1. Introduction

Nitrogen constitutes four fifth of the world's atmosphere, however, life on this planet except for few species and systems is unable to assimilate it in the gaseous form. These few species and systems able to fix nitrogen biologically include i) non symbiotic free living ones e.g. bacteria and blue green algae ii) symbiotic systems e.g. rhizobia legume, angiosperm-actinomycetes and blue green algae associations and iii) associative systems e.g. rhizosphere, phyllosphere and lichens. The rhizobia-legume symbiotic system contains bacteria from the genus Rhizobium which are able to form nodules on the roots of the plants of the family Leguminosae.

1.1 Family Leguminosae -

Leguminosae is one of the largest families of flowering plants, ranking third in size after Compositae and Orchidaceae. It is having about 16,000 to 19,000 species in about 750 genera. It has a worldwide distribution and its species occur in temperate zones, humid tropics, arid zones, high lands, low lands, and savannas. Leguminosae has been divided in three clearly distinct subfamilies on the basis of floral differences viz., Caesalpinioideae, Mimosoideae and Papilionoideae.

i. Caesalpinioideae - Contains about 2,800 species - trees, Shrubs, mainly trees of the forests of Africa, South America and Asia.

ii. Mimosoideae - Contains about 2,900 species - trees, shrubs, woody vines, few perennial herbs found in semi-arid, tropical and subtropical regions of Africa, North and South America, Asia and Australia.

iii. Papilionoideae - Contains about 14,000 species - trees, shrubs, herbs, annual or perennial, worldwide occurrance.

In human welfare, legumes form an important group of plants second only to grasses (Gramineae). Their seeds are rich in protein and oil and thus form an important part in the animal and human food. They are also commercially important and extensively used for the production of insecticides, viscous resins, gums, tannin, natural dyes, medicines and fibres, as a source of luxurious timbers, as ornamental flowering plants and as a honey source.

The most important characteristic of legumes is their symbiotic association with bacteria belonging to genus Rhizobium. This symbiotic activity culminates in the formation of nodules on the leguminous root system, which is an unique ecological character of family Leguminosae.

1.2 Nodulation status of Leguminosae -

A wide variation is seen in the nodulation ability of leguminous plants and majority of legume species belonging to Caesalpinioideae infact do not bear nodules on their root system.

Allen and Allen (1981) have compiled the nodulation survey data of Leguminosae as detailed in Table No. 1. According to them 48 per cent of the leguminous genera have been examined for nodulation, and out of these 86 per cent were found to be nodulated. At the generic level 83 per cent of Mimosoideae, 95 per cent of Papilionoideae and 40 per cent of Caesalpinioideae were nodulated.

Lim and Burton (1982) have put forward the following major difficulties responsible for the limited knowledge of nodulation status of Leguminosae.

i. Inaccessibility of specimens, many of which are found in areas which are not easily reached by collectors.

ii. Some genera and species are confined to certain parts of the world only.

iii. Woody tree genera are difficult to examine for presence or absence of nodules.

iv. Seeds of number of tropical legume species, which are required for confirming nodulating ability, are not available or difficult to obtain.

1.3 The genus Rhizobium -

In legume-rhizobia symbiosis, the legume is the bigger partner, while the Rhizobium is the smaller partner, often referred to

Table 1 : Summary of nodulation survey data. (Allen and Allen 1981.)

Subfamily	Number of genera	Number of genera reported				Estimated number of species	Number of species reported			
		+	+/-	-	Total		+	+/-	-	Total
Mimosoideae	66	18	8	5	31	2,900	351		37	388
Caesalpinioideae	177	13	13	39	65	2,800	72	6*	180	258
Papilionoideae	505	241	14	14	269	14,000	2,416		46	2,462
Total	748	272	35	58	365	19,700	2,839	6	263	3,108

* One species each of _Amherstia_, _Copifera_, _Eperua_, _Hymenaea_, _Mora_, and _Saraca_.

as the 'Microsymbiont'. Rhizobia are Gram negative rods, aerobic and chemoorganotrophs, do not form endospores and are motile with peritrichous or subpolar flagella.

