1. INTRODUCTION

“Nature selects those living beings which suit better to the newer extreme or stressful conditions”

“Survival of the fittest”

---- Charles Darwin

Bacteria, one of the living things on earth, can live virtually everywhere. Many of them survive and grow in environments which are extreme by our measures, but for them it is just a home. For example they can survive and grow in the environment with saturated NaCl solution Schubert et al. (2009), Vreeland et al. (2007), Mormile et al. (2003). High concentrations of salt in the environment and many other factors provide a “physical window frame” within which life can expand. The dimensions of this frame vary for every different group of living beings.

Though for higher living beings like plants and animals the frame appears to be very much restricted, for microbes these dimensions are very much wide and flexible. Environmental conditions outside this frame make the environment extreme. Extreme environments impose stress on living beings for their survival and growth. Any such condition of an environment which requires significant modification of organisms living in them from their immediate ancestors is a stressful condition and is an extreme environment. Those which do not get modified for these stresses, suffer and get extinguished. But those which undergo required modifications, appear fit to survive. Nature selects those living being which suit better to the newer extreme or stressful conditions.

Soil is one of the most diverse habitats and most complex environment on the Earth. This diverse ecosystem is assemblage of many physical, chemical and biological components including many micro as well as macro organisms. Soil environment is the most important environment that can affect agricultural production.
About 24% of earth’s land surface is covered by cultivated systems. But there is very much increased pressure on biodiversity from direct and indirect stress conditions. Although salt is a natural element of soil and water, soil salinity can be one of the very important stressful conditions affecting agricultural productivity. It is a condition in which the soluble salt content reaches to a level harmful to crops. Soil salinity reduces the osmotic potential of the soil solution. This makes water extraction impossible for some plants. Thus the productivity of agricultural land is decreased. Likewise soil salinity also affects the microorganisms. Although very diverse groups of microorganisms exist in soil, majority of them are also prone to the effects of high salinity. Under high solute concentration, water activity of a soil is reduced towards unfavorable level for soil microbes. Water activity of soil decreases with increase in solute or salt concentration.

Effect of high salt concentration in the soil may be due to the solute itself or due to its effect on water activity. In a diverse group of microorganisms there exist varieties based on their survival or growth response to salt concentrations in the soil. These groups include, non-halophiles, slight halophiles, moderate halophiles, borderline extreme halophiles, extreme halophiles, and halotolerant microorganisms (Kushner, 1978). Non-halophiles are those which grow best in media containing less than 0.2 M salt (1.16% NaCl). These include most of the natural eubacteria and most of the fresh water organisms. Slight-halophiles are those which grow best in media containing 0.2 – 0.5 M salt (1.16% to 2.9% NaCl). Many marine organisms are slight halophiles. Moderate halophiles which constitute bacteria and some algae are those which grow best in media containing 0.5 to 2.5 M salt. (2.9% to 14.5% of NaCl). Organisms able to grow in less than 0.1M salt (0.58% NaCl) are considered facultative halophiles. Borderline halophiles grow best in media containing 1.5 to 4 M salt (8.7% to 23.2% NaCl). Some of the borderline halophiles are Ectothiorhodospira halophila, Actinopolyspora halophila. Extreme halophiles are those which grow best in media containing 2.5M (14.5%) to 5.2M (30.16% NaCl) salt. (saturated). The red halophiles, halobacteria and halococci are extreme
halophiles. Halo-tolerant organisms are non-halophiles which can tolerate salt. If the growth range extends above 2.5M salt (14.5% NaCl), it may be considered extremely halotolerant. *Staphylococcus aureus*, and other Staphylococci, solute tolerant yeasts and fungi are some of the halo tolerant organisms.

Nitrogen is one of the most essential component for the plants in soil. It is available in atmosphere. This atmospheric nitrogen is not utilizable for plants. That needs to be fixed in to some available form for plants and then to animals.

Physical, chemical and biological processes are responsible for most of the nitrogen fixation in the biosphere.

Physical or atmospheric nitrogen fixation contributes some 5–8% of the total nitrogen fixed on the earth. Tremendous amount of energy of lightning and thunders make nitrogen molecules combine with oxygen in air forming nitrogen oxides. These are dissolved in rain, forming nitrates, and then these nitrates are carried to the earth.

In chemical or industrial nitrogen fixation, under great pressure, at very high temperature of about 600°C, and with the use of a catalyst, atmospheric nitrogen and hydrogen can be combined to form ammonia (NH₃). Ammonia can be used directly as fertilizer, but most of it is further processed to urea and ammonium nitrate (NH₄NO₃).

Although fixation of N₂ in to utilizable form can occur physico-chemically, one of the significant means of fixation of N₂ is by biological process.

In biological nitrogen fixation two moles of ammonia are produced from one mole of nitrogen gas, at the expense of 16 moles of ATP and a supply of electrons and protons.

\[
N₂ + 8H^+ + 8e^- + 16 \text{ ATP} = 2\text{NH}_3 + \text{H}_2 + 16\text{ADP} + 16\text{Pi}
\]

Gaseous nitrogen is bound to the nitrogenase enzyme complex, the Fe-protein is reduced by electrons donated by ferredoxin. Then the reduced Fe-protein binds to ATP and reduces the molybdenum-iron protein. The reduced molybdenum-iron protein then donates electrons to
N₂, producing HN=NH. In two further cycles of this process, HN=NH is reduced to H₂N-NH₂, and this finally is reduced to 2NH₃ (Figure No. 1.1).

![Diagram of nitrogen fixation process]

Figure No. 1.1 Schematic representation of mechanism of biological nitrogen fixation.

This reaction is performed exclusively in bacteria and related prokaryotic organisms. An enzyme complex termed nitrogenase which consists of an iron protein and a molybdenum-iron protein, is used in the process. Nitrogenase is a biological catalyst found naturally only in certain microorganisms such as the symbiotic Rhizobium and Frankia, or the free-living organisms like Azospirillum and Azotobacter. Approximately 90 million metric tons of N₂ is fixed biologically in soils annually by both symbiotic and non-symbiotic means. Symbiotic N₂ fixation contributes much in this process. The significant symbiotic association involves rhizobia as micro-symbionts and leguminous plants as macro-symbionts.

Salt affected soils may cause decrease in the symbiotic association between bacteria and plants. Some leguminous plants tolerate high salinity, but they may fail to establish symbiotic association for N₂ fixation. This may be mainly because of lack of proper salt tolerant rhizobia in such soils. However, it should be remembered that Nature is
the best biotechnologist. Accordingly nature itself will select rhizobia in these saline soils, which obviously will be salt tolerant ones.

1.1 ROLE OF BIOLOGICAL NITROGEN FIXATION IN SOIL FERTILITY:

In an atmosphere nitrogen is present in the form of nitrogen gas. The atmosphere contains about $10^{15}$ tones of N$_2$ gas, out of which about $3 \times 10^9$ tones of N$_2$ on a global basis is transformed by the nitrogen cycle per year (Postgate, 1982).

