2. REVIEW OF LITERATURE

2.1 Nodulation in Legumes

Fred (1932) gave credit to Fuchs for the first report on nodulation on the roots of *Aphaca, Vicia faba* and *Trigonella foenumgraecum*. Around 18000 species of the family Leguminosae were distributed in about 650 genera within three sub families namely, *Papilionoideae, Mimosoideae* and *Caesalpinioideae* (Polhill et al., 1981, Allen and Allen 1981). Except the subfamily Caesalpinioideae, in which only few species or genera were found nodulated, nodulation status was found common among the subfamilies namely, Mimosoideae and Papilionoideae (Sprent 2001).

2.1.1 Nodulation status in Caesalpinioideae –

In the earliest reports of Allen and Allen (1936a, 1936b) only 3.3 percent of the members of the subfamily Caesalpinioideae were found nodulated. Later, Allen and Baldwin (1954) reported nodulation in 33% of the members. In 1976 Allen and Allen made global survey on the nodulation status in the members of subfamily Caesalpinioideae and reported that 30 percent of the members of this subfamily were nodulated. Many workers then reported regarding the members not showing nodulation in the subfamily. This subfamily contains approximately 2500 - 2800 species (Rasanen, 2002). Reports of de Faria et al. (1989) and Giller (2003) indicate that twenty three percent members of this subfamily form nodules and all these are considered to be the most primitive groups. The nodulation was found 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*. Members of the *Chamaecrista* were found nodulated. Some have primitive nodule structures, while some have persistent infection thread in the nodules.

2.1.2 Nodulation status in Mimosoideae –

In the earlier reports Allen and Allen (1936a, 1936b, 1947) reported that 11.8 percent of the members of the subfamily Mimosoideae
showed nodulation. Bedle (1964) reported 68 species from the subfamily Mimosoideae and Papilionoideae as bearing nodules.

Grobbelaar et al. (1967) and Grobbelaar and Clark (1974) reported respectively 17 and 38 species of Mimosoideae as nodulated. Species of *Inga* and *Pentoclethra* also have been reported as nodulated by Norris (1969). In 1974, Corby reported that 62 out of 69 species of Mimosoideae were nodulated. Allen and Allen (1981) reported that 83% of genera and 90% of species of Mimosoideae were found nodulated. About 3000 species which include mostly small trees and shrubs of tropical and subtropical regions of Africa, North and South America, Asia and Australia (Rasanen, 2002) are the members of the subfamily Mimosoideae. Giller (2003) reported that ninety percent members of the subfamily Mimosoideae were found nodulated. The exception for nodulations are some of the species of the genus *Acacia*, specifically found in very arid environments, (Odee and Sprent, 1992; Sprent 1994).

**2.1.3 Nodulation status in Papilionoideae –**

The earliest reports of Allen and Allen (1936a, 1936b, 1947) and Allen and Baldwin (1954) indicated that 85 and 86% species respectively of Papilionoideae were nodulated. Allen and Allen (1961) have reported that 959 species distributed in 175 genera out of 1024 species in the subfamily Papilionoideae were nodulated. Grobbelaar et al. (1964) reported that 37 out of 44 species of Papilionoideae were nodulated. de Souza (1966) reported that all the 40 species of Papilionoideae bore nodules. Grobbelaar et al. (1967) reported that 217 species out of 247 were found nodulated. Norris (1969), Dubey (1972), Grobbelaar (1972), Lim and Ng (1977) and many workers have reported the nodulation in many species of the subfamily Papilionoideae. The subfamily Papilionoideae is the largest one including approximately 12000-13500 species (Rasanen, 2002, Sprent 2001) of which ninety seven percent members 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), Peanut (Arachis hypogaea), etc.

2.2 Work in India-

Joshi (1920) was the first to report nodulation in wild legume named Crotolaria juncea in India. Rangaswami and Oblisami (1962) have reported root nodulation in Crotalaria retusa and C. ternatea.

Varieties of legumes are grown in India under different agro-climatic conditions. Presence of native rhizobia was therefore anticipated in Indian soils. But Sundara Rao et al. (1969) and Subba Rao et al. (1972) have done an extensive survey of nodulation status of legumes, and have reported nodulation in chickpea, pigeon pea, moong bean, soybean and groundnut in India. Subba Rao (1972) have reported that 35 % of Caesalpinioideae, 90% of Mimosoideae and 95% of Papilionoideae were nodulated. Sinha et al. (1971) in their survey on nodulation in Caesalpinioideae in Burdwan and Houghly districts of West Bengal have reported that out of 40 species examined 24 were nodulated. Rewari (1979) also have surveyed nodulation status of pulses in India under ‘All India Coordinated Pulse Improvement Programme’ in 1979. All of them have reported that although presence of native rhizobial varieties was anticipated in Indian soils, at more than 50 % of the cases most of the legumes except groundnuts nodulated poorly.

Gandhi (1986) have reported nodulation in 31 wild legumes which included one species of Caesalpinioideae, seven of Mimosoideae and 23 of Papilionoideae. Paknikar (1987) have reported nodulation in Alysicarpus hamosus for the first time in India. Bajekal (1996) have reported nodulation in wild legumes at Ratnagiri sea cost in India.

Although the level of nitrogen currently needed by high yielding varieties is more than the amount of nitrogen accumulated by green manure crops, NIIR, Delhi (2004) have reported that legume plants like Dhaincha (Sesbania aculeata), Sunhemp, etc. undergo nodulation and can fix about 60 kg of nitrogen per hector of land. Effect has been successfully utilized in green manuring. NIIR (2004) have further reported that such a
green manuring could give higher yields of paddy in Haryana as compared to those from NPK alone.

Nautiyal et al. (1988) have reported that the plants of *Vigna unguiculata*, *Glycine max*, and *G. soja* were nodulated by *Bradyrhizobium* CC-1. Deka and Azad (2006) studied 157 pure isolates of *Rhizobium* obtained from 6 common pulses grown in 6 different places of Assam. They reported nodulation by only 15 isolates in those pulses.

### 2.3 Effect of salinity on life forms

Every living cell contains about 70 to 90% of water in its cytoplasm. It is the most essential constituent of the cell as it is a solvent for solublising all the nutrient without which metabolism is not possible. Concentration of the solute present in and out of the cell determines the colligative property known as osmosis. Increase or decrease in the concentration of the solute at either side can affect adversely the cell. They can affect enzyme actions, enzyme regulations, assembly and disassembly of organelles, membrane structures and functions. The solute concentration of any solution determines the amount of available water content in the system. This available water content can be expressed as water activity ($a_w$) value. Any little change in the water activity of the system can affect physiology of a cell (Kushner, 1978).

Cells exposed to hypo or hyper saline environments show change in their structure and function. Extreme hypo or hyper saline environments can totally deform the cells exposed to them. In hypo saline environments the cells burst, the effect is known as plasmoptysis while, in hyper saline environments, cells undergo shrinkage due to efflux of water from the cytoplasm, the effect is known as plasmolysis (Csonka, 1989), resulting in increase in the solute concentrations of the cell leading to reduced water activity. This brings about the cessation of the metabolic activities of the cell. The ionic species along with the pH change, makes the situation more complicated (Sprent, 1984).

However, some salt tolerant varieties of bacteria as well as plants respond positively to such variations in the salinity in the environments.
They concentrate some compatible solutes intracellularly. Cells also accumulate some osmo protectant solutes such as proline, betaines of glycine and proline, cholines and some structural analogues of betaines (Csonka, 1989).

Subba Rao (1976, 1979, 1982, 1984, 1995) and Alexander (1977) have clearly explained that Nitrogen is very essential and important constituent of the living world.

Sprent and Sprent (1990) have clearly mentioned in their reports that considering the proportion of saline soils in the world, very little efforts have been expended on examining the effect of salinity on nitrogen fixation.

Chien et al. (1992) in their studies related to the effect of salt on plants as well as rhizobia have reported that most crops are sensitive to relatively low levels of salinity. Further they have added by reporting that the effect can be even more in case of legumes as both the partners micro and macro symbiont are sensitive to salt. There are earlier reports by Zahran and Sprent (1986) that rhizobia and the legume plants are sensitive to salt at both the stages i.e. at free living and at symbiotic stage.

Reduced symbiotic nitrogen fixation due to the toxic effect of salts on rhizobia has been reported by Bernstein and Ogata, (1966) and Bhardwaj, (1975). Symbiotic nitrogen fixation is an associated activity involving a plant (especially leguminous) as macrosymbiont, a rhizobial variety as a microsymbiont and their unit activities like infection to root hairs, development of nodules, functioning of enzymes in the nodules. When all this is to take place in salt affected soil then every separate unit of a system may get affected with less or more degree.

2.4 Occurrence of legumes in saline soils.

Yadav and Singh (1970) have reported about the occurrence of nodulated species of *Acacia*, *Albezia*, *Butea*, *Dalbergia*, *Pithecellobium* and *Prosopis* in saline soils having up to 0.45% of salt. *Sesbania cannabina* and *S. aculeate* have been obtained from saline soils by Bhardwaj (1972,
There are also reports of Eskew and Ting (1978) regarding discovery of nodulated species of Prosopis in Sonoran desert in Colorado. Felker et al. (1981) have reported about the number of nodulated shrubs and trees of Prosopis growing in a variety of harsh saline environments like sea shores, salt lakes and salt deserts. Singleton et al. (1982) have found species of *Indigofera, Mimosa and Canavalia* in the beach sand of high salinity.

