# 2. Review of Literature

The work reported in the past on the symbiotic biological nitrogen fixation by wild legumes may be presented under four heads.

i. Nodulation survey of wild legumes.
ii. Cultural, biochemical and physiological characteristics of rhizobia.
iii. Serology of rhizobia.
iv. Symbiotic characteristics of rhizobia from wild legumes.

## 2.1 Nodulation survey of wild legumes -

Norton and Walls (1905) made a survey of wild leguminous plants of Maryland and suggested that nearly all of them are important agents for adding nitrogen and humus to the soil. Warren (1909) emphasized that wild legumes are abundant both in number of individuals and species on poor soils and slopes where grass grows scantily.

Erdman and Walker (1928) studied 63 native and cultivated species of legumes of Ames, Iowa of which six species were never found nodulated. Allen and Allen carried out extensive studies on the nodulation of wild legumes. In 1936 they surveyed 150 different species of leguminous plant from 35 genera for nodulation and concluded -

i. The old belief that all legumes invariably have nodules

and they fix nitrogen was incorrect.

ii. nodulation was least prevalent among species of Caesalpinioideae.

iii. Woody tropical species were nodulated as readily and effectively as tender herbaceous legumes of temperate zone.

iv. within the same genus, e.g. Cassia, the nodulating ability of some species was consistently negative while that of others was consistently positive.

They further (1947) showed that 887 leguminous plants from 167 genera were nodulated whereas 77 species were non-nodulated. Allen and Baldwin (1954) reported that out of 1063 leguminous species investigated by them for nodulation, all except 133 were found to be nodulated. Lange (1959) surveyed wild legumes from South Western Australia and reported nodulation for the first time on 137 species from 24 genera including eight new genera. Grobbelaar et al. (1964)showed the occurrence of nodules in 44 legume species comprising several species of Acacia & Albizzia of the subfamily Mimosoideae and a number of species belonging to subfamily Papilionoideae. They further (1967) reported nodulation on 246 legume comprising 17 spp. of Mimosoideae, 12 spp. of Caesalpinioideae and 217 spp.of papilionoideae.

Beadle (1964) found that out of 80 legumes surveyed by them in arid and semiarid regions of east Australia 68 were

nodulated. De Souza (1966) in Trinidad studied 79 legume species and found that 32 species from several genera were previously unlisted for nodulation. He first reported nodulation in three species of Swartizia. Corby (1974) surveyed 370 leguminous species from Rhodesia and observed nodulation on all except 13 leguminous species. Norris (1969) observed nodulation on 30 species of tree legumes out of 53 species studied during his survey in Amazonia and Guyana. Gallardo (1970) showed nodules on 32 genera of legumes out of 37 examined in Argentina. Barrios and Gonzalez (1971) found 109 legume species to be nodulated out of 129 species examined from Venezuelan Savannas.

Dubey et al. (1972) reported nodulation on 13 previously unrecorded species from Puerto Rico. Grobbelaar and Clarke (1972) found nodulation on 207 species, which had not been examined previously for nodulation from South Africa. They (1974) also reported nodulation for the first time on 76 legume species out of the 213 species studied. Yanasugondha et al. (1977) studied nodulation of 52 species of native legumes from various parts of Thailand including nine trees; thirty six herbs, five shrubs and two wood climbers.

Lim and Ng (1977) studied nodulation on legumes in Singapore and observed nodulation on 33 species out of 35 species examined belonging largely to Mimosoideae and Papilionoideae.

In this study nodulation on Delonix regia and Adenanthera pavonia was reported for the first time. These results were confirmed in subtropical Pakistan by Athar and Mahmood who reported nodulation on 52 species, five of which were new records (Lim and Burton 1982). de Faria et al. (1985) made an extensive search for active nitrogen fixing nodules in legume trees in South East Brazil. From 343 legume species observed by them, 171 were nodulated from which 86 had not been described as nodulating species before.