This transformation does not include exclusively biological transformation; the fertilizer industry also provides very important quantities of chemically fixed nitrogen. Out of total fixed nitrogen content of the world about 25% comes from chemical fertilizers and about 60% from biological processes (Aids, 1993).

For more than 100 years, biological nitrogen fixation (BNF) which has been exploited extensively in agricultural practice, has commanded the attention of scientists concerned with plant mineral nutrition (Burris, 1994; Dixon and Wheeler, 1986).

Although, very wide varieties of organisms have the nitrogen fixation ability (Table No. 1.1), only a very small proportion of species are capable of doing so. About 87 species in two genera of archaea, 38 genera of bacteria, and 20 genera of cyanobacteria have been identified as diazotrophs or organisms that can fix nitrogen Dixon and Wheeler (1986); Sprent and Sprent (1990); Zahran et. al. (1995).

This biological nitrogen fixation includes activities of symbiotic as well as non-symbiotic nitrogen fixers. One can exploit activities of symbiotic nitrogen fixers for reclaiming soil fertility. Symbiotic N2 fixation by rhizobia in legumes contribute substantially to total biological nitrogen fixation. Biofertilizer like “Rhizobium” can supply 20-25 kg N/ha (NIIR Board 2004).
Table No. 1.1: Nitrogen fixing organisms. (Nainawatee et. al., 1997)

<table>
<thead>
<tr>
<th>Groups of diazotrophs</th>
<th>Genera in the group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Free-living diazotrophs</td>
<td></td>
</tr>
<tr>
<td>A. Strict anaerobes</td>
<td>Methanosarcina,</td>
</tr>
<tr>
<td></td>
<td>Methanococcus</td>
</tr>
<tr>
<td></td>
<td>Bacillus, Clostridium,</td>
</tr>
<tr>
<td></td>
<td>Desulfotomaculum,</td>
</tr>
<tr>
<td></td>
<td>Desulfovibrio</td>
</tr>
<tr>
<td>B. Facultative anaerobic</td>
<td>Enterobacter, Escherichia,</td>
</tr>
<tr>
<td></td>
<td>Klebsiella</td>
</tr>
<tr>
<td>C. Micro aerobes</td>
<td>Aquaspirillum,</td>
</tr>
<tr>
<td></td>
<td>Arthrobacter, Azospirillum</td>
</tr>
<tr>
<td>D. Aerobes</td>
<td>Azotobacter, Beijerinckia,</td>
</tr>
<tr>
<td></td>
<td>Derxia</td>
</tr>
<tr>
<td>E. Photosynthetic bacteria</td>
<td>Rhodopseudomonas,</td>
</tr>
<tr>
<td></td>
<td>Chromatium,</td>
</tr>
<tr>
<td></td>
<td>Rhodospirillum</td>
</tr>
<tr>
<td>F. Cyanobacteria</td>
<td>Anabaena, Nostoc,</td>
</tr>
<tr>
<td></td>
<td>Calothrix,</td>
</tr>
<tr>
<td></td>
<td>Cylindrospermum</td>
</tr>
<tr>
<td>2. Symbiotic diazotrophs</td>
<td></td>
</tr>
<tr>
<td>A. Rhizobium- legume</td>
<td>Rhizobium,</td>
</tr>
<tr>
<td></td>
<td>Bradyrhizobium,</td>
</tr>
<tr>
<td></td>
<td>Azorhizobium</td>
</tr>
<tr>
<td>B. Actinomycetes</td>
<td>Frankia</td>
</tr>
<tr>
<td>C. Cyanobacterium</td>
<td>Anabaena</td>
</tr>
<tr>
<td>3. Associative diazotrophs</td>
<td>Azotobacter, Azospirillum</td>
</tr>
</tbody>
</table>

Atmosphere contains molecular nitrogen. Plants can not assimilate nitrogen in its molecular form. Nitrogen in soil is in the form of nitrate and nitrite. This is the available form of nitrogen to the plants. Symbiotic (e.g. *Rhizobium*) as well as free living nitrogen fixing microorganisms (e.g. *Azotobacter*) convert atmospheric nitrogen in nitrite and nitrate and thus fix it.
Rhizobia occur in soil, when leguminous plants grow in that soil rhizobia infect roots of these plants. This results in formation of nodules at their roots. If environment is suitable then rhizobia fix atmospheric nitrogen in the nodules. This nitrogen is then made available to the plants. Plants in turn support the rhizobia. Thus both grow in symbiotic association (Subba Rao 1976, 1979, 1982, 1984, 1995, Alexander, 1977).

1.1.1 A Microsymbiont-

One of the partners of symbiotic nitrogen fixation process is a microsymbiont, rhizobia. By end of the 19th century, it was realized that atmospheric nitrogen was being assimilated through the root-nodules of legume plants. In 1888, Beijerinck reported isolation of the root nodule bacteria and established that they were responsible for this process of nitrogen fixation. He named these bacteria as Bacillus radicicola. Later, Frank changed the name to Rhizobium with originally just one species, R. leguminosarum (Willems, 2006).

Rhizobium cells are small to medium sized (0.5-0.9 x 1.2–3.0 micron), Gram negative rods. Young cells are motile due to bi-polar, sub- polar or peritrichous flagella. Cells contain about 40-50 % of lipid granules. Most strains produce exopolysaccharide slimy material (Subba Rao, 1995).

Different species of Rhizobium are classified into two groups viz. (i) slow growing rhizobia (under the genus Bradyrhizobium) (ii) the fast growing groups (under the genus Rhizobium) These genera form nodules only at the roots of the concerned plant. However, there is also a third genus named Azorhizobium that forms nodules at the shoot of concerned plant. This nodule development by symbiotic diazotrophic soil bacteria on leguminous plants is a highly specific plant–microbe interaction (Spaink, 1994). Generally, only specific type of bacterial symbionts form nodules on restricted hosts. Hosts are also nodulated by restricted type of microsymbionts. Examples of the host leguminous plants and their corresponding rhizobial varieties are as shown in Table No. 1.2.
Table No. 1.2: Nodulation host range among rhizobia (Spaink, 1994).

