survival of bacteria influencing the physicochemical environment in proximity of the bacterial cell (Francius *et al.*, 2011). Moreover, they assist the microbial communities to endure extremes of temperature, salinity and nutrient availability. Changes in the EPS production, being observed with increase in NaCl concentration, could signify specific adaptive responses to salinity. Some bacterial cells produce complex extracellular polysaccharides (EPS) that are instrumental in imparting salt tolerance to bacterial cells (Potts, 1994).

In the third strategy of osmoadaptation, salt-tolerant AIMs exhibited higher potassium accumulation with increase in salt stress. In response to the supplementation of 10, 12.5, 15 and 17.5 per cent NaCl to the growth medium, the mean intracellular potassium content of salt-tolerant FP isolates was increased by 113.0, 185.4, 208.4 and 108.8 per cent over control medium respectively. Such an increase in intracellular potassium content resulted in maintaining the intracellular Na to K ratio within 0.5.

It has been reported that, turgor adjustment in bacteria exposed to hyperosmotic environments is generally achieved by the accumulation of very large amounts of a few organic solutes termed 'compatible solutes'. The amassing of these compounds counteracts cell dehydration and thus contributes to maintain an outwardly-oriented cytoplasmic pressure, which is the driving force for cell growth. Bacterial compatible solutes are accumulated either by *de novo* biosynthesis or by uptake from the environment so called osmoprotectants (Csonka and Hanson, 1991). These compatible solutes confer protection against the deleterious effects of low water activity, maintain the appropriate cell volume and protect intracellular macromolecules (Csonka and Epstein, 1996). Under osmotic stress, the accumulation of these osmoprotectants is achieved by either endogenous *de novo* synthesis or uptake mediated by specific transporters (Kempf and Bremer, 1998). It can also be due to triggering of rapid fluxes of cell water along the osmotic gradient out of the cell, thus causing a reduction in turgor and dehydration of the cytoplasm.

Although ethylene is essential for normal growth and development in plants, at high concentration it can be harmful as it induces defoliation and other cellular processes that may lead to reduced crop performance. Using their 1-amino cyclopropane- 1-carboxylic acid (ACC) deaminase activity, PGPR can divert ACC from the ethylene biosynthesis pathway. Thus, rhizobacteria assist in diminishing the accumulation of ethylene levels and re-establish a healthy root system needed to cope with environmental stress. The present investigation also demonstrated the presence of ACC deaminase activity in 57 out of 67 salt-tolerant AIMs which depicts their probable role in reducing the plant ethylene levels under salt stress. Nadeem *et al.* (2009) reported the role of ACC deaminase containing rhizobacterial strains such as *Pseudomonas*, *Flavobacterium* and *Enterobacter* in improving the growth of maize under salt-affected soils. Similarly, Bhattacharjee *et al.* (2012) observed ACC deaminase activity in *Rhizobium leguminosarum* bv. *trifolii* SN10 strain isolated from root nodules.

5.4 Plant growth promotional activity of salt-tolerant AIMs

Salinity adversely affects the growth and yield of several crop plants. However, interaction of PGPR with crop plants in saline conditions reduces the extent of poor growth and thus helps plants to survive in adverse conditions. Preliminary screening of salt tolerant AIMs under controlled conditions indicated their probable role in plant growth promotion. Inoculation of salt-tolerant isolates gave significant improvement in per cent seedling emergence, plant height as well as dry matter content of wheat as compared to un-inoculated control (UIC) at all the soil ECe levels tested in the present study. Previous studies have indicated that selected plant growth-promoting bacteria mitigated the deleterious effect of salinity on growth of tomato, pepper, canola and cotton (Glick *et al.*, 1997; Kang *et al.*, 2008). Soaking wheat seeds in either *Azotobacter vinelandii* (AZ) or *Streptomyces* sp. (ST) or both culture filtrates (AZ+ST) increased significantly wheat seed germination. Moreover, soil inoculations with the bacterial cells of AZ, ST or AZ+ST enhanced root depth, shoot length, dry weights of root and shoot and mineral and protein content of wheat in normal and saline conditions which have been ascribed to nitrogen fixation, production of auxins or unidentified compounds (Aly *et al.*, 2012). Increase in the biomass of wheat plants in the present investigation could be due to better nutrition of the plants under saline conditions. In addition, several physiological, enzymatic and biochemical changes in plant due to inoculation of plant growth-promoting bacteria have been suggested to help in alleviating the salt or drought stress (Han and Lee, 2005 and Barriuso *et al.*, 2008).

The present study also emphasized the importance of inoculation of salt-tolerant AIMs either individually or in consortia in improving the growth and development of wheat crop raised under graded soil salinity under field conditions. Our results suggest that inoculation of wheat with salt-tolerant AIMs viz., *Enterobacter aerogenes* S63(1)R, *Azospirillum irakense* S173E, *Enterobacter cloacae* S125R, *Pseudomonas* sp. S4(1)S, consortia of all four bacteria and *Aspergillus niger* WL9S (singly) promote good growth of wheat plants under saline soils at both lower as well as higher soil ECe levels compared to control.

Plants inoculated with consortia of four bacterial strains [*Enterobacter aerogenes* S63(1)R + *Azospirillum irakense* S173E + *Enterobacter cloacae* S125R + *Pseudomonas* sp. S4(1)S] showed good growth and higher salt tolerance than plants inoculated with individual strains and UIC plants at each of the soil ECe levels examined. Germination of wheat seeds was directly affected by increasing salinity levels.

Seedling emergence was substantially delayed in UIC with increase in soil salinity but inoculated treatments showed great improvement in seedling emergence at all the five soil ECe levels tested. Inoculation treatments also had positive impact on all other growth parameters. The plant height and root lengths were significantly increased with inoculation of salt-tolerant AIMs over UIC plants at all the soil ECe levels.