Bala et al. (1990) also have found nodulation in the varieties of legumes such as *Acacia, Dalbergia, Leucaena, Pithecellobium, Prosopis* and *Sesbania* in agricultural land rendered saline.

Mohite et al. (1990) reported that leguminous halophyte namely, *Derris trifoliata* showed nodulation in mangrove. Craig (1989) and Craig et al. (1991) also have reported nodulation in case of *Acacia* in the saline regions of Australia and also have indicated about their potential use in saline affected soils.

Rogers et al. (1997) have demonstrated that there is much variation in the range of salt tolerance of pasture legumes ranging from highly sensitive to moderately sensitive. Wooly clover (*Trifolium tomentosum*) and *Trifolium squamosum* were significantly more salt tolerant than berseem clover cv. BigBee and subclover cv. Clare. However, they reported that berseem clover is a major species widely utilized in salt prone areas in many overseas countries. Nobel (1987) also have reported that Persian clover is a marginally more salt tolerant than subclover.

Bajelkal (1996) found nodulated legume species of *Desmodium, Geissaspsis, Phaseolus* and *Zornia* capable of tolerating cvariable salinity at costal region at Ratnagiri.

Hall and Evans (2001) have found that *Trifolium clusii* and *Trifolium salomonium* were the two of the best performing species in acid and saline environments. They also have demonstrated potential use of *Trifolium incarnatum, Melilotus spp., Trigonella balansae*, spotted medic (*Medicago Arabica*) and *Trifolium isthmocarpum* in saline alkaline soils.

Naidu and Uday Bhaskar (1998) have shown that there are some pulses and legumes like peas, grams, which are low salt tolerant, but Dua
(1992) have reported that *Cicer arietinum* var kabuli has relatively more salt tolerance to salinity than the ‘desi’.

Rana et al. (1980) have indicated role of polyploidy breeding in evolving crop varieties suited to problem in saline soils and have reported that breeding crop varieties tolerant to salinity offers significant opportunities for better management of areas with salinity problem. Rao and Sharma, (1995) have reported that the rhizobial strains most effective in non-saline conditions were also the most effective in salt-affected soil.

Tilak et al. (2005) have reported that legumes like *Vicia faba*, *Phaseolus vulgaris* and *Glycine max* are more salt tolerant than others such as, e.g. *Pisum sativum* and so can occur in saline soils.

Bajekal (1996) isolated sixteen salt tolerant fast growing rhizobial strains from twelve different wild legume species collected from a saline environment along the Ratnagiri sea cost.

### 2.4.1 Effect of salinity on Leguminous Plants:

Many workers have worked for studying effect of NaCl on legumes. Most of them have reported adverse effect. Dua (1992) have reported it in chick-pea, Singleton and Bohlool (1984) in soybean and reports regarding faba-bean have been given by Zahran and Sprent, (1986) and Cordovilla et al., (1996).

Abrol et al. (1988) in their book on management of salt affected soils have clearly mentioned that although some amount of water remains in the root zone of crops, excess of soil salinity due to increased osmotic pressure of the soil solution renders less water available to plants. Not only this but excessive concentration and absorption of individual ions may prove toxic to the plants and may retard the absorption of other essential plant nutrients. All this causes adverse effect on growth of the plants and so on the yield. Extent of effect depends on the salinity of the soil. They have made a mention that the legume bean can give 25 % less yield in the soil with ECe 4 dS/m in cool location and 3 dS/m in hot location. Murthy and Janardhan (1971) in their studies on salt tolerance of plants have clearly indicated that the yield reduction of the crops with increasing salinity was much more in the dry than in the wet season.
Research with the plants in saline soil indicate that salinity can limit plant growth in case of legumes. Among some of them, Rout and Shaw (2001) and Rao et al., (2002) showed that the effect is due to both hyper-ionic and hyper-osmotic stress effects as well as due to depressed symbiotic performance; while Delgado et al., (1994); Soussi et al., (1998), (1999); Ferri et al., (2000) showed the same due to diminished photosynthetic efficiency, nitrogen fixation and carbon metabolism in legumes.

Murthy and Janardhan (1971) have worked on salt tolerance of plants and have indicated that the reduction in the crop yield can be due to salinity.

2.5 Occurrence of rhizobia in saline soils.

Brockwell et al. (1995); Peoples et al. (1995); Thies et al. (1995) have reported that under stressful conditions like salinity competitive and persistent rhizobial strain do not express their full capacity for nitrogen fixation.

Abdel-Wahab and Zahran (1981) and Cordovilla et. al. (1995) have reported sustenance of nitrogen fixation in salt tolerant strains of *Vicia faba* under saline conditions indicating occurrence of rhizobia in saline soils. Zahran and Sprent. (1986); El-Shinnawi et al. (1989); Zahran (1991) have reported that the nodule formation on legumes is more sensitive to salt or osmotic stress than are the rhizobial isolates.

Basha and Vivekanandan (2001) have reported that rhizobial strains from extreme environments like coastal saline soil in Tamil Nadu, India, were isolated and characterized through root nodule-trap method. The strains were tested in *Vigna radiata* and *V. unguiculata* against different levels of artificial salinity in pots. Saline soil rhizobia performed well against salinity as determined by nodulation test and total nodular nitrogen content. The presence of leg haemoglobin and nitrogen content of the nodules indicated that in spite of higher salinity, the rhizobial strains were able to fix some amount of atmospheric nitrogen. They further reported that rhizobial strains isolated from extreme environments may be
an ideal solution to overcome the salinity problem with simultaneous enrichment of soil nitrogen through symbiotic nitrogen fixation.

Chen et al. (1995) have reported about isolation and characterization of 20 different strains of root nodule bacteria isolated from an arid saline desert soil in the Xinjiang region of northwestern People's Republic of China.

Lloret et al. (1995) for his studies on osmotic pressure and ionic stress used salt tolerant *Rhizobium meliloti* isolated from nodules of a *Melilotus* plant growing in a salt marsh in Donvana National Park (southwest Spain). They reported this strain to be growing at NaCl concentrations of up to 500 mM.

Botsford (1984) and Breedveld et al. (1990) have reported several strains of Rhizobia along with *Rhizobium meliloti* to be growing at high concentration of salt.

Lal and Khanna (1994) have isolated 35 Rhizobial isolates of *Acacia nilotica* from different agro-climatic zones in 1994 and have reported that two isolates viz, ANG4 and ANG5, tolerated up to 850 mM NaCl, while ANG3, was sensitive to NaCl above 250 mM.

### 2.5.1 Effect of salinity on rhizobia:

Steinborne and Roughley (1975) also have shown that the growth of both *R. trifolii* and *R. meliloti* was slowed by the addition of salt in broth. Singleton et al. (1982) studied effect of NaCl on rhizobial isolates from legumes growing on beach sands and salt affected irrigated fields. They found that the growth of all but one of the rhizobial isolate was slowed by the presence of NaCl. Jenkins (2003) studied response of 16 rhizobial isolates to NaCl in YEM broth with 2mM, 100mM, 300mM and 500 mM (2.9%) of NaCl concentrations in it. He found that 8 rhizobial isolates out of 16 showed adverse effect on growth with increase in NaCl concentration, while six showed increase in extent of growth up to 300 mM (1.74%) NaCl. However, all the 16 isolates were sensitive to 2.9% of NaCl concentration. Nagales et al. (2002) and Thrall et al. (2008) also have reported that increasing salt concentrations have a detrimental effect on rhizobial populations.
Research of Aparicio-Tejo and Sanchez-Diaz (1982); Dejong and Phillips (1982); Huang et al. (1975) and Sprent (1972) had given a common observation that the adverse effects on di-nitrogen fixation capacity and hence the productivity of the whole legume plant is due to sensitivity of rhizobial isolates from soil to a soil water deficit.

Working on the effects of salinity and sodicity upon nodulation and nitrogen fixation in chickpea (Cicer arietinum) Rao et al. (2002) found that nodule number and nodule biomass in Cicer arietinum decreased very much with increasing salinity.

Bekki et al., (1987) reported considerable inhibition of nodulation and N2-fixation in case of legumes under saline conditions. According to Elsheikh and Wood (1990b), although legume plants are adversely affected by NaCl, nodulation and nitrogen fixation are apparently more salt sensitive than is growth. During their work they observed that nodulation and nitrogen fixation were reduced even by the low NaCl concentration, while plant dry weight was affected only by 100 mM.

The adverse effect resulting in to limited growth of the leguminous plant due to high salinity shown by Delgado et al. (1993) is due to depressive effect on nitrogen-fixation capacity which according to Georgiev and Atkins (1993) is actually due to decreased symbiotic development of root nodule bacteria. This shows that the adverse effect on the leguminous plant is also due to effect of salinity on the symbiotic partners of the plants. Both the symbiotic partners, a plant and the rhizobial microsymbiont are important in the symbiotic association. However, Zahran (1999) reported that legumes are more sensitive to salinity than their rhizobial counterparts and so the symbiotic association is more sensitive to salt stress than only free-living rhizobia.

Kulkarni and Nautiyal (1999) have reported about the rhizobia of P. juliflora who could be found in alkaline soil, and tolerate up to 32% NaCl for up to 8 h, 55°C up to 3 h, and 45°C with salt at pH 12. Kulkarni and Nautiyal (2000) have reported that high salt tolerance of rhizobia aids in tolerance to high pH and temperature also.