<table>
<thead>
<tr>
<th>Genus</th>
<th>Members</th>
<th>Host legumes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>Rhizobium</em></td>
<td><em>R. meliloti</em></td>
<td>Alfalfa, Sweet clover, Fenugreek</td>
</tr>
<tr>
<td></td>
<td><em>R. leguminosarum</em></td>
<td>Pea (<em>Pisum sativum</em>) Vetch, Sweet pea</td>
</tr>
<tr>
<td></td>
<td>bv. <em>Viciae</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>R. leguminosarum</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>bv. <em>phaseoli</em></td>
<td>Bean (<em>Phaseolus vulgaris</em>)</td>
</tr>
<tr>
<td></td>
<td>Bv. <em>trifoli</em></td>
<td>Clovers</td>
</tr>
<tr>
<td></td>
<td><em>R. loti</em></td>
<td>Trifole</td>
</tr>
<tr>
<td></td>
<td><em>R. fredii</em></td>
<td>Soybean (<em>Glycine max, G. soja</em>)</td>
</tr>
<tr>
<td></td>
<td><em>R. tropici</em></td>
<td>Bean (<em>Phaseolus vulgaris</em>)</td>
</tr>
<tr>
<td></td>
<td><em>R.sp.NGR 234</em></td>
<td>70 legume species including Leucaena, Cowpea (<em>Vigna unguiculata</em>), Siratro, Soybean (<em>Glycine max</em>), Parasponia (non legume)</td>
</tr>
<tr>
<td>2. <em>Bradyrhizobium</em></td>
<td><em>B. japonicum</em></td>
<td>Soybean (<em>Glycine max, G. soja</em>)</td>
</tr>
<tr>
<td></td>
<td><em>B. elkanii</em></td>
<td>Soybean (<em>Glycine max, G. soja</em>)</td>
</tr>
<tr>
<td></td>
<td>*B. sp. “Cowpea”</td>
<td>Cowpea (<em>Vigna unguiculata</em>), Mungbean (<em>Vigna radiata</em>), Pigeonpea (<em>Cajanus cajan</em>), Chickpea (<em>Cicer arietinum</em>), Peanut</td>
</tr>
<tr>
<td>3. <em>Azorhizobium</em></td>
<td><em>A. caulinodans</em></td>
<td>Root and stem nodules on Sesbania</td>
</tr>
</tbody>
</table>

The term rhizobia, refers to members of the genus *Rhizobium*. However, the term was in use for all the bacteria that were capable of nodulation and nitrogen fixation in association with legumes and that belonged to a genus that was at one time part of the genus *Rhizobium* or closely related to it. In the first edition of Bergey’s Manual of Systematic Bacteriology (Krieg and Holt, 1984) the family *Rhizobiaceae* constitutes
four genera namely, *Rhizobium*, *Bradyrhizobium*, *Agrobacterium* and *Phyllobacterium* (Jordan, 1984).

First genus namely *Rhizobium* (Frank, 1889) included only three species namely, *Rhizobium leguminosarum* (Frank, 1879) consisting of three biovarieties namely, trifolii, phaseoli and viceae. *Rhizobium meliloti* (Dangeard, 1926) and *Rhizobium loti* (Jarvis et al., 1982), the fast grower from the lotus plant. Rhizobia related to this species were those from *Lotus corniculatus*, *L. tenuis*, *Cicer arietinum*, *Leucaena leucocephala* and *Sophora microphylla*.

Second genus was *Bradyrhizobium* (Jordan, 1982). It was heterogeneous group of root nodule bacteria whose taxonomic relations were not clear. The only known designated species was *B. japonicum*. Fourth genus was *Agrobacterium* (Conn, 1942) whose members were not found nodulating roots of any plant as well as not found fixing nitrogen. The fourth genus was *Phyllobacterium* whose members nodulated on the leaves of certain plants.

In the beginning of 20th century it was observed by Fred et al. (1932) that the rhizobia from one plant can cross inoculate the other plants from the same group. Fred recognized six species in the genus *Rhizobium* viz, *R.lupini* (*Lupinus*), *R.meliloti* (*Melilotus*, *Medicago*, *Trigonelia*), *R.trifoli* (*Trifolium*), *R .leguminosarum*, *R .japonicum* (*Lathyrus*, *Lens*, *Pisum* and *Vicia*) and *R. phaseoli* (*Phaseolus*) based on their host range.

In the early 1960s, bacteriologists used a large diversity of morphological, nutritional, metabolic, serological and genetic characters in numerical taxonomy and demonstrated the relatedness of *Rhizobium* and *Agrobacterium* and led to a clear distinction between the fast and slow growing rhizobia (Graham, 1964), with the latter group subsequently placed in a separate genus, *Bradyrhizobium* (Jordan, 1982).

In the first edition of Bergey’s Manual of Systematic Bacteriology (Krieg and Holt, 1984) only two rhizobial genera (*Bradyrhizobium* and *Rhizobium*) were described.

Since then, extensive phenotypic and genotypic variations have been described in rhizobia. Rhizobia are devided in to three genera as *Azorhizobium*, *Bradyrhizobium* and *Rhizobium* on the basis of 16S r-RNA
sequence alignment. Sahgal and Johri (2003) outlined the current status of rhizobial taxonomy wherein they have enlisted 35 species which are further distributed among seven genera as shown in Table No. 1.3.

Although most *Rhizobium* isolates can nodulate more than one host species and also several different bacterial species are often isolated from a single legume, it is only from a few legumes that the symbionts have, so far, been investigated thoroughly (Young and Haukka, 1996).

Table No. 1.3: Current status of rhizobial taxonomy
(Sahgal and Johri, 2003)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Genus</th>
<th>Species</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Allorhizobium</em></td>
<td><em>A. undicola</em></td>
<td><em>Neptunia natans</em></td>
</tr>
<tr>
<td>2</td>
<td><em>Azorhizobium</em></td>
<td><em>Az. cauliodans</em></td>
<td><em>Sesbania rostrata</em></td>
</tr>
<tr>
<td>3</td>
<td><em>Bradyrhizobium</em></td>
<td><em>B. elkanii</em></td>
<td><em>Glycine max</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>B. japonicum</em></td>
<td><em>G. max</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>B. liaoningense</em></td>
<td><em>G. max</em></td>
</tr>
<tr>
<td>4</td>
<td><em>Mesorhizobium</em></td>
<td><em>M. amorphae</em></td>
<td><em>Amorpha fruticosa</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>M. chacoense</em></td>
<td><em>Prosopis alba</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>M. ciceri</em></td>
<td><em>Cicer arietinum</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>M. huakuii</em></td>
<td><em>Astragalus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>M. loti</em></td>
<td><em>Loti</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>M. mediterraneum</em></td>
<td><em>Cicer arietinum</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>M. plurifarium</em></td>
<td><em>Acacia, Leucaena</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>M. tianshanense</em></td>
<td><em>Glycyrrhiza, Sophora and Glycine</em></td>
</tr>
<tr>
<td>5</td>
<td><em>Methylobacterium</em></td>
<td><em>M. nodulans</em></td>
<td><em>Crotalaria pedocarpa</em></td>
</tr>
<tr>
<td>6</td>
<td><em>Rhizobium</em></td>
<td><em>R. etli</em></td>
<td><em>Phaseolus vulgaris</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>R. galegae</em></td>
<td><em>Galega</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>R. gallicum</em></td>
<td><em>P. vulgaris</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>R. giardinii</em></td>
<td><em>P. vulgaris</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>R. hainanense</em></td>
<td><em>Centrosema, Desmodium, Stylosanthes, Tephrosia</em></td>
</tr>
<tr>
<td>Rhizobium</td>
<td>Legume or Herb</td>
<td>Vicia, Medicago, Leucaena, P. vulgaris</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>---------------</td>
<td>--------------------------------------</td>
<td></td>
</tr>
<tr>
<td>R. huautlense</td>
<td>Sesbania herbacea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. leguminosarum</td>
<td>Trifolium, Vicia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. mongolense</td>
<td>Medicago ruthenica</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. phaseoli</td>
<td>P. vulgaris</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. sullae</td>
<td>Hedysarum hedysari</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. tropici</td>
<td>Leucaena, P. vulgaris</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. trifolii</td>
<td>Trifolium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. yanglingense</td>
<td>Amphicarpaea, Trisperma, Corollina varia and Gueldenstaedtia multiflora</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sinorhizobium</th>
<th>Acacia Senegal, Prosopis chilensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. arboris</td>
<td></td>
</tr>
<tr>
<td>S. fredii</td>
<td>G. max</td>
</tr>
<tr>
<td>S. kostiense</td>
<td>A. senegal, P. chilensis</td>
</tr>
<tr>
<td>S. medicae</td>
<td>Medicago spp.</td>
</tr>
<tr>
<td>S. meliloti</td>
<td>Medicago sativa</td>
</tr>
<tr>
<td>S. saheli</td>
<td>Sesbania</td>
</tr>
<tr>
<td>S. terangae</td>
<td>Acacia, Sesbania</td>
</tr>
<tr>
<td>S. xinjiangense</td>
<td>G. max</td>
</tr>
</tbody>
</table>