The greater root length of plants is probably due to the availability of higher auxin concentrations in inoculated treatments. PGPR are known to release IAA and the interaction of IAA with plants has been reported to result in higher root length (Patten and Glick, 2002; Rajkumar *et al.*, 2005 and Chakraborty *et al.*, 2006). Plants inoculated with salt-tolerant AIMs either individually or in consortia showed higher chlorophyll content in wheat plant at all the soil ECe levels tested.

Inoculated plants also showed a higher percentage increase in dry biomass under saline conditions than did uninoculated plants. Increased salinity reduced the mean dry matter contents in control plants, but in the presence of salt-tolerant AIMS, the dry matter contents were higher than in controls. Similar results were reported for salt tolerance in various plants induced by rhizobacteria (Mayak *et al.*, 2004; Saravankumar and Samiyappan, 2007 and Zahir *et al.*, 2009).

5.5 Alleviation of salt stress in wheat by salt-tolerant AIMS

5.5.1 Status of antioxidative enzymes

Salinity and drought are well established to induce oxidative stress in plants. During salinity-induced oxidative stress, availability of atmospheric CO₂ is reduced because of increased stomatal closure, and consumption of NADPH by the calvin cycle is decreased (Gill and Tuteja, 2010). To overcome salt-mediated oxidative stress, plants detoxify reactive oxygen species (ROS) by up-regulating antioxidative enzymes like superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), glutathione reductase and glutathione peroxidase. The harmonized activities of the multiple forms of these enzymes in different sub-cellular compartments achieve a balance between the rate of formation and removal of ROS and sustain H₂O₂ at the required levels for cell signaling (Mittler, 2002). It is now widely accepted that the degree of oxidative cellular damage in plants exposed to abiotic stress is controlled by the capacity of the antioxidative systems (Turkan *et al.*, 2009). A correlation between antioxidant capacity and salinity tolerance has been reported in several plant species such as wheat (Meneguzzo *et al.*, 1999) cotton (Gossett *et al.*, 1994), rice (Dionisio-Sese and Tobita, 1998), *etc.*

It was observed that, the Anti-oxidative enzyme (AOE) activities increased with salinity and decreased upon inoculation of salt-tolerant AIMS. Variation in terms of the AOE activity and the rate of its increase with an increase in soil ECE was observed.

The inoculated wheat plants showed less AOE activity as compared to un-inoculated plants at all the soil ECE levels tested. Plant growth promoting rhizobacteria (PGPR) have previously been shown to have the ability to elevate CAT and peroxidase activities in response to severe salinity (Kohler *et al.*, 2009). Furthermore, reactive oxygen species (ROS) quenching is also achieved by non-enzymatic reactions involving the activities of water soluble ascorbate (ASC) and glutathione (GSH) and the lipophilic α -tocopherol, which is also known as Vitamin E. The effect of α -tocopherol is worth mentioning, since it prevents the propagation of lipid peroxidation by scavenging lipid peroxyl radicals in thylakoid membranes (Munne-Bosch, 2005).

A study of the concentration change of α -tocopherol might provide a better link between the antioxidant system and membrane damage under salinity and PGPR treatment. Therefore, PGPR effects on the change of a range of ROS scavengers should be further studied to elucidate which chemical is closely related to ethylene signaling transduction and the mechanism of PGPR protection of plants from activated oxygen species.

Among all the AOE enzymes analyzed, the maximum activity was recorded with SOD followed by catalase and minimum with peroxidase at all the three sampling periods. Superoxide dismutase (SOD) is an important antioxidant enzyme and is the first line of defence against oxidative stress in plants. SOD causes dismutation of superoxide radicals at almost diffusion-limited rates to produce H₂O₂ (Salin, 1987).

Superoxide dismutase (SOD) activity is a widely used indicator of salt stress. When salt stress is imposed, superoxide anions (O₂⁻) that are formed as byproducts of the electron transport chain will increase due to the damage of chloroplast and mitochondria. SOD is the enzyme that catalyzes superoxide to H₂O₂.

If not detoxified by catalase and peroxidase, H₂O₂ would form extremely aggressive oxygen species hydroxyl groups ($\cdot$ OH) through fenton reaction. The efficient removal of superoxide by SOD is therefore very important to prevent the production of highly destructive hydroxyl groups. The induction of SOD is considered as one mechanism of salt tolerance in plants (Hernandez *et al.*, 2001).

It was also reported that, increase in soil salinity leads to a significant increase of catalase and peroxidase activities in salt-stressed leaves of barley. While inoculation of barley with *Azospirillum brasilense* was found to decrease the magnitude of increase in catalase and peroxidase activities as compared to uninoculated plants and significantly ameliorate the deleterious effects of salinity thus improves the crop productivity (Omar *et al.*, 2009). Similar findings were also reported wherein the activity of antioxidant enzymes (catalase, peroxidase and SOD) in wheat leaves decreased under salinity stress after co-inoculation with two salt-tolerant PGPR strains viz., *Bacillus subtilis* SU47 and *Arthrobacter* sp. SU18 when compared to uninoculated control. It was concluded that, co-inoculation with *B. subtilis* and *Arthrobacter* sp. could alleviate the adverse effects of soil salinity on wheat growth (Upadhyay *et al.*, 2011).