As for the biochemical and physiological characteristics of the rhizobia, there is no distinct variation between them and thus it becomes difficult to classify them based on these characteristics. However, their ability to infect the leguminous plants is highly specific and this host specificity is primarily used for classification of these organisms and Fred, Baldwin and McCoy (1932), for the first time, put forward the concept of cross inoculation groups. Under this system of classification seven cross inoculation groups are recognized.

Graham (1976) has pointed out the following major limitations of the cross inoculation concept of classification.

i. Cross infection - Cross infection is the nodulation of plants from one affinity group by rhizobia from another. Each of the species has now been shown to be cross infective to some degree.

ii. Insufficient nodulation data - Of the 14,000 or so known species of legumes, only 8-9% have been examined for nodules and only 0.3-0.4% have been studied with respect to their symbiotic relationships with nodule bacteria.

iii. Scarcity of biochemical data - Many of the biochemical studies involve only a few strains, and so it is difficult togeneralizeas to taxonomic meanings of biochemical differences.

According to Elkan (1981) the assumption, that each species of Rhizobium nodulates only plants within a specified cross inoculation group, has lost credibility.

According to Somasegaran and Hoben (1985), however, the host dependent cross inoculation group system is the best practical system currently available inspite of criticisms levied against it.

In the 8th edition of Bergey's manual of determinative Bacteriology (Jordan and Allen, 1974) all the root nodule bacteria were included in the genus Rhizobium and they were grouped in the part 7 under the family Rhizobiaceae along with genus Agrobacterium. The rhizobia were further divided into two broad groups on the basis of their growth rate.
Group I : Rhizobia showing rapid growthon Yeast extract mannitol media. It contains four species - R.leguminosarum, R.phaseoli, R.trifolii and R.meliloti.
Group II : Rhizobia showing slow growth on Yeast extract mannitol media. It contains 2 species - R.japonicum and R.lupini.

The genus *Rhizobium* consisted six species referred above plus a miscellaneous grouping known as cowpea miscellany. This versatile group comprised tropical and semitropical vine, shrub and tree species from various genera of three subfamilies of Leguminosae. Isolates from each of these hosts are able to nodulate cowpea (*Vigna unguiculata* L. Walp.). This broad spectrum cowpea miscellany has not yet been designated with a species epithet.

The authors at the same time have stated that the taxonomic position of Rhizobium is controversial and that the current classification can be regarded only as tentative.

A revised classification is proposed in the 9th edition of Bergey's Manual of Systematic Bacteriology (Jordan, 1984) in which the fast growing species *Rhizobium trifolii*, *Rhizobium phaseoli* and *Rhizobium leguminosarum* are combined as one species, designated *R. leguminosarum* (Frank) Frank, comprising three biovars (*trifolii*, *phaseoli* and *viceae*). *Rhizobium meliloti* Dangeard is retained as a separate species in the genus *Rhizobium* and fast growing lotus rhizobia and related strains are included into a new species, *Rhizobium loti* (Jarvis *et al*. 1982). The slow growing, non-acid producing root nodule bacteria are separated from the fast growing, acid producing strains and placed in a new genus *Bradyrhizobium* (Jordan, 1982).

The genus Bradyhizobium represents a heterogenous group of nodule bacteria within which the taxonomic relationships are not well understood. There is only one designated species, Bradyrhizobium japonicum. For the present, it is suggested that the members of the genus Bradyrhizobium other than B.japonicum be referred to as Bradyrhizobium sp. with the name of the appropriate plant in the parentheses immediately following e.g. Bradyrhizobium sp. (Vigna) or Bradyrhizobium sp. (Lupinus).