Most recent taxonomic studies are using a polyphasic approach with genetic, phenotypic, chemotaxonomic, phylogenetic data combined to establish a comprehensive picture of the relationships of the bacteria, and to propose a suitable classification (Graham et al., 1991; Vandamme et al., 1996).

The discovery of more diverse rhizobia, lead to a gradual increase in the number of genera. This is because of studies of the many different legume species and their rhizobia. So far, about 20% of the total of 18,000 species and 57% of 650 genera of legume plants have been studied for nodulation (Sprent, 1995). This leaves a large number of legume species yet to be studied and potentially many more species and genera of rhizobia to be described.
Currently, there are 44 recognized species of nodule bacteria on legumes classified in 11 genera, 9 belonging to α-proteobacteria namely, *Allorhizobium, Azorhizobium, Bradyrhizobium, Devosia, Mesorhizobium, Methylobacterium, Ochrobactrum, Rhizobium* and *Sinorhizobium*. Rhizobia have crossed the boundaries where they originally belonged, i.e. α-proteobacteria in the year 2001 when *Burkholderia* spp was described from the nodules of the South African legume *Aspalathus carnosa* and *Ralstonia taiwanensis* in *Mimosa* nodules from Taiwan. Tripathi (2002) also has reported *Ralstonia* from *Mimosa* nodules from India.

In parallel with increase in the number of genera of rhizobia, there has also been significant increase in the number of validly published species, which constitutes now recognized, 48 species of rhizobia as shown in table No. 1.4

Table No. 1.4: Rising number of species in the genera of the rhizobia

(Willems Anne, 2006)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Genus</th>
<th>Number of species before</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Rhizobium</em></td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td><em>Bradyrhizobium</em></td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td><em>Sinorhizobium</em></td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td><em>Azorhizobium</em></td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td><em>Mesorhizobium</em></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td><em>Allorhizobium</em></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>4</td>
</tr>
</tbody>
</table>
The genera *Azorhizobium*, *Bradyrhizobium* and *Mesorhizobium* which have been included in the Family Rhizobiaceae are allocated in the second edition of the Bergey’s Manual of Systematic Bacteriology in the new families Azorhizobiaceae, Bradyrhizobiaceae and Phyllobacteriaceae respectively of which first two families are related with each other but distant from the other families. Rhizobiaceae is a revised family which has two genera namely, *Rhizobium* and *Sinorhizobium*, and are genotypically related to Bartonellaceae, Brucellaceae and Phyllobacteriaceae. Now the genus *Rhizobium* has been extensively revised. All the species of genera *Agrobacterium* and *Allorhizobium* have been placed in the genus *Rhizobium* with amended names.

Up to September 2009 there were 76 validly published species names of rhizobia in 13 genera. Most of these bacterial species are in the Rhizobiaceae family in the alpha-proteobacteria and are in either the *Rhizobium*, *Mesorhizobium*, Ensifer, or *Bradyrhizobium* genera. However recent research has shown that there are many other rhizobial species in addition to these. In some cases these new species have arisen through lateral gene transfer of symbiotic genes (Weir, 2010).

**Rhizobium**

**Mesorhizobium**


**Ensifer (formerly Sinorhizobium)**

The *Sinorhizobium* and the genus *Ensifer* belong to a single taxon genus (Weir, 2010). *Ensifer* is the earlier heterotypic synonym and thus takes priority (Young, 2003). This means that all *Sinorhizobium* spp. must be renamed as *Ensifer* spp. according to the Bacteriological code. The taxonomy of this genus was verified by Martens et al., (2007). The genus currently consists of 15 species namely, *Ensifer abri*, *Ensifer americanum*, *Ensifer arboris*, *Ensifer fredii*, *Ensifer indiaense*, *Ensifer kostiense*, *Ensifer kummerowiae*, *Ensifer medicae*, *Ensifer meliloti*, *Ensifer mexicanus*, *Ensifer adhaerens*, *Ensifer saheli*, *Ensifer terangae*, *Ensifer xinjiangense*, and *Sinorhizobium morelense* has not yet been named as *Ensifer* Martens et al., (2007).

**Bradyrhizobium**

The *Bradyrhizobium* genus currently consists of 8 species namely, *Bradyrhizobium canariense*, *Bradyrhizobium elkanii*, *Bradyrhizobium iriomotense*, *Bradyrhizobium japonicum*, *Bradyrhizobium jicamae*, *Bradyrhizobium liaoningense*, *Bradyrhizobium pachyrhizi*, and *Bradyrhizobium yuanmingense*. 
**Azorhizobium**
The *Azorhizobium* genus currently consists of 2 species namely, *Azorhizobium caulinodans* and *Azorhizobium doebereinerae*.

**Methylobacterium**
The *Methylobacterium* genus currently contains only one rhizobial species namely, *Methylobacterium nodulans*.

**Burkholderia**
The *Burkholderia* genus currently contains seven named rhizobial members and others as *Burkholderia* sp. namely, *Burkholderia caribensis*, *Burkholderia cepacia*, *Burkholderia mimosarum*, *Burkholderia nodosa*, *Burkholderia phymatum*, *Burkholderia sabiae*, *Burkholderia tuberum*.

**Cupriavidus**
*Cupriavidus* formerly *Wautersia*, formerly *Ralstonia*, has recently undergone several taxonomic revisions (Weir, 2010). This genus currently contains a single rhizobial species namely, *Cupriavidus taiwanensis*.

**Devosia**
The *Devosia* genus currently contains only a single rhizobial species namely *Devosia neptuniae*.

**Herbaspirillum**
The *Herbaspirillum* genus currently contains a single rhizobial species namely, *Herbaspirillum lusitanum*.