5.5.2 Accumulation of protective compounds

Salinity causes an imbalance in the ratio of ion homeostasis in the plant system. Plants try to maintain low salt composition in the cytosol by extrusion through the plasma membrane using the Salt Overly Sensitive (SOS) pathway or by scavenging in the vacuole through NHX1 antiporters. Salinity impedes the ratio of Ca^{2+} and K^+ in the cell. However, an increase in K^+ concentration can alleviate the deleterious effect of salinity on growth and yield (Giri *et al.*, 2007). In the present study, a low Na^+ content and a higher K^+ content in wheat plants inoculated with salt-tolerant AIMs at both the stages of crop growth and at all the soil ECe levels tested was observed. Therefore, a higher K/Na ratio in plants under salt stress was due to the restricted Na^+ uptake and enhanced K^+ uptake.. Similarly, several PGPR are reported to reduce the salt toxicity in various other plants by lowering the Na^+ concentration and increasing the K^+ concentration (Hamdia *et al.*, 2004; Nadeem *et al.*, 2006; Yildirim *et al.*, 2006; Bano and Fatima 2009 and Kohler *et al.*, 2009).

Proline, glycine-betaine, polyols and total soluble sugars are very important biochemical indicators of salinity tolerance in plants (Ashraf and Harris, 2004). When plants face salt stress, proline accumulates in the cytosol and helps substantially in cytoplasmic osmotic adjustment (Leigh *et al.*, 1981). Proline also helps the plant cell by stabilizing subcellular structures such as membranes and proteins, scavenging free radicals and buffering cellular redox potential under salt stress (Ashraf and Foolad, 2007). In the present study, wheat plants inoculated with salt-tolerant AIMs showed low proline content at all the soil ECe levels tested compared to uninoculated plants. This phenomenon was therefore not very active under this experimental conditions.

Inoculation of salt-tolerant AIMs resulted in significant increase in glycine-betaine and total sugar contents of wheat plants. In general, consortia inoculated wheat plants showed maximum glycine-betaine and total sugar contents and were least in UIC plants at all the soil ECe levels tested. The glycine-betaine and total sugar contents of wheat plants were increased with increase in soil ECe level up to 11.2 dS/m and declined thereafter. Different amino acids and nitrogen-containing compounds are accumulated in higher plants under salinity stress (Gilbert *et al.*, 1998; Mansour, 2000 and Ashraf and Harris, 2004). Total free amino acids are reported to be high in the foliar parts of different salt-tolerant plants such as sunflower, safflower, *Eruca sativa*, *Medicago sativa* and *Lens culinaris* (Fougere *et al.*, 1991; Hurkman *et al.*, 1991; Ashraf, 1994; Ashraf and Fatima, 1995 and Ashraf and Tufail, 1995). In the current study, wheat plants inoculated with salt-tolerant AIMs showed enhanced protein content as compared to uninoculated plants at all the soil ECe levels tested. Higher protein contents were observed under drought stress in the presence of plant-growth-promoting *Pseudomonas* sp. (Sandhya *et al.*, 2010). Few of these proteins may have direct correlation with salinity tolerance. Similarly, many proteins directly or indirectly related to plant growth promotion were found differentially expressed in rice plants by the interaction of *Pseudomonas fluorescens* (Kandasamy *et al.*, 2009).

5.5.3 Enhancement of soil enzyme activities and microbial biomass content

A better understanding of the role of soil enzymes activity in the ecosystem will potentially provide a unique opportunity for an integrated biological assessment of soils due to their crucial role in several soil biological activities, their ease of measurement and their rapid response to changes in soil management practices (Dick, 1994; Dick, 1997 and Bandick and Dick, 1999).

In the present investigation, two soil enzyme systems were measured, firstly, the enzymes those are no longer associated with viable cells but stabilized in the soil matrix (urease and phosphatase) and secondly, the enzymes that occur in viable cells and not in stabilized soil complexes (dehydrogenase). Inoculation of different salt-tolerant AIMS was found to increase the dehydrogenase, urease and phosphatase activities by 40.5 to 90.1, 28.3 to 43.4 and 16.8 to 28.2 per cent over un-inoculated control (UIC) with increase in soil salinity from 2.4 to 14.3 dS m⁻¹ respectively. Similarly, increase in soil salinity resulted in decrease in the activity of soil enzymes. It was interesting to note that, the maximum effect was observed with dehydrogenase wherein the activity was decreased by 13.4 to 45.8 per cent with increase in soil ECe from 4.9 to 14.3 dS m⁻¹ respectively over lowest soil ECe of 2.4 dS m⁻¹. Similarly, the activities of urease and phosphatase were also affected by salinity decreasing by 6.3 to 34 and 4.6 to 26 per cent with increase in soil ECe from 4.9 to 14.3 dS m⁻¹ respectively over lowest soil ECe of 2.4 dS m⁻¹. Dehydrogenase, urease and phosphatase are known to play a vital role in C, N and P cycling in soil system respectively (Dick *et al.*, 1994). These enzymes are constantly being synthesized, accumulated, inactivated and/or decomposed in the soil, hence playing an important role in agriculture and particularly in nutrients cycling (Tabatabai, 1994 and Dick, 1997).

Many of the enzymes are extracellular, stable and form complexes with the organic and mineral colloids (Garcia *et al.*, 1994). The increase in conductivity depresses the clays (Diaz, *et al.*, 1994) and stable enzymes remain unprotected and, therefore, more susceptible to denaturation. The cell lysis resulting from a reduced osmotic potential can also explain this decrease in enzymatic activity. When cell lysis occurs, the enzymes are more susceptible to denaturation by proteolysis, which results in decreased enzymatic activity (Frankenberger and Bingham, 1982).