Surange et al. (1997) and Hafeez et al. (1991) have reported that rhizobia of Albizia lebbek could survive 50°C and 5% NaCl. They also
have reported that rhizobia of *Sesbania formosa*, *Acacia farnesiana* and *Dalbergia sissoo* could tolerate 5% of NaCl and could grow at pH 12.

Kulkarni and Nautiyal (2000) found that morphology of the *Rhizobium* can be changed due to salt stress. They further reported that the length of *Rhizobium* sp. in salt-stressed cells can be increased significantly. They also studied effect of salt and pH stress on temperature-tolerant *Rhizobium* sp. NBRI330 isolated from root nodules of leguminous plants growing in alkaline soil.

Bhardwaj (1975) showed that the salinity and alkalinity tolerance limits of *Rhizobium* and *Bradyrhizobium* spp. are much higher than those of the legume host and so the salt tolerance of the host plant decides the possibility of a successful symbiosis.

Tilak et al. (2005) have reported that slow growing rhizobia of *Glycine max* were inhibited at 100 mM NaCl, while various strains of *Sinorhizobium meliloti* and *R. leguminosarum* grow at more than 300 mM NaCl. They also have reported the tolerance of some tree rhizobia to up to 500 to 800 mM of NaCl.

Rai and Prasad (1986) have reported the NaCl tolerance of fast growing rhizobial isolates to up to 400 mM (2.32%) concentrations of NaCl.

Pillai and Sen (1973) have reported that the growth of rhizobia of *Dolichus lablab* got enhanced in presence of 1% of NaCl. Yadav and Vyas (1973) and Chaudri et al. (1992) have reported the maximum NaCl tolerance of some fast growing rhizobial isolates to up to 200 mM (1.16%) concentrations. Paknikar (1987) have reported tolerance to 2.5% of NaCl in rhizobia of wild legumes of *Vigna* species, while Lal and Khanna (1994) have reported salt tolerance up to 600 mM of NaCl in the rhizobia of *Vigna* species.

Bajekal (1996) reported that the NaCl tolerance limits of the sixteen fast growing rhizobial isolates of wild legumes from saline environments of Rantagiri sea coast were ranging from 3.5% to 7.0%.

### 2.6 Salinity and symbiotic nitrogen fixation by legume- rhizobial association.
Rhizobium and plant association needs development of nodules. Soil salinity can have effect on formation and functioning of nodules. Reports are conflicting, as some workers have shown adverse effects of salts on this property, while according to some workers the effect is positive or negligible. While working with *Rhizobium meliloti* and *Medicago sativa* Subba Rao et al. (1972) reported that nodulation is delayed at 0.4% of NaCl while is inhibited at 0.7% of NaCl. Similar results were obtained with KCl and MgCl$_2$. Subba Rao et al. (1974) found inhibition of nodulation in *Medicago sativa* by carbonate and bicarbonates even in low concentration. Balasubramanian and Sinha (1976) reported that although nodule number in case of *Medicago* and *Vigna sinensis* and *V. aureus* is decreased their size is increased. They further reported these nodules failed completely to fix nitrogen in chickpea and mungbean, but not in cowpea. Delgado et al. (1993) have further added in their conclusion saying that saline stress not only affects these physiological responses but also accelerates greening of the nodules due to lowering of the leg hemoglobin content in the nodules.

Due to low tolerance of grain legumes to salinity and high sensitivity of symbiotic nitrogen fixation to similar stress, the grain legumes are adversely affected in saline soils. About forty years ago Bernstein and Ogata, (1966) during their studies on effect of salinity on nodulation, nitrogen fixation and growth of soybeans and alfalfa have shown that activity of nodules, fully formed under non-saline condition, is retarded if the plant is grown under saline condition. His observations were supported by Yousef and Sprent, (1983) who studied effects of NaCl on growth of *Vicia faba* (L.) and have reported that the proportion of active nitrogen fixing nodules is decreased under saline condition. Singleton and Bohlool, (1984) also have studied the effect of salinity on nodule formation by soybean in detail and have reported that colonization of root surface by soil rhizobia is not affected but the initiation or growth of new nodules which include infection of root hairs by rhizobia and then subsequent nodule development are affected due to salinity.

Yousef and Sprent (1983), Zahran and Sprent (1986) and Sprent and Zahran (1988) worked on the effect of salt on legumes in Egypt. They
found that timing of exposure of legumes to salinity plays an important role. Similar results have been obtained by Udayasuriya et al. (1985) in *Glycine max* and by Bhardwaj (1974) and Rai et al. (1985) in case of *Lens esculenta*.

More than one abiotic factors like temperature, pH are also affecting nodulation process. While working with *Pisum sativum*, Kumar and Garg (1980, 1981) have reported combined effects of salinity and pH. They have shown decrease in nodulation with increase in salinity and pH. Even after plants are well nodulated, their nodules show early senescence when shifted to saline alkaline conditions. If pH is lowered then the effects are seen reduced considerably. Kumar and Promila (1983) in *Cicer* rhizobium and Siddiqui et al (1985) reported that rhizobia affected adversely are recovered faster after desalinization. Reports are also on symbiotic association in between *Cajanus cajan* and its rhizobium indicating that the association is sensitive to increasing salinity.

Lal and Khanna (1994) were working with three rhizobial isolates namely, ANG4 and ANG5, and ANG3. In all these isolates they found decrease in a nitrogenase activity with increasing concentration of NaCl up to 150 mM. They have even showed that the nodulation by ANG4 and ANG3 have been decreased to a 15 % and 100 % respectively at 75mM and 100 mM of NaCl. ANG4 and ANG5 could retain 25% and 15% nitrogenase activity respectively, even at 100 mM NaCl. They could further conclude that Salt-tolerant Rhizobium isolates can nodulate and fix N2 in saline soils.

Nosheen et al. (2004) tried growth of *Vigna radiata* under salt stress and showed that there is adverse effect on the root and shoot dry mass of the plant due to saline conditions. They reported this effect due to decrease in the number and the total weight of the nodules per plant of *Vigna radiata* (L.) with increase in NaCl concentration. By treatment with 0.2 % of NaCl they found that the nodule number is decreased to 54 to 36%. Although number was decreased they found increase in the size of the nodules. They also showed that due to substantial decrease in number and weight of pods per plant, the reproductive growth of Mung bean was affected adversely.
Yousef and Sprent (1983) grew *Vicia faba*, while Mirza and Tariq grew *Sesbania sesbanae* [1992] and *Cicer arietinum* and *Trifolium alexandrinum* [1993] under salt stress. All of them studied effect of salt stress on the growth of these plants in terms of number of nodules per plant. In their studies they found that the number is decreased very much in response to the increasing salt concentration. They further observed that the adverse effect of salinity on nodule number was partially compensated by an increase in nodule size.

While studying the effect of salt on different aspects of plants, Elshiekh and Wood (1990), Sheokand et al. (1995) and a majority of other workers have observed a reduction in extent of nodulation under salt stress. However, reports of Soussi et al. (1999), Cordovilla et al. (1999) and Garg and Singh (2004) indicated increase in nodule growth which subsequently led to an increase in nodule dry mass and all these effects were pertaining to salt tolerant varieties of legumes.

Work of Serraj (2002) and many others have pointed that symbiotic nitrogen fixation by legumes is sensitive to environmental stresses particularly salinity.

Velagaleti and Marsh, (1989) used ‘William’ a salt-sensitive soybean cultivar and salt tolerant and sensitive strains of *Bradyrhizobium* for studying the influence of host cultivars on the growth and symbiotic performance of soybean under salt stress. There they observed that irrespective of salt tolerant or sensitive strain of *Bradyrhizobium* used for inoculation of soybean, ‘William’ performed poorly in saline soils. Similarly El-Sheikh and Wood, (1995) also have shown that ‘Manchu’ a salt tolerant cultivar of soybean sustained nodulation irrespective of the salt tolerance of the strain of bacterium; and they did not notice any significant difference in shoot or root dry weight, nodule number or nodule weight after using a salt-sensitive *Bradyrhizobium* strain or a salt-tolerant *Rhizobium* for inoculation. But Saxena and Rewari (1992) during the studies on *C. arietinum* had concluded that the yields of the grain legume could be improved substantially in saline soils by selecting for both a salt-tolerant host and an appropriate *Rhizobium* strain. Rao and Sharma, (1995)
studied combination of a single host with eight individual strains of rhizobia and then concluded that the rhizobial strains most effective in non-saline conditions were also the most effective in salt-affected soil.

2.7 Saline Soils in India.

Naidu and Uday Bhaskar (1998) have taken a survey of Indian agricultural land and have reported that in India out of the total geographical area of 329 million hectares (Mha.), 175 Mha. is considered as affected, in which, alkali soils and saline soils including coastal areas account for 3.6 Mha. and 5.5 Mha. respectively. Madhya Pradesh, Rajasthan, Maharashtra, Karnataka, Andhra Pradesh, West Bengal, Tamil Nadu and Gujarat are the major states affected due to saline soils. Parts of black soil areas of Gujarat, Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu have alkali soils also.

Nearly 50 percent of the irrigated land in the arid and semi-arid regions have problems due to soil salinization. This figure indicates the magnitude of the problem that must be solved in order to meet future food needs of the world population.