**Ochrobactrum**
The *Ochrobactrum* genus currently contains two rhizobial species namely, *Ochrobactrum cytisi* and *Ochrobactrum lupine*.

**Phyllobacterium** The *Phyllobacterium* genus currently contains three rhizobial species namely, *Phyllobacterium trifolii*, *Phyllobacterium ifriqiyyense* and *Phyllobacterium leguminum*. 

15
**Shinella**

The *Shinella* genus currently contains a single rhizobial species namely, *Shinella kummerowiae*.

### 1.1.2 A Macrosymbiont

The other partner of symbiotic nitrogen fixation process is a macrosymbiont, a leguminous plant. Legumes are plants of the pea or bean family, the Leguminosae (*Fabaceae* in the USA). These plants are the members of the family Leguminosae. This is an extremely diverse and largest family comprising of 18,000 species classified into around 650 genera (Polhill, 1994), distributed throughout the world. The members include vegetables (Beans, Peas), ornamental trees (Bauhinia, Flamboyant, Cassia), fodder crops (Clover, Lucerne) and weeds (Vetches and Trefoils). Legumes are a significant component of nearly all terrestrial biomes and are used as crops, forages and green manures. They vary from ground cover and aquatic to shrubs, climbers and trees.

It is a family divided into three sub-families: *Papilionoideae*, *Mimosoideae* and *Caesalpinioideae* (Allen and Allen 1981). These subfamilies are sometimes recognized as three separate families: *Papilionaceae*, *Mimosaceae* and *Caesalpiniaceae*. Nodulation is common among the subfamilies Mimosoideae and Papilionoideae but within the Caesalpinioideae subfamily, only few species or genera can nodulate (Sprent 2001).

The subfamily Papilionoideae is the largest and the most widespread one with about two-thirds of all the genera and species of the family. The subfamily Papilionoideae contains approximately 12000-13500 species (Rasanen, 2002). They are mainly herbs, distributed worldwide (Sprent 2001). Ninety seven percent members of the family Papilionoideae were found nodulated (Giller 2003, de Faria et al., 1989). Members of the family Papilionoideae which do not get nodulated were found in primitive tribes. The Papilionoideae contains most of the
important leguminous crop species such as the Soya Bean (*Glycine max*), Common Pea (*Pisum sativum*), Chickpea (*Cicer arietinum*), French Bean (*Phaseolus vulgaris*), Lentil (*Lens culinaris*) and Peanut (*Arachis hypogaea*), etc. The majority of the species are herbaceous, although there are some trees and shrubs, e.g. *Laburnum* and *Gorse* (*Ulex*).

For the most part the Papilionoideae are easily recognized by their characteristic papilionaceous (butterfly-like) flowers. The flower is irregular and is made up of five petals; a ‘banner’ petal, two wing petals, and two petals partially fused together to form a boat-shaped keel. The keel encloses the stamens, which are not visible externally.

Majority of the Mimosoideae members are tropical or subtropical trees and shrubs. The subfamily Mimosoideae is characterised by their small, regular flowers crowded together, generally into spikes or heads. The stamens are the most attractive parts of the flower, the five petals inconspicuous. The leaves are predominately bipinnate. The subfamily Mimosoideae contains approximately 3000 species which are mostly small trees and shrubs of tropical and subtropical regions of Africa, North and South America, Asia and Australia (Rasanen, 2002). Ninety percent members of the subfamily Mimosoideae were found nodulated (Giller 2003). Some species of the genus *Acacia* from Mimosoideae, specifically found in very arid environments, are exception for nodulations (Odee and Sprent, 1992; Sprent, 1994). *Acacia* and *Mimosa* are the important genera within Mimosoideae subfamily. Certain *Acacia* species like *Acacia pycnantha, Acacia melanoxylon* and *Acacia senegal* are extremely important economically.

Most of the members of the subfamily Caesalpinioideae are tropical or subtropical trees and shrubs having irregular (zygomorphic) flowers with five petals which are not differentiated into standard, wings and keel. The stamens are visible externally. This subfamily contains approximately 2500 - 2800 species, which are mainly trees of the tropical savannahs and forests of Africa, South America and Southeast Asia (Rasanen, 2002). Twenty three percent members of this subfamily form nodules and all these are considered to be the most primitive groups (de
Within this family the nodulation is restricted to a few tribes a most notable of which is Cassieae. It forms a bridging group in between nodulating and non nodulating legumes. This tribe has three genera namely, Cassia, Senna and Chamaecrista. Species of the last genus are found nodulated. Some have primitive nodule structures, while some have persistent infection thread in the nodules. Several species in this subfamily are tropical ornamental plants such as Delonix regia and Caesalpinia pulcherrima. Senna alexandrina is a commercially grown medicinal plant, known for its purgative qualities. Several species in the subfamily Caesalpinioideae are well-known tropical ornamental plants. Some of the examples are Delonix regia and Caesalpinia pulcherrima. Senna alexandrina is a commercially grown medicinal plant (ILDIS 2006).

Many varieties of legumes grow naturally in the soil of variable nature in many different regions of the world. These types of legumes are known as wild legumes. Majority of them have a potential of forming symbiotic association with nitrogen fixing bacteria forming nodules at their roots. Such plants help in making barren soil fertile.

Some varieties of legumes are cultivated for some purpose like use as a food, fodder, etc. Such legumes are known as cultivated legumes, e.g. many pulse legumes.

1.2 PULSES, THE IMPORTANT CROPS OF THE LEGUMINOUS FAMILY:

Many plants of the Leguminosae (Fabaceae in the USA) family can form an essential item of the daily diet of the people as well as good forage and animal feed. Pulses constitute the group of such crops of the leguminous family.

Pulses being having comparatively very high protein content and amino acid content are significant food items for non-vegetarians (FAO, 1994). Many pulses are used as leafy vegetables.
Pulses are defined by the Food and Agricultural Organization of the United Nations (FAO) as annual leguminous crops yielding from one to twelve grains or seeds of variable size, shape and color within a pod. (FAO, 1994). The plants of Leguminosae family are of dual use, as a food or fodder and as a fertilizer in increasing soil fertility.