Temporal variation in microbial biomass (C and N) was observed among the treatments and soil ECe levels. Among the inoculation treatments, consortia recorded maximum mean microbial biomass (C and N) as compared to all other inoculation treatments and UIC. Irrespective of inoculation treatments, mean microbial biomass C and N were found to decrease with increase in soil salinity. Rietz and Hayness (2003) also found negative exponential relationship of microbial biomass carbon with soil EC. Reports in the literature indicate that MBC content ranged from 100 to 600 mg kg⁻¹ in non saline soils (Powlson *et al.*, 1987; Sparling, 1997 and Anderson, 2003) and 125 to 445 mg kg⁻¹ in saline soils (Tripathi *et al.*, 2006). Our results are in line with these findings. Tripathi *et al.* (2006) found that higher ECe (upto 16 dS m⁻¹ in coastal saline soils) caused significant decrease in MBC content. Soil desiccation due to dry weather (Van Gestel *et al.*, 1992) coupled with increase in soil salinity (Batra and Manna, 1997 and Rietz and Haynes, 2003) can decrease the MBC value. Sardinha *et al.* (2003) suggested that salinization has stronger effects on soil microbial properties than heavy-metal pollution observed at other sites (Chander *et al.*, 2001) and salinity is probably one of the most stressing environmental conditions for microbial growth and proliferation in soil. Microbial biomass decreased with increasing level of salinity, underlying the detrimental effect of salinity on soil microorganisms (Shah and Shah, 2011).

5.6 Genes encoding salt tolerance

The mechanism of stress tolerance exists in all living systems with minor modifications from bacteria to higher plants. Most bacteria possess ability to tolerate salt stress for a short period, whereas some others can grow at high salt concentration. The mechanisms responsible for salt stress tolerance and growth under high salt conditions range from induction of general stress tolerance proteins, Na⁺/H⁺ efflux pumps, modification of membrane composition and fluidity to increased production and/or uptake of compatible solutes (Kapardar *et al.*, 2010). Various genes responsible for providing salt tolerance and ability to grow at elevated salt concentration have been identified from different bacteria including the genes involved in synthesis and uptake of various compatible solutes (Naughton *et al.*, 2009).

In the present study, a total of 21 salt-tolerant strains (6 each of free-living N₂ fixers, PSB and FP and 3 associative N₂ fixers) were screened for the presence of four gene clusters viz., *proBA*, *ectABC*, *otsAB* and *gbsTIBA*.

All the 21 salt-tolerant AIMs were found positive for both *proAB* and *gbsTIBA* gene clusters whereas, only 12 strains (6 FP, 4 PSB and 2 free-living N2 fixers) for *ectABC* gene cluster. *otsAB* gene cluster was detected in most of the salt-tolerant AIMs except three strains viz., ASPSBXI S156R, ASPSBXI S189E and ASFPV Dr16E. The experimental evidence presented herein confirms that the presence of all or any of the four gene clusters confers tolerance to rhizobacteria at various salt concentrations and hence there was no relationship between the salt tolerance property of the isolates and presence of number of genes responsible for salt tolerance.

Among the compatible solutes, proline appears to be the most widely distributed osmolyte accumulated under stress conditions not only in plants but also in eubacteria. The results suggest that unabated production of proline and presence of *proAB* gene cluster in all the test isolates indicates its role for survival of the NaCl stressed cells. Genes encoding proline biosynthesis and involved in salt tolerance have been isolated and characterized in several halophilic and halotolerant bacteria (Chen *et al.*, 2007).

Presence of expected amplicon of the *betTIBA* gene cluster in all the twenty one AIMs depicts its role in osmoregulation. The molecular details of the choline to glycine betaine synthesis pathway encoded by the *betTIBA* gene cluster have been fully characterized in *Escherichia coli* (Lamark *et al.*, 1991). The genes encoding the glycine-betaine biosynthesis were also analyzed in other bacteria and shared many characteristics with their homologs found in *E. coli* (Rokenes *et al.*, 1996).

Trehalose, a non-reducing disaccharide is another major compatible solute, which maintains fluidity of membranes and protects the biological structure of organisms under stress. It was observed that, *otsAB* gene cluster was detected in most of the salt-tolerant AIMs except three strains viz., ASPSBXI S156R, ASPSBXI S189E and ASFPV Dr16E. The gene cluster *ectABC* coding for diaminobutyric acid (DABA) acetyltransferase (EctA), DABA aminotransferase (EctB) and ectoine synthase (EctC) was isolated and characterized by several researchers (Canovas *et al.*, 1998; Ono *et al.*, 1999 Kuhlman and Bremer, 2002) and reported to have role in salt tolerance of bacteria.

Similarly, 12 strains (6 FP, 4 PSB and 2 free-living N2 fixers) were found positive for the presence of *ectABC* gene cluster while it was not detected in nine salt-tolerant AIMs (3 ANF, 4 FNF and 2 PSB isolates). It could be due to preferential accumulation of osmolytes by microorganisms hence ectoine synthesis might be a strategy to tolerate higher salt concentration. Presence of *ectABC* gene cluster in several bacteria and its role in trehalose biosynthesis has been reported by various researches (Avonce *et al.*, 2006; Elbein *et al.*, 2003 and Schiraldi *et al.*, 2002). They also reported that, existence of four types of trehalose biosynthetic pathways in bacteria till date. However better analysis can be made only after the analysis of the transcriptome under salt stress.

Future line of work

1. Analysis of the possibility of application of microbial derived organic osmolytes in enhancing salt tolerance in different crops.
2. Studies on isolation, identification and characterization of genes encoding salt-tolerance.
3. Understanding the effect of salt stress on catalytic activities of enzymes involved in dinitrogen fixation, mineral phosphate solubilization and phytohormone production.
4. Studies on regulation and expression of genes involved in salt stress signaling pathways.

6. SUMMARY AND CONCLUSIONS

Salt-tolerant agriculturally important microorganisms (AIMs) isolated from stress-ecosystems of Karnataka and maintained in the culture bank of the Department of Agricultural Microbiology, UAS Dharwad were subjected to functional analysis and molecular characterization. The biochemical basis for salt tolerance in these isolates was also analyzed under salt stress. Besides, their plant growth promotional activities were assessed at different soil ECe levels in wheat under green house as well as field conditions. The results obtained from the present study are summarized below.