Recently a new species Rhizobium fredii is erected for fast growing rhizobia from soybean (Scholla and Elkan, 1984).

It is thus seen that in this classification also a species epithet is not given to members of 'Cowpea miscellany'. Trinick (1982) states that unfortunately there has been a tendency to use this group as a dumping ground for all unspecialised and specific group of legumes not falling into other cross inoculation group and he suggests that before devising a classification system combining as much of the bacterial genome as possible, a tremendous effort is required to study the cowpea miscellany. According to Elkan (1981) there is a dearth of biochemical and broad spectrum serological tests useful for classification and identification of rhizobia. Therefore, it is necessary to carry out detailed nodulation studies involving additional plant species.

1.4 The root nodule bacteria associated with wild legumes -

The research on the root nodule bacteria done so far primarily concerns the agriculturally important legumes occurring in temperate regions of Europe and America. It, however, does not cover the entire Leguminosae family because a large number of them found in tropical zone have not been included in this study. As compared to tropical regions fewer legumes occur in temperate regions and the flora is less predominant in terms of number of genera and species. Only 12% of legume genera are typical of temperate climates (Eli, 1977). The tropical legumes on the other hand form an important group with reference to their nitrogen fixing ability as they can leave upto 30% of fixed nitrogen in soil for succeeding crops (Oke, 1966) as against 10-15% contributed by temperate legumes (Parker, 1977).

In the tropics also much of the early knowledge of nodulation status of legumes and associated rhizobia is confined to species of the few genera which are useful in agriculture and species that are grown as cover crops, as green manures and in crop rotation. Out of 18,000 species of leguminous plants only 100-150 species are actually cultivated (Lim and Burton 1982). Thus the uncultivated wild legumes, which comprise large portion of Leguminosae, are neglected. Mac Connel and Bond (1957) have defined wild legume as a legume of no agricultural significance or one which though used in agriculture, is growing in a natural community.

Lim and Burton (1982) suggest that there is a large scope in the study of nodulation of tropical wild legumes and it is highly likely that more instances of nodulating species both at generic and specific level await discovery. According to Allen and Allen (1981) this study is important and urgent because of rapid landscape changes and exploitation of tropical regions and should be carried out before these important resources are destroyed forever.

In the tropical environment the protein shortage is a prominent feature and tropical soils often lack available nitrogen. The legume Rhizobium symbiosis is the most promising plant-bacteria association for immediate increases in protein yeild. Under pressure of population increases and shortages of energy and fertilizers, it is important to increase food production without expending large quantities of energy. Use of inorganic fertilizers is becoming increasingly difficult because of the high cost and depletion of fossil fuels which become precursors in the production of fertilizers. The industrial process requires about 6,000 Kilocalories of energy per Kilogram of nitrogen fixed (Delwiche, 1970). In addition, environmental pollution resulting from the excessive use of fertilizer nitrogen brings out the greater need for exploitation of biological nitrogen fixation.

Larsen (1979) suggests on a global basis that 140 million tons of nitrogen are fixed biologically each year and about 80% of this i.e. 112 million tons is fixed by nodulated leguminous plants. The world has a factory production of about 50 million tons of anhydrous ammonium a year and more than 40 million tons of this is used as fertilizer. The biological process appears to fix three times the amount produced by chemical fixation.

For increasing world food supplies, through more extensive utilization of legume-Rhizobium association, both symbionts must be studied in detail. According to Lim and Burton (1982) with 18,000 species of leguminous plants, the potential for Rhizobium germplasm appears enormous. Allen and Allen (1981) suggest that just as crop breeders today use a huge collection of seeds of wild and cultivated species to select germplasm for improved crops, Rhizobium workers too should launch a parallel effort in search of new nitrogen fixing systems within the Leguminosae.

Thus the studies on the legumes and status of wild arboreal and herbaceous legumes and cultural, biochemical, physiological, symbiotic and serological characteristics of root nodule bacteria associated with them, assumes special importance.