Naidu and Uday Bhaskar (1998) have given some approaches for reclaiming saline soils in India. In their report on reclamation of saline soils, they have clearly mentioned some of the reasons of degrading soils of India. According to them one of the reasons for becoming soils saline is use of salty water for irrigation purpose. The extent of damage to soil depends upon the quality and quantity of salts and on the type of soils on which the water is to be used. In Maharashtra one more reason is an excessive applications of irrigation water that permits continuously the accumulation of salts at the surface due to capillary action and evaporation. If the quality of the subsurface water is saline, the accumulation rate would be much faster. Many saline soils are formed due to inadequate and impaired drainage. They have further mentioned that if the electric conductivity (EC) of saturated soil extract is more than 4 dS/m, exchangeable sodium percentage (ESP) is less than 15 and pH is less than 8.5 then such soils are called “saline soils” or “white alkali” or “Solonchack” soils. The EC values are indicative of the severity of the
problem. In alkali soils or sodic soils, the exchangeable sodium percentage (ESP) is greater than 15 as a result the pH is more than 8.5 and the electric conductivity (EC) is below 4 ds/m. If EC value is above 4 ds/m then the soils are known as saline alkaline soils. While discussing on amendments on the reclamation of saline soils Abrol et al. (1988) have mentioned that saline soils are dominated by neutral soluble salts. At high salinities sodium chloride is most often the dominant salt although calcium and magnesium are present in sufficient amounts to meet the plant growth needs. Graham and Parker (1964) while studying salt tolerance of rhizobia observed high leaching losses of nitrogen as NO₃ and decreased nitrification rates due to high salinity, influence the nitrogenous fertility for crops grown in saline soils. Michiels et al. (1994) have studied Effects of temperature Stress on Bean-nodulating Rhizobium Strains. In that they used heat-tolerant (CIAT899) and a heat-sensitive (CNPAP512) strains of bean-nodulating Rhizobium. They have reported that heat tolerant strain could tolerate 45°C. Bundela et al. (2009) have reported that the extent of Salt Affected Soils in India assessed by conventional and remote sensing approaches ranged from 6.0 to 26.1 Mha. and 1.2 to 10.1 Mha. respectively.

2.7.1 Nodulation in saline soils-
Rao et al. (2002) have worked on the effects of salinity and sodicity upon nodulation and nitrogen fixation in chickpea (Cicer arietinum) and reported that the nodulation property of these plants can be affected adversely by increase in soil salinity.

Subba Rao et al. (1972) have worked on effect of NaCl, KCl as well as MgCl₂ on the nodulation in by Rhizobium meliloti in Medicago sativa and have reported that nodulation in these plants is delayed at 0.4% while is inhibited at 0.7% of salt. Subba Rao et al. (1974) have also found inhibition of nodulation in Medicago sativa by carbonate and bicarbonates even in low concentration. Balasubramanian and Sinha (1976) have studied nodulation in case of Medicago and Vigna sinensis and V. aureus and have reported that nodule number in these plants in decreased under
saline environment but their size is increased. They also have worked on the nitrogen fixation ability under saline conditions and have reported that in chickpea and mungbean nitrogen fixation can not occur in saline environments but in case of cowpea it can occur.

Kumar and Garg (1980, 1981) have reported adverse effect of salinity on nodulation in *Pisum sativum*. They also have reported that even after nodulation salinity can cause early senescence.

Lal and Khanna (1994) have reported decrease in the levels of nitrogenase in rhizobial isolates namely, ANG4 and ANG5, and ANG3 due to increase in salinity up to 150 mM of NaCl. They also have reported 100% inhibition in the nodulation at 100 mM of NaCl in case of ANG4 and ANG3. They could also report that Salt-tolerant rhizobia could nodulate and fix nitrogen in saline soils.

Arun and Sridhar (2004) have obtained salt tolerant rhizobia of *Vigna unguiculata* as well as of *Vigna mungo* from the coastal sand of west coast of India. Bhardwaj, (1974) have reported that rhizobia having tolerance to Saline-alkaline soil of pH 10.3 could be obtained from Indian clover (*Medicago parviflora*), Dhaincha (*Sesbania aculeata*), Berseem (*Trifolium alexandrium*), Guar (*Cyamopsis tetragonoloba*), Cowpea (*Vigna sinensis*) and lentil (*Lens esculenta*).

Garg and Singh (2004) worked on desi and kabuli cultivars of chickpea and have reported that in salt-affected soils, salt tolerant cultivars have more efficient nodulation and support higher rates of symbiotic nitrogen fixation than the sensitive cultivars. They have further stressed the need of introducing salt-tolerant varieties of legumes for improving agricultural production in saline soils.

Rao et al. (2002) also have reported about salt tolerant as well as salt sensitive varieties of Chickpea (*Cicer arietinum*) in India at Central Soil Salinity Research Institute (CSSRI, Indian Council of Agricultural Research), Haryana. They also have studied on nodulation and nitrogen fixation in Chickpea (*Cicer arietinum*).

2.8 Pulse legumes.
Pulses are important food crops due to their high protein and essential amino acid content. Like many leguminous crops, pulses play a key role in crop rotation due to their ability to fix nitrogen.

Most of the pulses are the members of family Leguminosae (Fabaceae), and have been classified in to sub family Papilionoideae (Faboideae). This sub family is the largest of the three sub families not only in the number of the genera and species but also in the number of the members forming nodules (Allen and Allen, 1981; de Faria et al., 1989)

Almost all the pulses are classified under the sub family Faboideae which was previously known as Papilionoideae. As given by Aids (1993) pulses under study are classified as given in introduction.

2.9 Salinity and pulse legumes.

Maas and Hoffman (1977) have made a review of data on tolerance of crops in relation to growth stage and showed that the salt tolerance of soybean could either increase or decrease between germination and maturity depending on the crop variety.

Efforts are being made in different parts of the world to induce, by various means, tolerance to salinity in some field crops. Rana et al. (1980) indicated the promising role of polyploidy breeding in evolving crop varieties suited to problem soils. Breeding crop varieties tolerant to salinity offers significant opportunities for better management of areas with salinity problem.

Indicating wide variation in the ability of plants to tolerate salts in the soil, Abrol et al. (1988) have clearly indicated the need of salt tolerant crop plants to be used in saline soils. Ratings of salt tolerance of plants are based on yield reduction on saline soils when compared with that on similar non-saline soils. The plants to be grown in saline soil should have at least some salt tolerance. However, tolerance to salinity is not a fixed property of a species and is dependant up on factors like climatic conditions, growth stage and species difference of the crop.
Although many workers have reported presence of wild legumes in saline soils, not many reports are available on the cultivation of pulse leguminous crops in them. However some research on the salt tolerant pulse legumes is being done. Majority of this type of work is being carried out in arid and semi-arid regions of the world.

Dua (1992) reported that the ‘kabuli’ variety of chickpea is more tolerant to salinity than the ‘desi’. while, Naidu and Bhaskar (1998) reported that legumes like peas are low salt tolerant,

Garg and Singh (2004) worked for comparing the relative salt tolerance of both desi and kabuli cultivars of chickpea in terms of nitrogen fixation and carbon metabolism. Their results suggest that in salt-affected soils tolerant cultivars have more efficient nodulation and support higher rates of symbiotic nitrogen fixation than the sensitive cultivars.

Along with many other pulses chickpea is also an important legume crops for human nutrition grown in arid and semi-arid regions. Ashraf and Waheed, (1993) reported this chickpea to be a salt-sensitive variety. Van der Maesen, (1987) reported that in India two types of cultivars are grown, a native (desi) type and a mediterranean (kabuli) type. As the chickpea is indigenous to arid areas, some genotypes may have some degree of salt adaptation. Soussi et al., (1999); Rao et al., (2002), reported the same from their studies. Serraj, (2002) reported that there is difference in salt tolerance not only in different species but also in different genotypes of the same species. Genotypic variability amongst the desi and kabuli cultivars, with kabuli showing higher tolerance, have been reported recently by Rao et al. (2002).

Growth of salt sensitive genotype of *C. arietinum*, which was used by Lauter *et al.* (1981) could be inhibited even at 20 mM NaCl (EC approx. 2 dS m\(^{-1}\)). Rao et al. (2002) have used a salt tolerant variety of *Cicer arietinum* CSG 8962 in their studies. They observed the threshold salinity level in the form of ECe value (highest ECe at which growth is not significantly depressed with respect to a non-saline control) was 6.2 dSm\(^{-1}\). It means there can be salt tolerant pulse legumes. Salt tolerant as well as salt sensitive varieties of Chickpea (*Cicer arietinum*) are available in India at Central Soil Salinity Research Institute (CSSRI, Indian Council of
Agricultural Research), Haryana. Rao et al. (2002) for their work on studies on effects of Salinity and Sodicity upon Nodulation and Nitrogen Fixation in Chickpea (*Cicer arietinum*) have collected seed samples from the same.

Naidu and Bhaskar (1998) have suggested some approaches for reclamation of saline soils. Those include leaching, drainage and management practices.

Tilak et al. (2005) have reported that response of legumes as well as rhizobia to salinity varies greatly. Some legumes like *Vicia faba, Phaseolus vulgaris* and *Glycine max* are more salt tolerant than others such as, e.g. *Pisum sativum*.

Zahran (1999) in his studies on “*Rhizobium*–legume symbiosis and nitrogen fixation”, have mentioned several *Rhizobium* species from salt-stressed soils in India and around the world. Following is the table No.2.1 indicating some of the hosts from which rhizobia, tolerating different abiotic factors, are mentioned.