A total of 195 salt-tolerant AIMs were examined for morphological, biochemical and physiological characteristics and identified tentatively using Bergey's Manual of Determinative Bacteriology. Similarly, 91 isolates were also identified using 16S rRNA sequencing.

- Of the 195 salt-tolerant AIMs, 140 isolates were identified upto species level (23 as *Pseudomonas aeruginosa*, 18 as *Azotobacter chroococcum*, 16 as *Azospirillum irakense*, 15 as *Pseudomonas fluorescens*, 12 as *Azospirillum brasilense*, 11 each as *Pseudomonas aureofaciens* and *Azotobacter vinelandii*; 10 as *Azotobacter paspali*, eight as *Azospirillum halopraeferens*, seven as *Pseudomonas pseudomallei*; four as *Azotobacter nigricans*, three as *Pseudomonas mendocina* and one each as *P. putida* and *P. vesicularis* and 55 were tentatively identified only upto genus level (17 as *Bacillus*, 14 as *Pseudomonas*, five as *Staphylococcus*, four as *Aminobacter*, three as *Escherichia*, two each as *Glucanobacter*, *Acetobacter* and *Enterobacter* and one each as *Alcaligenes*, *Flavimonas*, *Rhizomonas*, *Rhizobacter*, *Xanthobacter* and *Xanthomonas*).
- Out of 91 salt-tolerant AIMs identified using 16S rRNA sequencing, 61 isolates represented the bacterial class γ -Proteobacteria, 8 to α -Proteobacteria and 22 to Bacilli. Out of 61 salt-tolerant strains representing the class γ -Proteobacteria, 12 were identified as *Pseudomonas nitroreducens*, 16 as *Pseudomonas aeruginosa*, 7 as *Pseudomonas* sp., six each as *Azotobacter* (3 as *A. vinelandii*, 2 as *A. chroococcum* and one as *A. salinestris*) and *Enterobacter* (five as *Enterobacter aerogenes* and one as *E. oyzae*), four as *Klebsiella* (*K. oxytoca* and one each as *K. pneumoniae* and *Klebsiella* sp.), three each as *Pseudomonas putida* and *P. chlororaphis* sub sp. *aureofaciens*, two as *Enterobacter cloacae* and one each as *Pseudomonas monteilii* and *P. fluorescens*. Out of the eight salt-tolerant strains of α -Proteobacteria, six were identified as *Azospirillum* (3 as *A. irakense* and 1 each as *A. brasilense*, *A. halopraeferens* and *Azospirillum* sp.) and two as *Agrobacterium* (*A. tumefaciens*). Of the 22 isolates representing class Bacilli, 17 were identified as *Bacillus* (9 as *B. endophyticus*, 5 as *B. megaterium* and 2 as *B. subtilis*) and five as *Staphylococcus* sp.

Wide variation in functional properties and salt-tolerance was noticed among all the isolates. Plant growth promotional traits (N_2 fixation, P-solubilization, phytohormone production) of each isolate were found to be affected with increased addition of NaCl to the growth medium.

- Of the 195 isolates, only 79 were found to be positive for N_2 fixation (43 free-living and 36 associative N_2 fixers). The amount of N_2 fixed by the isolates, in general, was found to decrease with increase in NaCl concentration. All 79 isolates fixed N_2 at 5% NaCl while, only 32, 9, 2 and one isolate showed N_2 fixation at 7.5, 10, 12.5 and 15 per cent NaCl in the medium respectively. Among the isolates, three isolates of ANF (ASANFX S145E, ASANFX S148E and ASANFXI S173E) were found to fix N_2 even at 10% NaCl whereas two isolates of FNF [ASFNFIV S75(1)R and ASFNFIV S63(1)R] fixer N_2 at 12.5 and 15% NaCl respectively.
- A total of 81 salt-tolerant AIMs (78 bacterial and 3 fungal isolates) were found to exhibit P-solubilization. In general, the amount of P_i released from TCP was found to increase with increase in NaCl concentrations up to 7.5 per cent and declined thereafter.
- Out of 61 FP isolates, 18 exhibited inhibition of five fungal pathogens. Out of 18 antagonistic isolates, 6 were inhibitory to *Alternaria carthami*, 17 to *Fusarium oxysporum* f. sp. *carthami*, 11 to *Sclerotium rolfsii*, 5 to *Rhizoctonia bataticola* and 11 to *Pyricularia oryzae*.

Of the 18 isolates showing antagonistic activity, five isolates (ASFPV S94(2)E, ASFPXI S134(3)S, ASFPXI S158(1)R, ASFPXI S174(1)R and ASFPXI S187S) showed inhibition potential against all the five pathogens tested. Two isolates were inhibitory to three pathogens each and 8 inhibited two pathogens each.

- All the 195 salt-tolerant AIMs were found positive for IAA and GA production *in-vitro*. It was observed that, all the isolates showed IAA and GA production in the presence of 5% NaCl while 148, 105, 37, 17 and six isolates produced IAA and GA at 7.5, 10, 12.5, 15 and 17.5% NaCl respectively. The mean amount of IAA produced by salt-tolerant ANF, FP and PSB isolates was found to increase with increase in NaCl concentration up to 5, 7.5 and 10 per cent respectively and declined thereafter. Whereas the mean amount of IAA produced by salt-tolerant FNF isolates was found to decrease with increase in NaCl concentration. The mean amount of GA produced by the ANF and FNF isolates was decreased with increase in NaCl concentration. Whereas, the GA producing ability of FP and PSB isolates was found to increase with increase in NaCl concentration up to 7.5 and 10 per cent and declined thereafter.