Work done in India : The work done in India on nodulation of wild legumes spreads over last 3 decades barring isolated reports like the one by Joshi (1920). Following are important reports on nodulation of wild legumes. Rangaswami & Oblisami (1962) showed nodulation on Crotalaria retusa and Clitoria ternatea. Satyanarayan and Gaur (1965) studied nodulation status of some arid zone legumes in their natural state in Rajasthan and found that species like Atylosia scaraboides, Rhynchosia minima & Tephrosia purpurea are either devoid of root nodules or contain very few. Sinha et al. (1971) surveyed wild legumes in Hooghly and Bardwan districts of West Bengal for occurrence of nodules and found nodulation of 24 species out of 40 species examined. Nodulation was not observed on all the species of Caesalpinioideae. Bhelke (1972) reported occurrence of nodules on 10 legume species

from Western Maharashtra for the first time, including one new genus Geissapsis. Kulkarni (personal communication) reported nodulation for the first time on Indigofera anabaptista and Taverniera cunnifolia. Basnyat (1979) carried out survey in the hilly tract of Western Maharashtra and reported nodulation on 13 leguminous species.

## 2.2 Cultural, Biochemical and Physiological characteristics of rhizobia :

Beijerinck, the pioneer in the field of soil microbiology was the first to isolate root nodule bacteria. Allen and Allen (1936) and Norris (1956) found that the rhizobia associated with wild tropical legumes were slow growers. Lange (1961) isolated rhizobia from wild legumes indigenous to South Western Australia and found that all of them were slow growing type. Graham and Parker (1964) studied the colonial characteristics of 7 species of Rhizobium and showed that R.trifolii, R.meliloti, R.phaseoli and R.leguminosarum were fast growers and produced colonies with 4 mm diameter after 7 days. The colonies were circular, convex, white and glistening with an entire margin. Colonies produced by R.lupini, R.japonicum and Rhizobium sp. (cowpea) on the other hand were smaller than 1 mm after 7 days. They were circular, white and glistening and had an entire margin, finely granular texture and convex elevation.

Generally the rhizobia associated with the wild tropical legumes are slow growers but fast growing rhizobia have also

been isolated from some of them. e.g. species of Samanea, Andura, Albizzia and Cytisus (Allen and Allen 1936), Psoralea (Norris 1965), Leucaena leucocephala, Acacia farnesiana, Mimosa invisa, Mimosa pudica (Trinick 1968, 1980a), Sesbania grandiflora, Acacia pennatula and Glyricidium sepium (Roskoski and Wood, 1984), Prosopsis chilensis, Acacia cyanophylla A.melanoxylon (Herrera & Olivares, 1984) (Johnson and Allen, 1952). Recently Lim and Ng (1977) have isolated fast growing strains from many tropical legumes from Singapore which were previously reported to have slow growing symbionts. Johnson and Allen (1952) divided 39 strains isolated from Sesbania spp. into two well defined groups. The one from S.macrocarpa, S.longifolia was culturally similar to the fast growing rhizobia from clover, peas and beans, the other from S.grandiflora, S.tomentosa, S.aculeata and S.sesban fell into the slow growing group. Basnyat (1979) isolated 7 fast growing and 7 slow growing rhizobia from the wild and cultivated legumes from Western Maharashtra.

According to Dommergues et al. (1984) nitrogen fixing trees can be classified into 3 broad groups according to nodulation response patterns with fast and slow growing tropical strains of rhizobia.

Group 1 - which nodulates with fast growing rhizobia.

Group 2 - which nodulates with both fast and slow growing rhizobia.

Group 3 - which nodulates with slow growing rhizobia.

Somasegaran and Hoben (1985) state that the classification of rhizobia is becoming increasingly complex because of new findings of fast growing, acid producing rhizobia from some tropical legumes and the taxonomic status of these organisms may be resolved in future.

The important biochemical and physiological characteristics used in the study of root nodule bacteria are production of acid or alkali on YEMA, litmus milk reaction, utilization of carbohydrates, effect of pH and salt concentration on the growth of rhizobia and tests viz., Nitrate reductase, Gelatinase, Penicillinase, Urease, Catalase, Starch hydrolysis and $H_2S$ production.