**Table No. 2.1.** Tolerance of rhizobia to abiotic stresses (Tilak et al. (2005))

<table>
<thead>
<tr>
<th>Stress</th>
<th>Host from which isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline-alkaline soil, pH 10.3</td>
<td>Cowpea (<em>Vigna sinensis</em>) and lentil <em>Lens esculenta</em> (<em>Bhardwaj, 1974</em>)</td>
</tr>
<tr>
<td>Saline soil</td>
<td>Soybean (<em>El-Sheikh and Wood, 1995</em>)</td>
</tr>
<tr>
<td>Tolerant to 3% NaCl</td>
<td>Chickpea (<em>Cicer arietinum</em>) (<em>Rao and Sharma, 1995</em>)</td>
</tr>
</tbody>
</table>

**2.10 Salinity and rhizobia associated with pulse legumes.**

Initially many workers have reported that rhizobia do not tolerate salt. Amongst some of them are Pillai and Sen (1966) who reported the inhibition of growth of *Rhizobium trifolii* by 0.5 to 0.7 % of NaCl. Similiar
results for *Rhizobium leguminosarum* have been obtained by Pandher and Kahlon (1978).

Tilak et al. (2005) during their studies on salt tolerance of different rhizobia have reported that the tolerance differs with the rhizobial species. Number of strains of *Bradyrhizobium japonicum* are inhibited at less than 100 mM NaCl, various strains of *Sinorhizobium meliloti* and *R. leguminosarum* grow at more than 300 mM NaCl. Rhizobia isolated from woody legumes like *Hedysarum, Acacia, Prosopis* and *Leucaena* can tolerate up to 500 to 800 mM of NaCl. In the studies on effects of salt and pH stress on temperature-tolerant *Rhizobium*, Kulkarni and Nautiyal (2000) have reported that high salt tolerance aids in tolerance to high pH and temperature also.

Arun and Sridhar (2004) have obtained salt tolerant rhizobia of *Vigna unguiculata* as well as of *Vigna mungo* from the coastal sand of west coast of India. They have isolated salt tolerant rhizobial isolate of four cultivated legumes, cowpea (*Vigna unguiculata*), green gram (*Vigna radiata*), black gram (*Vigna mungo*) and horse gram (*Macrotyloma uniflorum*), from the coastal sand of west coast of India. They have assessed symbiotic efficiency of these isolates. Several *Rhizobium* species have been reported by Zahran (1999) from salt-stressed soils in India and around the world.

Yadav and Vyas (1971a) studied response of root nodule bacteria of black gram (*Vigna mungo*) and green gram (*Vigna radiata*). They reported that salt resistant strains of black gram and green gram were sensitive to 6% of NaCl. Yadav and Vyas (1971b) studied effect of salts and pH on the growth of root nodule rhizobial strains of gram (*Cicer arietinum* L.) and found that rhizobial strains of gram were tolerating 3% of Cl⁻ of Na⁺. They also have studied effect of salts and pH on the growth of root nodule rhizobial strains of cowpea (*Vigna catjang* Walp) and found that strains of cowpea were tolerating 3% of Cl⁻ of Na⁺. El-Sheikh (1992) also have worked on the effect of salinity on growth and nitrogen yield of inoculated and N fertilized chickpea (*Cicer aritinum*) and reported that *Rhizobium* sp. of *Cicer* strain Ch191 tolerated up to 0.43 M (2.5 gm%) of NaCl. Total of 142 rhizobial isolates, nodulating *Cicer* plant were studied
for their NaCl tolerance in broth by El-Sheikh and Wood, (1989a/b, 1990b) and Rai and Prasad (1986). In their studies 27, 27, 27, 25, 18 and 18 isolates were found tolerating respectively 0.0, 50, 100, 200, 300 and 400 mM (2.32%) concentrations of NaCl. None of the isolate was found tolerating 600 mM (3.48%) and above concentrations of NaCl.

Yelton et al. (1983) have studied characteristics of fast and slow growing strains of *Rhizobium japonicum* in the medium with NaCl concentrations of up to 0.4 M (2.32 gm%) in broth. El-Sheikh and Wood, (1989a/b, 1990, 1995), Yelton et al. (1983), Sadowsky et al. (1983), Fujihara and Yonemyama (1993) studied in total of 44 strains of *Rhizobium fredii* for their salt tolerance. The NaCl tolerance of these isolates was found varying from less than 50 mM (0.29%) to up to 600 mM (3.48%). All the isolates were found sensitive to 800 mM (4.64%) of NaCl.

In all 103 strains of *Rhizobum leguminosarum* biovar trifolii have been studied for their NaCl tolerance in broth by Graham and Parker (1964) and Yadav and Vyas (1973), Chaudri et al. (1992) and Bernard et al. (1986). In their observations the maximum NaCl tolerance was seen up to 200 mM (1.16%) concentrations.

Payakapong et al. (2006) studied 373 rhizobial isolates from the nodules of the wild bean *Phaseolus lathyroides* that grew in the saline uncultivated areas for their ability of growing in YEM broth containing 300 mM (1.74g%) NaCl. They found that eight isolates could grow well at this concentration. The maximum NaCl tolerance found was to be up to 600 mM (3.51g%) in YEM broth. Shamseldin and Werner (2005) studied seven *Rhizobium etli* strains of *Phaseolus vulgaris* and have reported that isolate EBRI 21 and EBRI 26 tolerated NaCl up to 4%. In all 36 different strains of *Rhizobium leguminosarum* biovar *phaseoli* have been studied for their NaCl tolerance in broth by Amara and Miller (1986), Graham and Parker (1964) and Yadav and Vyas (1973) Maximum tolerance reported by them was up to 600 mM (3.51%) of NaCl.

Mensah et al. (2006) conducted an experiment to find out the effects of varying concentrations of NaCl ranging from 0.005 M to 0.200 M (1.16%) on the growth of rhizobium as well as cowpea associated with
rhizobium and reported that rhizobia were capable of tolerating relatively high salt concentration of up to 0.200 M (1.16%) NaCl. Paknikar (1987) have reported tolerance to 2.5% of NaCl in rhizobia of wild legumes of *Vigna* species. 182 fast growing rhizobial isolates of *Vigna* have been reported to be tolerating 0.0 to 600 mM (3.48%) of NaCl by Lal and Khanna (1994) and Zou et al. (1995). Zou et al. (1995) studied two fast growing rhizobial isolates of *Vigna* spp. and have reported them to be tolerating up to 800 mM (4.6%) of NaCl.

Sadowsky et al. (1983) and Stowers and Eaglesham et al. (1984) have reported that salt tolerance is a characteristic of fast growing rhizobial cells. Fujihara, (2009) have taken comparative account of fast and slow growing rhizobia and have stated in the review that salt tolerance is a property of fast growing rhizobia. Hua et al. (1982) reported that rhizobium species obtained from saline regions some times grow in concentrations approaching sea water and the rhizobial strains isolated from saline soils are typically more tolerant to salts than isolated from non-saline soils. Kucuk and Kivanc (2008) have characterized total of 28 *Rhizobium* strains from chickpea nodules. They reported that 15 strains out of those 28 could tolerate NaCl up to 0.5 M. Pillai and Sen (1973) studied salt tolerance of rhizobia of *Dolichus lablab* and reported that their growth got enhanced in presence of 1% of NaCl. Ali et al. (2009) studied salt tolerance of fast growing rhizobia from wild legumes. They reported that there is a decreased growth of rhizobial isolates with increasing salt concentration however they reported that six rhizobial isolates tolerated NaCl concentration up to 4.5% in broth.

Michael and Gerald, (1984) have studied fast growing *Rhizobium fredii* that nodulated soybean. They have reported that these fast growing rhizobia of soybean could tolerate up to 2% of NaCl on YEMA, while at 3% NaCl concentration the growth was inhibited.

There is some research on the effect of KCl on rhizobia. Yadav and Vyas (1971b) studied effect of salts and pH on the growth of root nodule rhizobial strains of cowpea (*Vigna catjang* Walp), and gram (*Cicer arietinum* L.) and found that strains of cowpea and gram were tolerating 3% of Cl− of K+. Mary et al. (1986) studied growth response of rhizobia in
relation to KCl concentration in the medium and have reported that that 590 mM (4.4%) of KCl completely inhibited the growth of rhizobia. Upchurch & Elkan (1977) found that KCl was more inhibitory than NaCl at the same concentrations to four strains of *R. japonicum*. Mary et al. (1986) studied growth status of rhizobia in relation to their tolerance to NaCl and KCl and have reported that NaCl and KCl have similar toxicities.

Botsford (1984) and Sprent (1984) have reported that carbonates are more toxic than other salts to rhizobia. This high sensitivity of rhizobial isolates to carbonates and bicarbonates have been reported by Yadav and Vyas (1973) also who reported that 0.2–0.8% NaHCO₃ was lethal for all the strains. Yadav and Vyas (1971b) studied effect of NaHCO₃ and pH on the growth of root nodule rhizobial strains of cowpea (*Vigna catjang* Walp), and gram (*Cicer arietinum* L.) and found that NaHCO₃ was toxic to all the strains.

Pandher and Kahlon (1978) have studied pH and sodium bicarbonate tolerance of *Rhizobium leguminosarum* isolated from pea (*Pisum sativum* L.) and found that sodium bicarbonate was inhibitory even at low concentrations to the isolates.

Reports by many workers then could show tolerance of rhizobia to salt. Rai and Prasad (1983) showed tolerance up to 1.5% of NaCl. Rai (1983) reported tolerance of Rhizobia of *Lens* spp. up to 2% of NaCl. Yadav and Vyas (1971a, 1971b) reported tolerance up to 6% in Rhizobia of luceren and pea. Some of the rhizobial strains obtained by Guan et al. (1992) could tolerate NaCl up to 1030 mmol.