FAO (1994) recognizes 11 primary pulses

- **Dry beans** (*Phaseolus spp.* including several species now in *Vigna*)
  - Kidney bean, haricot bean, pinto bean, navy bean (*Phaseolus vulgaris*)
  - Lima bean, butter bean (*Vigna lunatus*)
  - Azuki bean, adzuki bean (*Vigna angularis*)
  - Mung bean, golden gram, green gram (*Vigna radiata*)
  - Black gram, Urd (*Vigna mungo*)
  - Scarlet runner bean (*Phaseolus coccineus*)
  - Rice bean (*Vigna umbellata*)
  - Moth bean (*Vigna acontifolia*)
  - Tepary bean (*Phaseolus acutifolius*)

- **Dry broad beans** (*Vicia faba*)
  - Horse bean (*Vicia faba equina*)
  - Broad bean (*Vicia faba*)
  - Field bean (*Vicia faba*)

- **Dry peas** (*Pisum spp.*)
  - Garden pea (*Pisum sativum var. sativum*)
  - Protein pea (*Pisum sativum var. arvense*)

- **Chickpea**, Garbanzo, Bengal gram (*Cicer arietinum*)

- **Dry cowpea**, Black-eyed_pea, blackeye bean (*Vigna unguiculata ssp. dekindtiana*)

- **Pigeon pea**, cajan pea, congo bean (*Cajanus cajan*)

- **Lentil** (*Lens culinaris*)

- **Bambara groundnut**, earth pea (*Vigna subterranea*)

- **Vetch**, common vetch (*Vicia sativa*)

- **Lupins** (*Lupinus spp.*)

- **Minor pulses include:**
- Lablab, hyacinth bean (*Lablab purpureus*)
- Jack bean (*Canavalia ensiformis*), sword bean (*Canavalia gladiata*)
- Winged bean (*Psophocarpus tetragonolobus*)
- Velvet bean, cowitch (*Mucuna pruriens var. utilis*)
- Yam bean (*Pachyrizus erosus*)

1.3 SALINE SOILS IN INDIA:

Due to increased population and food demand, need for increasing the agricultural productivity made Government of India to improve the facilities of irrigation by constructing dams on different rivers. This resulted in availability of water for agriculture tempting the farmers in many places for over irrigation.

Problems due to over irrigation of agricultural lands were first time reported in 1858 by Punjab Government and were referred to as ‘reh‘ and ‘usar‘ tracts. In Indian terminology Reh means salt efflorescence, and Usar which has been derived from the Sanskrit word “ustra” means infertile soil. Another alarm regarding the disastrous spread of reh in some other parts of northern India was raised in 1876 (Sharma and Gupta, 1986).

Indiscriminate use of water through the flush system of irrigation and no provision for drainage were some of the causes mentioned in Reh committee report in the 1886. Later on this problem of increasing salt concentrations in soil was seen spread all over the country. In India saline and alkaline soils prevail in Indo-Gangetic basin in the north and are not suitable for raising food and fodder legumes (Subba Rao, 1995). In Maharashtra, the problem has become very severe. The agricultural land of Krishna river basin has been affected due to accumulation of excess of salts in it.

In India out of total geographical area of agricultural land of 329 Mha., 175 Mha has been reported as affected. This include alkali soils and saline soils of the coastal areas also. Agricultural land in various states such
as Madhya Pradesh, Rajasthan, Maharashtra, Karnataka, Andhra Pradesh, West Bengal, Tamil Nadu and Gujarat is affected due to salinity.

Bundela et al. (2009) have reported that the national extent of Salt Affected Soils SAS for India over the last four decades was assessed by conventional and remote sensing approaches using diverse methodologies and class definitions and ranged from 6.0 to 26.1 million hectares (Mha) and 1.2 to 10.1 Mha, respectively.

The Net Cropped Area is 174.73 lakh ha. Much of the agricultural land in Maharashtra is becoming infertile due to the problems of salinity. The Gross Cropped Area in Maharashtra is 225.56 lakh. The state has a large area under alkaline soil and saline soil which is 4.23 lakh ha and 1.84 lakh ha respectively (Compendium, Maharashtra Govt, http://rkvy.nic.in/download/compendium/Maharashtra.pdf, download: 9th Dec. 2010). Agricultural land in the Krishna river basin is the prominent amongst them.

Krishna river originates just north of Mahabaleshwar at Maharashtra in the Western Ghats at an elevation of about 1337 m and flows for about 1400 km and meats into the Bay of Bengal.

Nearly 8% of total geographical area of the country is occupied by the Krishna river basin, which is 10.4% (i.e. 20.3 million ha) of the culturable area of the country. The average total area of the basin of about 258.948 km$^2$, containing mainly black soils, red soils, laterite and lateritic soils, alluvium, mixed soils, red and black soils and saline and alkaline soils lie in the states of Maharashtra, Karnataka and Andhra Pradesh (http://www.indianetzone.com/32/krishna_river_basin.htm).

Sangli and Satara districts from Maharashtra State have appreciable salt affected agricultural lands in the Krishna river basin.

1.4 SYMBIOTIC NITROGEN FIXATION IN SALINE SOIL:

Growth and activity of the N$_2$-fixing plants may occur in varieties of environments. However, extremities of those environments can be limiting factors for the nitrogen fixing ability (Brockwell et al., 1995).
In the *Rhizobium*-legume symbiosis, which is a N$_2$-fixing system, the process of N$_2$ fixation is strongly related to the physiological state of the host plant which is dependant on the environmental stress that plant experiences. Therefore in presence of limiting factors such as salinity, unfavorable soil pH, nutrient deficiency, mineral toxicity, temperature extremes, insufficient or excessive soil moisture, inadequate photosynthesis and plant diseases; a competitive and persistent rhizobial strain is not expected to express its full capacity for nitrogen fixation (Brockwell et al., 1995; Peoples et al., 1995; Thies et al., 1995).

Some of the typical environmental stresses faced by the rhizobium and legume symbiotic partners may include water stress due to marginal lands with low rainfall and poor water-holding capacity, acidity due to acidic soils of low nutrient status, salinity, soil nitrate, extremes of temperature, heavy metals, biocides, etc. (Bottomley, 1991; Walsh, 1995.)

Any one environmental stress can have more than one effect on the total symbiotic nitrogen fixation ability of the plant rhizobial system for e.g. stress due to saline environment can have effect in the form of direct toxicity as well as in the form of osmotic stress, water stress etc..

Some leguminous plants like *Vicia faba*, *Phaseolus vulgaris*, and *Glycine max* have been seen tolerating more salt than legumes like *Pisum sativum* (Zahran, 1991) Salt tolerance of the plants was seen varying from plant to plant. Abdel-Wahab and Zahran (1981) and Cordovilla et. al (1995) have reported that some salt tolerant strains of *Vicia faba* sustained nitrogen fixation under saline conditions.

The legume-*Rhizobium* symbioses and nodule formation on legumes are more sensitive to salt or osmotic stress than are the rhizobia (Zahran and Sprent. 1986; El-Shinnawi et al. 1989; Zahran 1991).
Salt stress caused by 170 mM NaCl affects the nitrogen fixation by causing curling or deformation of soybean root hairs, while NaCl with the concentration 210 mM could completely suppress the nodulation with *Bradyrhizobium japonicum* (Tu, 1981).

Due to salt stress a nodule is distorted which then causes reduction in the rate of N2 fixation by legume (Sprent and Zahran, 1988; Zahran, 1999) in turn reducing the dry weight and N2 content of the shoot.

### 1.5 Rhizobial Occurrence in Saline Soil:

Varieties of rhizobia are found in soil with or without their respective legumes. In symbiosis with the leguminous plants at their root nodules, the macrosymbiont, legume is the bigger partner, while the “microsymbiont” rhizobium is the smaller one. After a period of nitrogen fixation, nodules become senescent and the decay of tissue sets in liberating motile forms of rhizobia into soil. These liberated rhizobia then serve as a source of inoculum for the succeeding crop of a given species of legumes (Subba Rao, 1995).