Graham and Parker (1964) have studied most of the above mentioned characteristics of the rhizobia from the seven cross inoculation group. The cultural, biochemical and physiological characteristics of rhizobia from wild as well as cultivated legumes were studied by Rangaswami and Oblisami (1962). Raju (1938) studied variations in the fermentation characters of different strains of nodule bacteria of the cowpea, Cicer and dhaincha groups.

Norris (1965) tested 717 strains of rhizobia for growth on Yeast extract Mannitol medium containing bromothymol blue. Strains of R.trifolii, R.meliloti, R.leguminosarum and R.phaseoli produced acid in this medium while most other strains gave on alkaline reaction.

Some of the biochemical and physiological characteristics studies include those with rhizobia from Egyptian clover (Essawi and Abdel Ghaffer 1967), rhizobia associated with some tree legumes (Basak and Goyal 1980), fast and slow growing rhizobia associated with soybean (Sadowsky et al. 1983), chick pea rhizobia (Gaur and Sen 1981), temperature sensitive and tolerant Rhizobium strains (Jain and Rewari 1983), fast growing rhizobia from Lablab purpureus, Leucaena leucocephala, Mimosa spp., Acacia farnesiana and Sesbania grandiflora (Trinick 1980). The characteristics like utilization of carbohydrates and production of acid or alkali in the medium by fast and slow growing Rhizobium spp. nodulating Cajanus cajan and Cicer arietinum were studied by Bromfield and Kumar Rao (1983). Konde and Moniz (1967) studied biochemical characteristics of nodule bacteria from wal and methi. Acid production by rhizobia from Trifolium and Lotus was studied by Brockwell et al. (1966) and by Jones and Burrows (1969). Yanasugondha et al. (1977) studied physiological characteristics like reaction in litmus

milk, ability to use sugars,of the rhizobia from 52 species of wild and cultivated legumes from Thailand.

Shinde (1976) studied biochemical and physiological characteristics of rhizobia from 24 wild legumes while Basnyat (1979) studied characteristics of 7 fast growing and 7 slow growing strains from wild and cultivated legumes from Western Maharashtra.

Singh et al. (1976) studied physiological characteristics of the rhizobia from wild species of Arachis and found no significant correlation between any of the physiological properties of the strains and their symbiotic efficiency.

2.3 Serology of rhizobia :

The earliest serological studies with the root nodule bacteria is that of Zipfel (1912) who showed a relation between rhizobia nodulating Pisum sativum and Phaseolus vulgaris. Vogel and Zipfel (1921) observed that the serological properties were stable characteristic of the strain. Stevens (1923) using a collection of 55 strains from seven host groups classified them into 18 distinct groups. Bushnell and Sarles (1939) found no correlation between the ability of strains to cross inoculate and cross agglutinate when working with rhizobia from cowpea, soybean and lupine groups. Hansen and Tanner (1932)found three

agglutination groups in six rhizobial cultures of the cowpea group.

Graham (1963) used antisera prepared against 58 strains of root nodule bacteria and noted three serologically distinct groups : i) R.phaseoli, R.trifolii and R.leguminosarum; ii) R.lupini, R.japonicum and Rhizobium spp. and iii) R.meliloti. Later studies on serology with agglutination technique included those of Johnson and Means (1963), Koontz and Faber (1961), Skrdleta (1965), Holland (1966), Sidhu et al. (1977), Date and Decker (1965) and Chahal et al. (1978).

The application of the Ouchterlony gel immunodiffusion technique for the detection of soluble antigems (Dudman 1964) has assisted in more direct strain recognition and the demonstration of antigens not detectable by agglutination. The immunodiffusion technique was further used in the studies of Rhizobium japonicum (Skrdleta, 1969; Dudman 1971), R.trifolii (Humphrey and Vincent, 1965; Pankhurst, 1974; Dazzo and Hubbel, 1975), cowpea rhizobia (Dadarwal et al. 1977) slow growing rhizobia (Vincent et al. 1973), Lotus rhizobia (Pankhurst, 1979), rhizobia from Psophocarpus tetragonolobus (Ikram and Broughton, 1980), rhizobia from some pulse crops (Dadarwal and Sen 1973), Green gram rhizobia (Dadarwal et al. 1979), R.meliloti (Humphrey and Vincent, 1975; Sinha and Petersen 1980; Fuquay et al. 1984), Cicer rhizobia (Gaur

and Sen, 1984), rhizobia from some Australian woody legumes (Lawrie 1983).