El-Sheikh and Wood (1990a/b) reported that fast growing rhizobia are better tolerant of salts than slow growers. Observations by Fujihara and Yoneyama (1993) are in accordance with above. They found that *Rhizobium fredii* a fast growing micro symbionts of soybean tolerates up to 400 m mol of NaCl, while *Bradyrhizobium japonicum* a slow growing symbionts of the same could not tolerate even up to 150 m mol.

Pillai and Sen (1969) reported that in case of salt tolerant rhizobia colony morphology is changed from slimy to rough in response to increasing salt concentration in the medium. Upchurch and Elkan (1977)
reported that slime producing rhizobia are more tolerant to salt than non producing one. Graham and Parker (1964) observed that normal rhizobia associated with pea can tolerate a maximum salinity up to 4.5 dS/m.

Abdel Wahab and Zahran (1979) have observed tolerance of five rhizobial isolates to five different levels of NaCl concentrations. They found *Rhizobium leguminosarum* and *R. meliloti* were tolerant to high levels of salinity than *Rhizobium japonicum*, cowpea Rhizobium, and *R. trifolii*. They further showed that there is strong retardation of growth with increasing salt concentration. They further showed that rhizobial sensitivity to salts may be partly responsible to the inhibition of nitrogen fixation by legumes growing under salt stress.

While studying on effects of salt and pH stress on temperature-tolerant rhizobia, Kulkarni and Nautiyal (2000) used *Rhizobium* sp. NBRI330 isolated from root nodules of leguminous plants growing in alkaline soil. They worked for finding individual stress survival limits of the isolates to various factors. Their isolate could survive 32% (w/v) of salt for 8 h. Although the isolate could tolerate 45°C temperature they could survive at 55°C for about 3 h. They further observed that high temperature (45°C) tolerance of the isolate was in the presence of salt at pH 12, as compared with pH 7.

Working with nodulation and nitrogen fixation in soybean inoculated with salt-tolerant and salt-sensitive symbiotic nitrogen fixers in saline soil El-Sheikh and Wood (1995) reported that out of many environmental stresses salt stress is the major one which adversely affects legumes in arid and semi-arid regions, particularly because the nitrogen requirements of these plants is dependent on symbiotic nitrogen fixation.

There is much difference in between the micro-symbionts and their legume partners with respect to tolerance to salinity and alkalinity. Bhardwaj (1975) showed that the salinity and alkalinity tolerance limits of *Rhizobium* and *Bradyrhizobium* spp. are much higher than those of the legume host. This shows that it is predominantly the tolerance of the host plant that decides the possibility of a successful symbiosis. Similar reports are also obtained by Lauter et al (1981), Singleton et al. (1982) and Kassem et al (1985).
El-Sheikh and Wood (1989a, b) reported that there is more effect of salt at alkaline pH and at low temperature.

Shamseldin and Werner (2005) have tried for isolating rhizobia that can tolerate different environmental stresses. They found a high degree of diversity in the tolerance of *Rhizobium etli* strains isolated from Egyptian soils. Two highly tolerant strains EBRI 21 and EBRI 26 from their isolates were tolerating NaCl concentration up to 4%. Shamseldin and Werner (2005) in their studies with respect to pH, they further reported that stress of alkalinity had a less detrimental effect on nodulation and N2 fixation than stress of salinity. They also showed a positive correlation in between the salt tolerance of rhizobial strains and their adaptation to alkaline pH 9.

If bacteria are non tolerant to salt then presence of higher amount of salt is a stress condition, and for salt tolerant the same condition may not be equally stressful. Many workers while working with salt tolerant rhizobia have substantiated the observations of Csonka (1989) that is there can be active response by salt tolerant bacteria to presence of salt. The effect can be seen in the form of change of colony morphology or in the shape and size of the cell or in biochemical properties.

Lloret et al. (1995) observed that the colony morphology of the rhizobial isolates is changed due to growth on salt supplemented solid medium. The colonies resembled semi-rough. Kulkarni and Nautiyal (2000) found that morphology of the *Rhizobium* is changed due to stress. During their studies they reported that the length of *Rhizobium* sp. in salt-stressed cells increased significantly to 3.04 μm while for pH stressed cell it declined to 1.40 μm from 1.75 μm of non-stressed control cells at the same time the shape of temperature-stressed cells changed to spherical (0.42 μm).

Breedveld et al. (1990, 1991) showed that property of production of capsular substance is affected due to presence of salt. They reported decrease in the extracellular polysaccharide. In case of *Rhizobium meliloti* and *R. leguminosarum* biovar trifolii the reports show change in the type of capsular polysaccharide from high molecular weight to low one. Accumulation of trehalose also has been reported by them.
2.11 Isolation of Rhizobia:

Subba Rao (1995) have made a mention of Beijerink in Holland who was the first to isolate and cultivate a microorganism from the nodules of legumes in 1888 and to name it as *Bacillus radiocola* which is now placed in Bergey’s Manual of Determinative Bacteriology under the genus *Rhizobium*.

During isolation of rhizobia from soil with some extreme conditions such as salinity or acidity, rhizobia can grow better in a medium modified to resemble closely the soil conditions Date (1982). The same fact was reported by Date and Halliday (1979) during isolation of acid tolerant rhizobia. The modified medium, simulating similar stress condition to that found in soil, then can become selective to those bacteria which can tolerate the stress and can automatically eliminate non-tolerant varieties during isolation.

Zahran et al. (1995) reported that the majority of *Rhizobium* strains isolated from saline soils were salt tolerant, being able to grow in media containing more than 510 mM (2.9835%) NaCl. Work of Shamseldin and Werner (2005) is indicating occurrence of salt tolerant rhizobia as they have reported that symbiotic effectiveness of salt tolerant rhizobial isolates is better than salt sensitive ones in saline soils. Extreme condition like increased salinity and or alkalinity of a soil is known to limit nodulation and nitrogen fixation, but Lie et al. (1987) suggested that the centers of diversity of legume hosts were also likely to be the centers of diversity of their compatible rhizobia. Date (1982) reported that if isolation of rhizobia is tried in a medium resembling conditions of its habitats then they grow better. This fact had already been reported for acid tolerant rhizobia by Date and Halliday (1979).

Chen et al. (2004) used the standard plant trap method to isolate indigenous rhizobia of *Glycine max* from soil samples. They used four different trap plants including wild soybean, and Chinese cultivated soybean (*Glycine max*). After inoculation with a soil sample, the plants were grown for 4 weeks. They isolated root nodule bacteria from the root nodules of these plants. Root nodule bacteria of soybean were isolated by
Annapurna et al. (2007) from field soils of different soybean-growing locations by a plant trap method. They used soils from various sites in India as inoculum in sterilized pots filled of vermiculite and sand. Surface sterilized soybean seeds were sown in the pots. Plants were harvested at 4 weeks after emergence and a single pink nodule from each plant of the pot was removed and isolations of root nodule bacteria was carried out as described by Vincent (1970). Rosenblueth and Martinez-Romero (2004) have used bean plant as a trap plant to isolate *Rhizobium etli* strains from soils.

2.12 Characterization of root nodule bacteria.

2.12.1 Morphological characteristics:

As given in *Bergey’s Manual of Systematic Bacteriology* (1984), *Rhizobium* and *Bradirhizobium* are the genera of gram negative, rod shaped bacteria fixing nitrogen symbiotically with especially leguminous plants. Size range of the cells is 0.5 – 0.9 x 1.2 – 3.0 micrometers. Bacteria may be pleo-morphic appearing swollen, globular, ellipsoidal, club-shaped or branched. Due to presence of beta hydroxy butyric acid granules cells from old culture can show uneven staining. Cells are motile with the help of single polar or sub-polar flagellum or with 2-6 peritrichous flagella. Cells of *Bradirhizobium* are motile with monotrichous polar or sub polar flagellation.

2.12.2 Cultural characteristics:

As given in Bergey's Manual of Systematic Bacteriology (1984), cells of *Rhizobium* can grow on yeast mannitol- mineral salts agar giving circular, convex, semitransparent (translucent), raised and mucoid colonies of 2-4 mm in size within 3 to 5 days. Vincent (1970) has described that when rhizobia grow on agar surface they give discrete, round less convex to dome shaped, some times conically elevated colonies. Subsurface growth is always typically lens-shaped. Colonies appear white opaque to milky or watery semi-transparent. Bhardwaj (1972) had reported that Rhizobia isolated from legumes of saline alkaline soil give red, orange
or yellow pigmented colonies. The colonies are usually mucoid appearing opaque with little firm gum, or translucent appearing glistening or dull with gummy, soft slime. They may or may not be with opaque centers (Jordan 1984). Vincent (1954, 1962) reported that rhizobia can produce low, medium or high gum. As cells do not take congored, on the media containing congored colonies appear light pink or whitish. Trinick (1982) have described rhizobial colonies to be white, milky or creamy, convex, glistening or dull with a regular entire margin.

Fast growing rhizobia usually form larger colonies in comparison with that of slow growing. Colonies of *Rhizobium trifolii*, *Rhizobium leguminosarum* and *R. phaseoli* produce very large, gummy colonies on yeast extract mannitol agar. Slow growing rhizobia produce colonies of hardly 1 mm in diameter within about 10 days. *Bradyrhizobium* form circular, opaque, white, convex, granular colonies of the size less than 1 mm even within about 7 days of incubation. The colonies are tough, making preparation of their suspension little difficult.