The biochemical basis for salt-tolerance in 67 AIMs (18 ANF, 14 FNF, 23 PSB and 12 FP isolates tolerating 5 to 10, 10 to 15, 10 to 15 and 15 to 17.5% NaCl respectively) was analyzed. All the isolates were found to accumulate higher amounts of compatible solutes (proline and glycine betaine) and sugars in response to increasing NaCl concentrations in the growth medium as compared to control (without added salts), besides maintaining their intracellular Na to K ratio between 0 to 0.5.

- Out of 67 salt-tolerant AIMs, 57 isolates (15 ANF, 14 FNF, 19 PSB and 9 FP) were found to possess ACC deaminase activity *in-vitro*. While, all the 57 isolates showed ACCD activity at 5 and 10% NaCl while, only 45 isolates possessed ACCD activity at 10% NaCl but was reduced significantly with increase in NaCl concentrations.
- All the isolates accumulated osmoprotectants at 7.5% NaCl, whereas 44, 37, 17 and 6 isolates at 10, 12.5, 15 and 17.5% NaCl respectively. The amount of osmoprotectants accumulated in salt-tolerant N_2 fixers, P-solubilizers and fluorescent pseudomonads, in general, were found to increase with increase in NaCl concentrations upto 7.5, 12.5 and 15% respectively and declined thereafter.
- The amount of proline produced by 67 salt-tolerant AIMs under salt stress ranged from 0.0 to 46.7 μ g, 0.0 to 56.2 μ g, 0.0 to 69.2 μ g, 0.0 to 90.0 μ g, 0.0 to 75.5 μ g and 0.0 to 62.4 μ g per mg protein in the presence of 5, 7.5, 10, 12.5, 15 and 17.5% NaCl respectively as compared to control (2.5 to 21.6 μ g per mg protein). The amount of glycine-betaine produced by salt-tolerant AIMs under salt stress ranged from 0.05 to 0.62 μ mol, 0.10 to 1.42 μ mol, 0.00 to 1.85 μ mol, 0.00 to 2.81 μ mol, 0.00 to 1.49 and 0.00 to 0.80 μ mol per mg protein at 5, 7.5, 10, 12.5, 15 and 17.5 per cent NaCl respectively as compared to control medium (0.00 to 0.34 μ mol per mg protein).

The plant growth promotional activity of nineteen salt tolerant AIMs *viz.*, 4 associative N_2 fixers, 5 free-living N_2 fixers, 5 P-solubilizing bacteria (4 bacteria and 1 fungi) and 5 fluorescent pseudomonads were assessed in wheat (cv. DWR 162) at different soil ECe levels (0.37, 2.32, 4.08, 5.38, 7.21 and 7.55 dS/m) against un-inoculated control in environmental growth chamber. Among the isolates, irrespective of soil ECe levels, inoculation of salt-tolerant AIMs showed significant improvement in seedling emergence (60.0 to 95.6%), plant height (16.1 to 26.1 cm) and dry matter content (40.6 to 62.1 mg/plant) over UIC (56.7%, 14.2 cm and 32.6 mg/plant respectively). The field performance of salt-tolerant AIMs was assessed in wheat (cv. DWR-162) under gradient of soil salinity at ARS, Gangavati during Rabi 2010-11. Plant growth promotional activity as well as the stress-alleviation effects of salt-tolerant AIMs in wheat was examined and results obtained from the present study are summarized below.

- In general, the inoculation treatments significantly enhanced both growth and yield parameters over uninoculated control. Among the inoculation treatments, consortia [*Azospirillum irakense* S173E, *Enterobacter aerogenes* S63(1)R, *Enterobacter cloacae* S125R and *Pseudomonas* sp. S4(1)S] recorded significantly higher plant height, dry matter content, tillers count, grain and straw yield as compared to other inoculation treatments and UIC.

It was followed by *Aspergillus niger* WL9S, *Enterobacter aerogenes* S63(1)R, *Enterobacter cloacae* S125R, *Azospirillum irakense* S173E and *Pseudomonas* sp. S4(1)S. Similar trend was observed in almost all the observations recorded.

- Among the treatments, wheat crop inoculated with consortia recorded the highest mean grain and straw yields (39.5 and 55.9 q/ha respectively) as compared to UIC (25.9 and 39.4 q/ha respectively). The grain and straw yields of wheat was found to decrease with increase in soil ECe levels.
- In general, inoculation of salt-tolerant AIMs resulted in significant improvement in uptake of all three nutrients (N, P and K) at all the soil ECe levels tested. While, increase in soil ECe levels over and above 4.9 dS/m was found to have negative impact on uptake of nutrients by wheat crop. Among the treatments, wheat crop inoculated with consortia recorded the highest mean total uptake of N, P and K (122.8, 24.3 and 72.4 kg/ha respectively) as compared to other inoculation treatments and UIC.
- Activity of antioxidative enzymes (AOE) viz., catalase, superoxide dismutase and peroxidase were decreased with inoculation but increased with soil salinity. Among the treatments, irrespective of soil ECe levels, UIC plants showed highest AOE activity as compared to inoculation treatments. Among the inoculated treatments, consortia recorded the lowest AOE activity as compared to other inoculants where in *Pseudomonas* sp. S4(1)S inoculated plants showed the highest AOE activity.
- Wheat plants inoculated with salt-tolerant AIMs recorded significantly lower proline and polyols contents as compared to UIC at all the soil ECe levels examined. Among the inoculants, consortia recorded the lowest proline and polyols content as compared to other inoculants while the contents were highest in UIC plants. It was also observed that, the proline and polyols contents were found to increase with increase in soil ECe up to 11.2 dS m⁻¹ and decrease thereafter.
- Inoculation of salt-tolerant AIMs resulted in significant increase in glycine-betaine and total sugar contents of wheat. In general, consortia inoculated wheat plants showed maximum glycine-betaine and total sugar contents and were least in UIC plants at all the soil ECe levels tested. The glycine-betaine and total sugar contents of wheat plants were increased with increase in soil ECe level up to 11.2 dS/m and declined thereafter.
- It was observed that, the sodium content of wheat plants continued to increase with increase in soil ECe level upto 14.3 dS/m, while the potassium content was increased only up to 8.7 dS/m and declined thereafter. The maximum K to Na ratios were recorded at 4.9 dS/m which however declined thereafter with increase in soil ECe. Wheat plants inoculated with salt-tolerant AIMs recorded significantly higher potassium content and K to Na ratio but lower Na content as compared to UIC plants.
- All the inoculation treatments recorded significantly higher soil dehydrogenase, urease and phosphatase activity over UIC at all stages of crop growth. However, increase in soil salinity from 2.4 dS m⁻¹ to 14.3 dS m⁻¹ adversely affected the activities of all the soil enzymes.
- Temporal variation in microbial biomass (C and N) was observed among the treatments and soil ECe levels. Among the inoculation treatments, consortia recorded the maximum mean microbial biomass (C and N) as compared to all other inoculation treatments and UIC. Irrespective of inoculation treatments, mean microbial biomass C and N were found to decrease with increase in soil salinity.