Serological methods using crushed nodule preparations are widely used in strain identification of rhizobia from nodules formed as a result of inoculation (Means et al. 1964, Parker and Grove 1970, Dudman and Brockwell 1968, Chahal and Sharma 1982, Somasegaran et al. 1983).

## 2.4 Symbiotic characteristics of rhizobia from wild legumes :

Carrol (1934) studied cross inoculation relationships of 41 leguminous species in 14 genera from Florida and included 31 species into the cowpea cross inoculation group, three to clover and one to the pea group. Conklin (1936) studied rhizobia from ten wild legumes for cross inoculation characteristics and found rhizobia from eight species related to cowpea and soyabean organisms. On the basis of reciprocal cross inoculation with cowpea, Allen and Allen (1936a) added 87 tropical leguminous species from Hawaii (34 from Mimoseae, 3 from Caesalpinieae and 50 from Papilionaceae) to the cowpea group. Bushnell and Sarles (1937) added newly ten species to the cowpea group from Wisconsin. Allen and Allen (1939) carried out cross inoculation tests for 54 strains isolated from 28 leguminous species in Hawaii, using 20 hosts of the cowpea group and noted variations in infectiveness and effectiveness in fixing

nitrogen. On the basis of the cross inoculation studies with cowpea, McKnight (1949) suggested the additions of eight species from Jacksonia, Platylobium, Mirbelia, Pultenaea and Acacia to the cowpea cross inoculation group. Bhide (1956) found Desmodium diffusum to be nodulated by rhizobia from five authentic members of the cowpea group while Habish and Khairi (1968) placed eight nodulated species of Acacia from Sudan into the cowpea group.

Rangaswami and Oblisami (1962) made detailed cross inoculation studies with the rhizobia from Sesbania speciosa, Crotalaria retusa and Clitoria ternatea and showed that all the three isolates could readily nodulate several known hosts of cowpea group and vice versa. Shinde (1979) added 24 new hosts from Western Maharashtra to belong to the cowpea cross inoculation group. Gaur et al. (1974) showed that rhizobia from 51 wild leguminous species were able to nodulate groundnut plants. Singh et al. (1976) showed that several strains from wild species of Arachis efficiently nodulated Arachis hypogaea. Lim and Ng (1977) found slow growing rhizobia associated with wild legumes belonging to cowpea group. Yanasugondha et al. (1977) showed that several rhizobial strains from wild and cultivated legumes from Thailand effectively nodulated Mungbean. Ramachandran et al. (1980) found that strain from wild sannhemp gave significantly higher dry weights of cowpea plants in a pot culture experiment. Basak and

Goyal (1980)showed that six rhizobial isolates from tree legumes, when tried on green gram and black gram,were superior to the uninoculated control and in some cases performed better than the specific rhizobia. Srivastava and Tewari (1981) showed effective nodulation of cowpea by rhizobia from two wild legumes Uraria picta and Zornia diphylla. Trinick (1980) found effective nodulation on Vigna unguiculata and V.unguiculata ssp. sesquipedalis by fast growing rhizobia from Acacia farnesiana, Mimosa spp. and Sesbania sp. Nodulation tests carried out by Johnson and Allen (1952) showed that six Sesbania rhizobia formed nodules and brought about ineffective growth responses in cowpeas. Basnyat (1979) showed that seven fast growing and seven slow growing isolates from wild and cultivated legumes were able to nodulate cowpea plants.

Balaji and Rangarajan (1985) showed that rhizobial strains isolated from Acacia dealbata, A.nilotica and Samanea cyclocarpum increased the shoot dry weight and plant nitrogen of three tree legumes i.e. A.nilotica, S.saman and Leucaena leucocephala.