Lim and Ng (1977) while working on legumes in Singapore isolated fast growing rhizobia from 14 legumes previously reported to have slow growing rhizobia. However, Trinick (1982) confirmed the presence of slow growing rhizobia with the same legumes. Occurrence of slow and fast growing rhizobia was known in wild legumes also. Gandhi (1986) isolated 17 fast growing and 14 slow growing strains of rhizobia from wild legumes.

### 2.12.3 Biochemical and physiological characteristics:

Rhizobia are actually identified on the basis of their ability of nodulating respective leguminous plants. Members of the genus *Agrobacterium* also can infect plants. Dye, (1979) have given some cultural, biochemical and physiological characteristics with the help of which one can differentiate *Rhizobium* and *Agrobacterium* from each other.

Graham and Parker (1964) recommended some characteristics of rhizobia which can be used as diagnostic features. These characters include-
1. Reaction yeast extract mannitol agar,
2. Reaction in Litmus milk,
3. Utilization of carbohydrates and organic acids,
4. Enzymatic activities for- urease, Catalase, oxidase, gelatinase, amylase, penicillinase,
5. Response to- temperature, pH, salt concentration, biotin, pantothenate, thiamine


Shinde (1976) isolated some cowpea rhizobia from some wild legumes and studied them for utilization of carbohydrates and amino acids, antibiotic sensitivity, litmus milk reaction, gelatinase, amylase, nitrate reductase H₂S production and found dissimilarities among the strains. Variation in the physiological characters of root nodule bacteria of cowpea, gram and dhaincha were observed by Raju (1938). Jadhav (1969) and Rangaswami and Oblisami (1962) also have studied some cultural and physiological characteristics for rhizobia of some wild and cultivated legumes. Nimbalkar (1986) studied these characters in 14 slow growing and 5 fast growing rhizobia.

Johnson and Allen (1952) and Konde (1975) have recommended crystal violet sensitivity test for rhizobia. Paknikar (1987) and Gandhi (1986) have studied these tests for many rhizobial isolates from some wild legumes.
2.12.4 Symbiotic characteristics:

Sindhu and Dadarwal (1997) mentioned that symbiotic association in between rhizobia and leguminous plants begins when specific substances produced by the plant give a characteristic signal to rhizobia that results in to nodulation. Vincent (1970) have given methods for studying cross inoculation abilities of the rhizobia with different leguminous plants, as well as methods for studying symbiotic effectiveness of rhizobia on leguminous plants.

2.12.4.1 Cross inoculation:

Mahmood and Athar (2008) performed cross inoculation experiment in between the rhizobia of tree legumes and Vigna mungo. After about six weeks of cultivation they reported that tree legume rhizobia formed globose nodules on the roots of Vigna mungo. They further reported that rhizobia of Albizia lebbek a tree legume failed to nodulate plants of Vigna mungo as well as Vigna radiata. Abaidoo et al. (2000) reported that Bradyrhizobium spp. nodulating new soybean cultivars in Africa are diverse and distinct from bradyrhizobia that nodulate North American soybeans. Hungria and Bohrer (2000) worked for identifying better soybean cultivar. During their studies they used three Bradyrhizobium elkani strains established in Brazilian soils cultivated with soybean. Symbiotic performance of 152 cultivars was evaluated. They reported variation in the property of nodulation among the cultivars with preference of cultivars for specific strains. Yelton et al. (1983) performed experiments on cross inoculation of Rhizobium japonicum strains 191 and 110 with soybean (Glycine max) and cowpea (Vigna unguiculata) plants. They reported that two strains were effective on both the hosts and nodules on both the legumes were similar. Farrukh et al. (2008) conducted a nodulation experiment using four strains of rhizobia of pea plant (PS-1, PS-2) and Lentil plant (LC-31 and LC-12) with three varieties of Pisum sativum plants. They reported that the rhizobial isolate namely, PS-1 and PS-2 could form nodules on pea plants but the rhizobial isolates of Lentil namely, LC-31 and LC-12 could not form nodules on any of the pea plant. Martinez-Romero (2009) reported that many plants in the Phaseoleae tribe are nodulated by bradyrhizobia, however they show some preferences for
bacterial symbionts. He further reported that this includes the species of *Vigna*. He further reported that the species of *Phaseolus* are nodulated by *Rhizobium etli* a rhizobium of *Mimosa*. He also has stated that plasmid plasticity of rhizobia probably leads to the generation of new symbiotic plasmid such as phaseoli, which are better suited to bean nodulation. Martinez-Romero et al. (1991), reported that beans are nodulated by diverse bacteria in introduced areas. Ampomah et al. (2008) assessed the host range, symbiotic effectiveness and competitiveness for nodule occupancy among five (AII-2-1, AII-5-2, AI-4-3, AII-3-4 and BIII-2-2) indigenous cowpea rhizobial isolates with the plants cowpea, groundnut, soybean and mungbean. LBG13 was the fast growing rhizobial strain of groundnut rhizobium used as reference strain in the studies. They reported that all the rhizobial isolates could nodulate cowpea, groundnut and mungbean, while only three namely, AII-2-1, AII-3-4 and BIII-2-2 nodulated soybean. Zhang et al. (1991) studied diversity of rhizobia form root nodules of leguminous trees. They performed a cross inoculation experiment with total of 122 rhizobial strains including 25 strains of representative *Rhizobium* and *Bradyrhizobium* species and 8 tree leguminous plants. They reported that *Rhizobium leguminosarum* isolates from *Pisum sativum* and *Phaseolus vulgaris* did not nodulate any of the 8 tree leguminous plants. *Bradyrhizobium* strains isolated from *Vigna radiata* could nodulate *Macroptilium atropurpureum*. Yelton et al. (1983) characterized fast growing strains of *Rhizobium japonicum*. They checked effectiveness of *Rhizobium japonicum* strain 110 and 191 on *Glycine max* and *Vigna unguiculata* (L) Walp. *Pisum sativum* and other soybean cultivars. After 28 days of cultivation they reported that both the rhizobial isolates could nodulate the plant of *Glycine max* and *Vigna unguiculata*, while the *R. japonicum* strain could not nodulate *Pisum sativum* plants. Michael and Gerald (1984) reported that the *Rhizobium fredii* strains of *Glycine max* formed nodules on *Glycine max*, *Vigna unguiculata* and *Cajanus cajan* (L.)Millsp. But the rhizobial isolate could not nodulate the plants of *Vigna radiata* and the United States cultivars of *Glycine max*. Research has also been done on the cross inoculation of wild legume rhizobia with cultivated leguminous plants. Bajekal (1996) studied cross
nodulation of coastal salt tolerant rhizobia with cultivated leguminous plants and reported that 16 fast growing salt tolerant rhizobia from sea coast could nodulate *Vigna unguiculata* plants. Shinde (1976), Gandhi (1986), Lim & Ng (1977), Ramchandran et al. (1980), Nimbalkar (1986), Paknikar (1987), Shinde (1976), Jadhav & Moniz (1972), Subba Rao et al. (1981) all these scientists have worked for testing cross nodulation ability of rhizobia of wild legumes on cultivated legumes.