A total of 21 salt-tolerant strains (6 each of free-living N₂ fixers, PSB and FP and 3 associative N₂ fixers) were screened for the presence of four gene clusters viz., *proBA*, *ectABC*, *otsAB* and *gbsTIBA*. All the 21 salt-tolerant AIMs were found positive for both *proAB* and *gbsTIBA* gene clusters whereas, only 12 strains (6 FP, 4 PSB and 2 free-living N₂ fixers) were found positive for *ectABC* gene cluster. *otsAB* gene cluster was detected in 18 salt-tolerant AIMs. In conclusion, these data suggest that the salt-tolerant functional groups of AIMs tested in the present study can play an important role in conferring salt tolerance in crops. It is evident that, salt-tolerant AIMs inoculated either individually or in consortia promotes plant growth under salt stress. Therefore, it is plausible to mention that salt-tolerant rhizobacterial strains could be used as an effective strategy for improving the growth of salt-sensitive plants and alleviating the impact of salt stress on tolerant crops for sustainable agriculture.

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Appendix I. Composition of different growth media/reagents/indicators used

Growth medium	Qty. (g/l)	Growth medium	Qty. (g/l)
A) Czapeck's solution		H) N-free malate semisolid medium	
NaNO ₃	2.00	Malic acid	5.0
K ₂ HPO ₄	1.00	KOH	3.0
MgSO ₄	0.50	K ₂ HPO ₄	0.5
KCl	0.50	FeSO ₄ .H ₂ O	0.1
FeSO ₄	0.01	MnSO ₄	0.1
Sucrose	30.00	MgSO ₄ .7H ₂ O	0.1
Yeast extract	1.00	NaCl	0.02
B) Glucose broth		CaCl ₂ .2H ₂ O	0.01
Peptone	5.00	Na ₂ MoO ₄	0.002
Beef extract	3.00	Agar	1.75
Glucose	5.00	BTB (0.5% in alcohol)	2 ml
BCP solution	15 ml	pH	6.5
C) Iodine solution		I) Nitrate broth	
Iodine	0.50	Peptone	5.00
Potassium iodide	1.00	Beef extract	3.00
D) King's B agar		KNO ₃	1.00
Peptone	16.00	pH	6.8-7.0
MgSO ₄	1.60	J) Norris N-free agar	
K ₂ HPO ₄	1.60	K ₂ HPO ₄	1.00
Glycerol	10 ml	MgSO ₄	0.20
Agar	18.00	CaCO ₃	1.00
pH	7.0	NaCl	0.20
E) Luria agar		Glucose	10.00
Tryptone	10.00	Agar	18.00
Yeast extract	5.00	K) Pikovskaya's agar	
NaCl	5.00	Glucose	10.00
Agar	18.00	MgSO ₄ (2.5%)	10.00 ml
F) Nutrient agar		CaCl ₂ (1%)	10.00 ml
Peptone	5.00	Tricalcium phosphate (TCP)	5.00
Beef extract	3.00	Agar	18.00
Sodium chloride	5.00	pH	7.0
Agar	18.00	L) MR-VP broth (g/l)	
pH	6.8-7.2	Peptone	7.0
G) Dubo's cellulose agar		Dextrose	5.0
NaNO ₃	0.5	K ₂ HPO ₄	5.0
K ₂ HPO ₄	1.0	pH	6.9
MgSO ₄ .7H ₂ O	0.5		
KCl	0.5		
Fe ₂ (SO ₄) ₃ .7H ₂ O	Traces		
Cellulose powder	12		

Contd.....