Although, native rhizobia are present in soil, all of them are not capable of forming nodules on particular plant. The exo-polysaccharides secreted by rhizobial cells at their surfaces along with some plant and bacterial proteins are important for surface interaction during nodule initiation and nodule invasion (Leigh and walker, 1994; Breedveld and Miller, 1994).

Rhizobia occur in soil and in the rhizosphere region of legumes as well as non-legumes. Legumes tend to promote multiplication of rhizobia in soil. In absence of legumes, population of rhizobia in soil declines. In spite of they being non-spore formers, rhizobia survive for 19 to 45 years (Subba Rao, 1995), but the native rhizobial flora gradually lose their efficiency of nodulating and fixing nitrogen in a particular plant. Some of the strains are highly effective in this respect while others are partially or completely ineffective. Certain nitrogen-fixing agents do not perform well and so their nitrogen fixing efficiency remains less though their abundance
is much. Hence, the artificial inoculation with tested effective strains, should be taken up as a means for obtaining optimum yield. This causes enrichment of soil with effective strains which is much beneficial for the crop yield. The results of experiments done on all India level have highlighted that Soybean responds spectacularly to rhizobial application and grain yields are often increased up to 50% over un-inoculated controls since our soils are deficient in specific bacteria capable of nodulating soybean (Subba Rao, 1995).

The environmental conditions of the soil have long been known to be influencing symbiotic nitrogen fixation. Even mild adverse conditions in the environment disturb the delicate balance between the host plant and the symbiont. Out of the major types of environmental stress, salt stress is the one affecting adversely the legume production in arid and semi-arid regions, particularly because these plants depend on symbiotic N2 fixation for their nitrogen requirements (El-Sheikh and Wood 1995).

Increased salinity of soil can limit agricultural productivity by adversely affecting the growth of the host plant as well as the symbiotic development of root-nodule bacteria (Georgiev and Atkins 1993), and thus finally, the nitrogen-fixation capacity (Delgado et al., 1993).

Legumes are more sensitive to soil salinity than their rhizobial counterparts and, consequently, the symbiosis is more sensitive to salt stress than free-living rhizobia (Zahran 1999). Nevertheless, rhizobial tolerance to salinity is also important for the symbiosis (Khadri et al., 2001).

Root nodule forming members of the genera Rhizobium and Bradyrhizobium show marked variation in salt tolerance. NaCl in 100 mM concentration can inhibit growth of a number of rhizobia (Yelton et al., 1983), while some strains of Rhizobium meliloti, were tolerant to 300 to 700 mM NaCl (Embalomatis et al., 1994; Helemish, et al., 1991; Mohammad et al.1991, Sauvage et al., 1983). Some strains of Rhizobium leguminosarum have been reported to be tolerating NaCl concentrations up to 350 mM NaCl in broth culture (Abdel-Wahab and Zahran 1979;
In these cases fast-growing strains showed more
tolerance than slow-growing strains (El-Sheikh and Wood 1990).
Rhizobial strains of soybean and chickpea were tolerant to 340 mM NaCl,
and some strains of *R. leguminosarum* nodulating *Pea* (*Pisum sativum*),
Vetch, Sweet pea have been found to be tolerating NaCl concentration up
to 350 mM (Abdel-Wahab and Zahran 1979), while those of *Vigna*
*unguiculata* were tolerant up to about 450 mM NaCl (Mpepereki et al.,
1997). Rhizobia from woody legumes like *Acacia, Prosopis*, and *Leucaena*
showed very high salt tolerance, tolerating about 500 to 850 mM NaCl

Soybean could be nodulated by *Rhizobium* as well as
*Bradirhizobium* species, while Chickpea could be nodulated by
*Bradirhizobium* species. Fast growing strains of these plants were
observed to be tolerating NaCl concentration up to about 340 mM (El-
Sheikh and Wood 1990). *Vigna unguiculata* was also nodulated by fast as
well as slow growing rhizobia, wherein some of the fast growers could
tolerate NaCl concentration up to 5.5% in broth slow-growing peanut
rhizobia are less tolerant than fast-growing rhizobia (Ghittoni and Bueno
1996).

Many reports have shown that high-salt tolerant strains of rhizobia
are symbiotically more efficient than salt-sensitive ones under saline
conditions (Chien et al., 1992).

As saline soils have their unique microenvironment, this would
work more effectively if the rhizobia tolerating comparatively high salt are
screened from saline soil and are used as legume inoculants in the same
soil along with the pulse legumes.

1.6 RECLAMATION OF SALINE SOILS:

Increase in the salinity of soil in India is a very serious problem
with respect to its use in agricultural productivity. Many workers are
trying to reclaim such soils. Many attempts are being undertaken to
reclaim saline soils. The ways include use of some physico-chemical processes, which being very expensive are not affordable to farmers. The technology but taking some crops along with reclamation is affordable technology to farmers.

1.6.1 Use of pulse legumes:

Age old practice of crop rotation is helping us to increase and to maintain fertility of an agricultural land under use for very long time. A crop of monocotyledon is usually followed by the crop of dicotyledonous plants. If monocotyledonous crops are taken continuously, then because of only excess use of soil nitrogen by these plants, nitrogen content of soil goes on decreasing. To increase the nitrogen content in the same soil it is an age old practice of taking leguminous crops in the same field. Dicotyledonous plants mainly of a leguminous variety were being used during crop rotation. Leguminous plants have an association with nitrogen fixing bacteria forming nodules at their roots.

One of the several ways that can be used for reclamation of saline soil is to grow or to propagate most essential leguminous commodities and through symbiotic nitrogen fixation and green manuring, increase the fertility of the soil. Use of legume is the best way for the enhancement of bio-productivity as well as for the reclamation of the marginal lands. This requires a symbiotic pair of pulse legume and the respective rhizobial partner resistant to existing salinity.

Although pulses were reported as low salt tolerant, Tilak et al. (2005) has reported more salt tolerance in Glycin max and Phaseolus vulgaris ‘Kabuli variety of chickpea has been found to be comparatively more salt tolerant than ‘desi’. Salt tolerance has been seen increased in soybean. Various efforts are being made to increase salt tolerance in some field crops (Rana et al., 1980). This research is definitely offering some good opportunities for better management of salinity problems.

Like many leguminous crops, pulses play a key role in crop rotation due to their ability to fix nitrogen. Pigeon pea in some areas are an
important crop for green manure. They can provide more than 60 kg of nitrogen per hectare.

NIIR Board (2004) estimates of amount of nitrogen fixed (kg/hectare) by various legume constitutes as by Alfalfa (100-300), Berseem (120-150), Chickpea (26-63), Clusterbean (37-196), Clover (100-150), Cowpea (53-85), Blackgram (38-50), Greengram (50-55), Groundnut (112-152), Lentil (35-100), Peas (59-80), Pigeonpea (60-200), Soybean (49-130) and Sesbania (69-90).