### 2.12.4.2 Symbiotic effectiveness

Abd-Alla et al. (1998) conducted two experiments to evaluate salt tolerance of various genotypes of soybean plants. For this they performed pot culture experiments with *Bradyrhizobium japonicum* and the evaluation was done after four weeks of cultivation on the basis of nitrogenase activity, nodule number, and nodule, root and shoot dry weights. They reported that the number of the nodules on the plants at zero mM NaCl was varying from minimum of 69 to maximum up to 306, while the super nodulating variety formed 609 nodules per plant. Nodule dry weight was ranging from 59 mg to 209 mg per plant, while the dry weight of the nodules in super nodulating plant was 196 mg per plant. Singleton and Bohlool (1984) studied effect of salinity on nodule formation in soybean. In the experiment they used eight soybean seeds per pot and *Rhizobium japonicum* USD A110 strain. After 54 days of planting the plants were harvested and analyzed for nodule number, shoot dry weight and nitrogen content. They reported that in the control (without NaCl treatment) plants, the nodule number per pot was 1229, the nodule dry weight was 2.97 gm per pot. In the reports of symbiotic studies of six rhizobial strains namely, AII-2-1, AII-5-2, AI-4-3, All-3-4, BIII-2-2 and LBG 13 with cowpea, mungbean and soybean plants. Ampomah et al. (2008) performed symbiotic studies of five rhizobial strains with cowpea, mungbean and soybean plants. They reported symbiotic effectiveness as the number of nodules formed by the rhizobial strains AII-2-1, AII-5-2, AI-4-3, All-3-4, BIII-2-2 and LBG13 on mungbean *Vigna radiata*, on *Glycine max* and *Vigna unguiculata*, ranged from 18 to 108. Meghvansi et al. (2005) identified pH tolerant starin of *Bradyrhizobium japonicum* and studied their symbiotic effectiveness in
Glycine max plants. In the experiment they used 8 rhizobial strains namely, Bj-1 to Bj-8. After 45 days of cultivation they reported that the number of nodules developed on soybean plants was ranging from 17 to 38 per plant, while the shoot dry weight was ranging from 2.4 gm to 4.4gms per plant. Yelton et al. (1983) characterized fast growing strains of Rhizobium japonicum. During characterization they checked effectiveness of Rhizobium japonicum strain 110 and 191 on Clark soybean (Glycine max) and cowpea (Vigna unguiculata Walp.) alfa alfa, peas and other soybean cultivars. After 28 days of cultivation they reported that the total plant dry weight of soybean plant was from 3.19+/− 0.35 gm per plant to 4.0+/− 0.2 gms per plant. El-Sheikh, (1998b), studied response of legume rhizobium symbiosis to salinity in Sudan. They reported that in uninoculated plants of Glycine max cultivar 814-49E the shoot dry weight was 3.5gm per plant, while with the rhizobium inoculation the dry weights was 5.5gm per plant. Farrukh et al. (2008) studied interactions of Pisum sativum under different environmental stress, During his studies they used four strains (PS-1, PS-2, LC-31 and LC-12) of Rhizobium leguminosarum and three varieties of Pisum sativum. They reported that the isolate namely, PS-1 formed high number of nodules up to 20 on all the three varieties of pea plants and PS-2 could form very less number of nodules. LC-31 and LC-12 were the isolates obtained from Lentil plants and they could not form nodules on any of the pea plant. Lawn and Bushby (1982) performed some experiments on reciprocal grafting between four Asiatic Vigna species to examine the control of growth, nodulation and nitrogen accumulation in the presence of several strains of Rhizobium. In the experiment they used four Vigna species as V. angicularis cv. Kotobuki, V. mungo cv. Regur, V. radiata cv. Celera and V. umbellate. All the plants were inoculated with cowpea-type Rhizobium strain CB 756. After 39 days of cultivation the plans were analyzed for nodule number, nodule fresh wt, and acetylene reduction activity of nodules, leaf area and shoot and root dry wt per plant. They reported that the shoot dry weight of Vigna mungo was 1025 mg per plant. The nodule numbers were 118mg, and the nitrogen contents were 58 mg to 80mg per plant. Mahmood and Athar (2008) during performing cross inoculation experiment in between the rhizobia of tree legumes and Vigna mungo reported that globose nodules are formed on the roots of Vigna mungo and the number was ranging from 4 to 7.
The nitrogen content per plant was ranging from 0.76 gm to 3.98 gm. Nosheen et al. (2004) while studying nodulation of mungbean reported that size of the nodules on the roots of *Vigna radiata* plant was 1.38 +/- 0.3 mm. During reciprocal grafting experiment between four Asiatic *Vigna* species and cowpea type *Rhizobium* strain CB756 Lawn and Bushby (1982) reported that after 39 days of cultivation the plants of *Vigna radiata* formed 117 nodules per plant and the nitrogen content was from 51 to 62 mg per plant, while the shoot weight was 1025 mg per plant. Hafeez et al. (1998) studied effect of salinity and inoculation on nitrogen fixation of *Vigna radiata*. They conducted nodulation experiment with the inoculum of mixture of five rhizobial strains as Ml 1, Ml 7, TAL-441, TAL-420 and TAL 169. Dry weights of the plants harvested from non saline pots after 33 days were 0.5 gms and 1.1 gms in uninoculated and inoculated plants respectively. After 45 days the weights were 2.1 and 2.7 gms per uninoculated and inoculated plant respectively, while after 65 days the weights were 2.4 and 3.1 gms respectively. They further reported that after 30 days of cultivation the number of nodules formed on the plant was 14 and 20 after 33 and 45 days of harvesting respectively, the shoot dry weight was 901 mg per plant. The total nitrogen content in the mature plant was 50.7 mg per gm of dry plant in uninoculated while it was 59.4 mg per gm of dry weight of plant. El-Sheikh, (1992), studied effect of salinity on growth and nitrogen yield of inoculated and N fertilized chickpea (*Cicer arietinum*). In the study he used *Cicer* rhizobium strain Chl91 and the chickpea cultivar ILC 482 and ILC 1919. The plants were harvested after 4, 6 and 8 weeks and then analyzed for total nitrogen contents of shoot and roots. He reported that the nitrogen contents of the shoot of uninoculated chickpea plants were 24.3 mg, 30.87mg, and 40 mg per plant after 4 weeks, 6 weeks and 8 weeks of harvesting while of the inoculated plants the nitrogen contents were 67.53mg, 157.13mg, 184.93mg per plant after 2 weeks, 6 weeks and 8 weeks of harvesting. El-Sheikh, (1998b), studied response of legume rhizobium symbiosis to salinity in Sudan. They reported that n uninoculated plants of *Cicer arietinum* cultivar NEC1101 and ILC482 the shoot dry weight was 0.81 and 0.8 gm per plant, while with the rhizobium inoculation the dry weights were 1.55gm and 0.9gm respectively. Soussi et al. (1998) studied effect of salt stress on the nitrogen fixation in chickpea. (*Cicer arietinum* L.) They used Chickpea cultivar ILC1919 and *Mesorhizobium ciceri* strain ch-191. The
plants were harvested after 21, 24, 38 and 42 days of cultivation and analyzed for total plant dry weight, nodule dry weight and acetylene reduction test. They reported that the total dry weight of the plants was 437 mg, 768 mg, 942 mg and 1077 mg per plant after 21, 24, 38 and 42 days of cultivation respectively. Rao et al. (2002) studied effects of salinity and sodicity on nodulation and nitrogen fixation in *Cicer arietinum*. In the experiment they used salt sensitive, salt tolerant, high nodulating and the test plant varieties. After 40 days of nodulation experiment they reported that the nodule number was 1.7 to 4.5 per plant, while the shoot dry weight was 0.21 to 0.36 gm per plant and the shoot nitrogen content was 6.8mg to 12.4 mg per plant. Zablotowickz and Focht (1981) performed studies on the physiological characteristics of cowpea rhizobia in that they evaluated symbiotic efficiency of *Vigna unguiculata* using rhizobial strains namely, 22, 28, 30 and 32 of *Vigna unguiculata* Walp. and cowpea cultivar California no. 5 black eyes. After 40 days of cultivation they reported that all the strains of rhizobia were capable of nodulating the host plants but there was significant effect of the rhizobial strain on nodule mass, nitrogenase activity and plant yield. They reported that the nodule mass was in the range of 175 to 270 mg per plant, and the nitrogen fixation efficiency was from 91% to 97.5%. The number of the nodules formed was ranging from 3.4 to 24.2 per plant. They further reported that there was no correlation of plant yield with nodule mass, nitrogenase specific activity or nitrogen fixation efficiency. Weaver and Wright (1987) studied effectiveness of rhizobia during culture and in nodules. Nodulation experiment was conducted in vermiculite container with Siratro plant and twenty ‘single colony’ *Bradyrrhizobium* isolates of TAL 309 strain of *Vigna*. All the strains they reported to be nodulating *Vigna unguiculata*, *Arachis hypogaea* and *Macroptilium atropurpureum* plants. After 5 weeks of cultivation they reported that the shoot dry weights of the plants ranged from 111 to 273. Yelton et al. (1983) characterized fast growing strains of *Rhizobium japonicum*. After 28 days of nodulation experiment with *Rhizobium japonicum* strain 110 and 191 on cowpea (*Vigna unguiculata* Walp.) they reported that the total plant dry weight of *Vigna unguiculata* plant was from 3.96+7- 0.04 gm per plant to 4.43+7- 0.57 gm per plant and the nodule weigh was 0.365+7-0.05 to 0.435+7-0.046 gm per plant. Mensah et al. (2006) studied effect of salt concentration and
pH on the association of cowpea plant and its rhizobium. After 21 days of nodulation experiment they reported that in the oil with 0.0 M NaCl added the cowpea plants developed 52.7 nodules per plant and the plants showed 4.4gm of dry weight per plant. In another experiment performed at different pH values of soil they reported that the number of nodules formed on the plants at pH 7 was 60.8 per plant, while the shoot dry weight was 10.8gm per plant. Payakapong et al. (2006) have performed a plant experiment with seeds of *Phaseolus lathyraldes* and salt-tolerant *Sinorhizobium* strain BL3. They reported that the number of nodules formed on the *Phaseolus* plant was 50 per five plants and the plant dry mass was 0.21 gms per five plants. Tejera et al. (2004) performed some experiments on the nodulation using *Phaseolus vulgaris* and salt tolerant *Rhizobium tropici* strain CIAT899 along with the decreased salt tolerant mutant strains namely, HBB, HB10, HB12 and HB13. They reported that at 0% of NaCl concentration the plant dry weight of uninoculated plant was 1.35 mg per plant, while of the plant nodulated by salt tolerant as well as decreased salt-tolerant mutant rhizobial isolates was 1.3mg per plant to 2.71 mg per plant after 32 days of harvesting. Tepe et al. (2004) while studying effect of different control methods on weeds, yield components and nodulation in *Lentil* plants conducted some field trials. In the trials, the inoculation material produced from the mixture of nodule-forming strains in lentil by the bacteria strains, *Rhizobium leguminosarum*, and the lentil plants were used. The plants were harvested after 35 to 40 days and analyzed for root dry weight and the nodule number. They reported that in weed free control set the plants formed 6.3 nodules per plant. Md. Harun-or Rashid et al. (2009) performed studies on molecular characterization of root nodulating rhizobia isolated from Lentil plant. During their studies they conducted symbiotic effectiveness test using lentil seeds and six rhizobial isolates of lentil. In the plants harvested after four weeks of cultivation they reported that in the uninoculated plants the shoot dry weight was 13 mg per plant while, in the inoculated plants the shoot dry weight was ranging from 13 mg to 22 mg per plant. The nodule number in the inoculated plant they reported was ranging from 7 to 23.