Growth medium	Qty. (g/l)	Growth medium	Qty. (g/l)
M) Nutrient gelatin (g/l)		Q) Skimmed milk agar	
Peptone	5.00	Skim milk powder	10.00
Beef extract	3.00	Peptone	5.00
Gelatin	120.00	Agar	18.00
pH	6.8-7.0	pH	7.2
N) Starch agar		R) Trypticase soy agar	
Peptone	5.00	Tryptone	17.00
Beef extract	3.00	Soyatone	3.00
Starch solution	10.00 ml	Glucose	2.50
Agar	18.00	Agar	18.00
pH	7.2	S) Urea broth	
O) Tributryn agar		Yeast extract	0.10
Peptone	5.00	KH ₂ PO ₄	9.10
Beef extract	3.00	Na ₂ HPO ₄	9.50
Tributryn	10.00 ml	Urea	20.00
Agar	18.00	Phenol red	0.01
pH	7.2	pH	6.8
P) Potato dextrose agar		Tryptone glucose yeast extract agar	
Peeled potato	200.00	Tryptone	5.0
Dextrose	20.00	Glucose	5.0
Agar	18.00	Yeast extract	3.0
Q) SIM agar		K ₂ HPO ₄	0.7
Peptone	30.00	MgSO ₄ .7H ₂ O	0.25
Beef extract	3.00	Agar	18.0
Ferrous amm. Sulphate	0.20	Simmon's citrate agar	
Sodium thisoulphate	0.025	NH ₄ H ₂ PO ₄	1.0
Agar	18.00	K ₂ HPO ₄	1.0
pH	7.3	NaCl	5.0
Minimal medium (M9)		Sodium citrate	2.0
Sterile distilled water	780 ml	MgSO ₄ .7H ₂ O	0.2
5x M9 salts	200 ml	BTB	0.08 ml
C source (20% solution)	20 ml	pH	6.9
Composition of 5x M9 salts (g/l)			
Na ₂ HPO ₄	64.0		
KH ₂ PO ₄	15.0		
NaCl	2.5		
NH ₄ Cl	5.0		

Appendix II. Reagents used for total DNA isolation for molecular characterization

Stock solutions

1M Tris (pH 8.0)

Tris	121.1 g
Water	8000 ml
pH (adjusted with conc. HCl)	8.0
Total volume	1000 ml

EDTA 0.5 M (pH 8.0)

EDTA	18.6 g
Water	80 ml
pH (adjusted with NaOH)	8.0
Total volume	100 ml

Sodium chloride 1M

Sodium chloride	58.44 g
Water	800 ml
Total volume	1000 ml

Lysozyme: 50 mg/ml

Dissolved 50 mg of lysozyme in 1 ml of T₁₀E₁ (pH: 8.0) and stored at -20° C

Proteinase K: 20 mg/ml

Dissolved 20 mg of proteinase K in 1 ml of sterile distilled water and stored at -20° C

DNase free RNase: 10 mg/ml

Dissolved 10 mg of DNAase free RNAase in 1 ml of sterile distilled water and stored at -20° C

2 per cent Sarkosyl

Dissolved 2 g of Sarkosyl in 100 ml T₅₀E₂₀ (pH: 8.0) as and when required and used fresh.

Sodium acetate 3M

Dissolved 408.3 g of sodium acetate in 800 ml of distilled water, adjusted pH to 8.00 with glacial acetic acid and made up the volume to 1 litre.

Phenol: chloroform

Mix equilibrated phenol (pH > 7.8), chloroform and isoamyl alcohol in the ratio 25:24:1 (v/v), store at 4° C.

Composition of the PCR reaction mix (25 µl tube⁻¹) for 16S rRNA amplification

10 x assay buffer with MgCl ₂	2.5 µl
dNTPs (2.5 mM)	1.0 µl

Contd.....

Primer (10 pmoles)	1.0 μ l
Template DNA (40 ng)	1.0 μ l
Taq DNA polymerase (3U/ μ l)	0.33 μ l
Sterile double distilled water	19.17 μ l

Loading dye composition

Loading dye (6x)	0.25% bromophenol blue, stored at 4 ⁰ C
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Recipe for 1 per cent agarose gel (40 ml)

Agarose	400 mg
1x TAE	40 ml
EtBr (10 mg/ml)	2 μ l

50x TAE buffer composition

Tris base	242 g
Glacial acetic acid	57.1 ml
0.5 M EDTA (pH 8.0)	100 ml
Total volume 1000 ml with double distilled water	

FUNCTIONAL ANALYSIS AND MOLECULAR CHARACTERIZATION OF SALT-TOLERANT MICROORGANISMS OF AGRICULTURAL IMPORTANCE

2012

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ABSTRACT

Salt-tolerant agriculturally important microorganisms (AIMs) isolated from stress-ecosystems of Karnataka were identified and assessed for their functional properties under salt stress. The biochemical basis for salt tolerance was also analyzed under salt stress. Besides, their plant growth promotional activities were assessed at different soil ECe levels in wheat under green house as well as field conditions. Plant growth promotional traits (N_2 fixation, P-solubilization, phytohormone production) of each isolate were found to be affected with increased addition of NaCl to the growth medium. The amount of N_2 fixed by the isolates, in general, was found to decrease with increase in NaCl concentration. While, the amount of Pi released from TCP was found to increase with increase in NaCl concentrations up to 7.5 per cent and declined thereafter. It was observed that, all the isolates were found to accumulate higher amounts of compatible solutes (proline and glycine betaine) and sugars in the presence of increasing NaCl concentrations as compared to control (without added salts), besides maintaining their intracellular Na to K ratio between 0 to 0.5. The field performance of salt-tolerant AIMs was assessed in wheat (cv. DWR-162) under gradient of soil salinity. In general, the inoculation treatments significantly enhanced both growth and yield parameters over uninoculated control (UIC) at all the soil ECe levels tested. Field efficacy of salt tolerant AIMs gave 6.5 to 27.8% increase in dry matter production and 13.9 to 80.5% increase in grain yield of wheat over UIC at soil EC of 2.4 - 14.3 dS/m. It was also observed that, activity of antioxidative enzymes (AOE) viz., catalase, superoxide dismutase and peroxidase were decreased with inoculation but increased with soil salinity. Inoculated wheat plants recorded significantly lower proline and polyols content but higher glycine-betaine and total sugar contents as compared to UIC plants. Similarly, inoculated plants also recorded significantly higher potassium content and K to Na ratio but lower Na content as compared to UIC plants. It was also observed that, inoculation of salt-tolerant AIMs recorded significantly higher microbial biomass (C and N) and soil enzyme activities over UIC. In conclusion, these data suggest that the salt-tolerant functional groups of AIMs tested in the present study can play an important role in conferring salt tolerance in crops.