Nearly half the annual quantity of biologically fixed nitrogen entering soil ecosystems is contributed by approximately greater than 100 agriculturally important legumes in symbiotic association with their rhizobia (Tate, 1995).

About 23 % of agricultural land in India is under the cultivation of pulse crops. Pulse crops cultivated after cultivation of monocotyledonous plants in such soils not only helps as a diet supplement but also help in restoring soil fertility.

In current studies legume-rhizobium association was taken in to account for reclamation of saline soils and emphasis was given to salt tolerant rhizobia associated with various pulse legumes like pigeon pea (*Cajanus cajan*, syn. *Cajanus indicus*), Common bean (*Phaseolus vulgaris*), Cowpea (*Vigna unguiculata*), lentil (*Lens culinaris*), Green gram, known as mung (*Vigna radiata*), chickpea known as bengal gram or channa (*Cicer arietinum*), White lentils, urd bean known as urid or black gram (*Vigna mungo*), garden pea (*Pisum sativum*), moth bean or known as moat bean (*V. aconitifolia*) and soybean (*Glycine max*).

**Pigeon pea:** The pigeon pea (*Cajanus cajan*, syn. *Cajanus indicus*) is a member of the family Fabaceae. Other common names are red gram, toovar, toor, togari, gandul, congo pea, gungo pea, and no-eye pea. Being a legume, pigeon pea enriches soil through symbiotic nitrogen fixation.

**Common bean:** *Phaseolus* is a genus in the family Fabaceae of about fifty plant species. Common bean is one of the most domesticated species of the genus *Phaseolus* for their beans. *P. vulgaris* is cultivated worldwide in
tropical, semitropical and temperate climates. As a legume, beans provided the nitrogen fixing bacteria which supply essential nutrients to the other crops.

**Cowpea:** The Cowpea (*Vigna unguiculata*) is one of several species of the widely cultivated genus *Vigna*. Four cultivated subspecies are recognized:

A drought tolerant and warm weather crop, cowpea is well-adapted to the drier regions of the tropics, where other food legumes do not perform well. It also has the useful ability to fix atmospheric nitrogen through its nodules, and it grows well in poor soils with more than 85% sand and with less that 0.2% organic matter and low levels of phosphorus. In addition, it is shade tolerant, and therefore, compatible as an intercrop with maize, millet, sorghum, sugarcane, and cotton. This makes cowpea an important component of traditional intercropping systems.

**Lentil:** The lentil (*Lens culinaris*) is a brushy annual plant of the legume family, grown for its lens-shaped seeds. Lentils are relatively tolerant to drought and are grown throughout the world. About half of the worldwide production of lentils is from India, most of which is consumed in the domestic market.

**Mung bean:** Green gram, known as mung in Hindi, is the seed of *Vigna radiata* which is native to India. It is also known as golden gram, green soy and mung bean(s).

**Chickpea:** The chickpea, chick pea, garbanzo bean, ceci bean, bengal gram, chana or channa (*Cicer arietinum*) is an edible legume. Chickpeas need a subtropical or tropical climate and more than 400 mm annual rain. They can be grown in a temperate climate, but yields will be much lower. There are two types of chickpea: Desi - with small, dark seeds and a rough coat (prevailing in the Indian subcontinent, Ethiopia, Mexico, Iran) and Kabuli- with light-coloured, larger seeds and a smoother coat (mainly grown in S Europe, N Africa, Afghanistan, introduced to India only in the 18th cent., Chile) The Desi form is also known as Bengal gram or chana. The Kabuli form is the kind grown e.g. in the Mediterranean today. The desi-type is probably the earlier form.
**Urd bean:** White lentils, Urd, urd bean, urad, urid, black gram, black lentil or white lentil (*Vigna mungo*) is a bean grown in southern Asia. Black gram originated in India where it has been in cultivated from ancient times and is one of the most highly prized pulses of India. It has also been introduced to other tropical areas mainly by Indian immigrants. It is an erect, sub-erect or trailing, densely hairy annual herb. The tap root produces a branched root system with smooth, rounded nodules. The pods are narrow, cylindrical and up to 6 cm long.

**Garden pea:** A pea is the small, edible round green bean which grows in a pod on the leguminous vine *Pisum sativum*, or in some cases to the immature pods. The pea plant is an annual plant, with a lifecycle of a year.

**Moth bean:** The moth bean (pronounced "moat bean") *V. aconitifolia*, also called mat bean or Turkish gram, is a small, drought-resistant annual trailing herb with small yellow flowers and deeply lobed leaves. These legumes are grown especially in dry parts of South Asia for its tiny (3-4 mm) edible beans. Seeds range in color from light brown to dark reddish brown.

**Soybean:** The soybean (*Glycine max*) is an important global crop. Beans are classed as pulses whereas soybeans are classed as oilseeds. Soybeans can grow in a wide range of soils, with optimum growth in moist alluvial soils with a good organic content. Like most legumes, they perform nitrogen fixation by establishing a symbiotic relationship with the bacterium *Bradyrhizobium japonicum* (syn. *Rhizobium japonicum*; Jordan 1982).

1.6.2 Use of rhizobia:

Many microorganisms present in the soil are of great use as potential biofertilisers. Soil microorganisms is a dynamic resource for agricultural production. Diversity and composition of the living community in the rhizosphere is however influenced by both, plant and soil type (Latour et al. 1996).

Depending on the nature and concentrations of organic constituents of exudates at the roots of plants in the rhizosphere, and the corresponding
ability of the bacteria to utilize these as sources of energy, the bacterial community develops in the rhizosphere. Selective enrichment of plant-species-specific microflora can take place in rhizospheric atmosphere. Some of the bacteria penetrate themselves in the roots of the plants and get their requirement of organic compounds and thus they protect themselves also from the competition in the rhizosphere. This phenomenon is seen exploited in soil during nitrogen limiting conditions in soil by legumes from the point of view of N2-fixation (Coutinho et al., 1999, Gualtieri and Bisseling 2000, Lafay and Burdon 2001, Sessitsch et al. 2002). Biological nitrogen fixation through the application of biofertilizers in the field is the only viable alternative key to sustain agricultural productivity.

Biofertilizers are the products containing living cells of different types of microorganisms which have an ability to mobilize nutritionally important elements from non usable to usable form through biological process.

One of the biofertilizers is composed of microbial inoculants or groups of microorganisms which are able to fix atmospheric nitrogen. Use of salt tolerant rhizobia as a bio-fertilizer can be of much help in reclaiming the soil fertility.

So far, saline soils have been studied for their physico-chemical characteristics, and no much work has been undertaken to screen the salt tolerant rhizobia from the salt affected lands from Krishna river basin in Maharashtra that can nodulate pulse legumes in this region. Nodulated leguminous plants do occur in such soils, and many rhizobia nodulating leguminous plants are known to be able to form nodules on the roots of pulses. Hence this project was undertaken to screen salt tolerant rhizobia from the same saline soils, which can nodulate pulse leguminous plants.