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Rhizobium association in legumes which grow in Rajasthan

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ABSTRACT: *Rhizobia* are vital for nitrogen input, fertility of soil and legume plant growth. Knowledge on *rhizobial* diversity from arid and semiarid areas is important for dry land agriculture in the context of climatic change and for economic utilization. This study provides morphological, biochemical, stress tolerance and plant growth promoting characteristics of fifteen *rhizobial* isolates from the nodules of same number of wild legumes and one isolate from cultivated *Arachis hypogea* from dry region of Rajasthan. Also, an attempt has been made to evaluate the effect of abiotic constraints (salt, pH and temperature) on the growth of *rhizobia* isolated from *Leucaena leucocephala*, *Tephrosia purpurea* and *Crotalaria medicaginea* grown in arid and semiarid regions of Rajasthan with a view to screen out stress tolerant isolates. The stress tolerant traits of these *rhizobia* are of potential value from the point of view of biofertilization of legume seedlings during a forestation of degraded areas in arid and semi-arid tropics of Rajasthan. Additionally, occurrence of nodulated legumes in the arid and semi-arid areas of Western Rajasthan and to characterize their associated symbiotic bacteria. Legumes are drought escaping (annual species), drought tolerant perennials or trees possessing deep root systems and other adaptations to arid conditions. Nodulation was recorded in all members of the Papilionoideae and Mimosoideae, but only one species of Caesalpinioideae. Internal structure of nodules varied within these groups, especially with respect to the presence or absence of uninfected cells in the infected region. Full 16S rRNA gene sequencing revealed that the nodules harboured a range of nodulating bacteria belonging to the genera *Sinorhizobium*, *Rhizobium* and *Bradyrhizobium*, within which they formed separate sub clades. Symbiotic nitrogen fixation (SNF) is a characteristic feature of nodulating legumes. The wild legumes are comparatively less explored for their SNF ability; hence, it is essential to study nodulation and identify the microsymbiont diversity associated with them. The *Rhizobium*-legume symbioses have received most attention and have been examined extensively. The behavior of some N₂-fixing systems under severe environmental conditions such as salt stress, drought stress, acidity, alkalinity, nutrient deficiency, is reviewed.

I. INTRODUCTION

Rhizobia/Bradyrhizobia have the ability to fix atmospheric nitrogen in symbiotic association with legumes and certain non-legumes like *Parasponia* and *Tremma*. They normally enter the root hairs, multiply there and form nodules. The amount of nitrogen fixed varies with the strain of *Rhizobium*, the plant species and environmental conditions. Not all legumes fix nitrogen. The capacity to form nodules appears to be absent from the majority of the segregate family Caesalpinaceae. On the other hand nodulation, if not nitrogen fixation, with root nodule bacteria appear almost universal in the segregate family Mimosaceae and other Fabaceae. Legumes play unique role in plant kingdom and rank third amongst flowering plants in number of species they contain. Most of the species are trees, shrubs and woody vines. The cultivated species include mainly food, forage and pasture plants. It is believed that legume symbiosis contributes at least 70 million metric tonnes N per year; approximately one half of which is derived from the cool and warm temperature zones and the remainder from the tropics.[1]

Rhizobium is the bacteria that live in symbiotic association with the root nodules of the leguminous plants. Fixation of nitrogen cannot be done independently. That is why *rhizobium* requires a plant host. *Rhizobium* is a vital source of nitrogen to agricultural soils including those in arid regions. They convert dinitrogen into ammonia. Ammonia, being toxic in nature, is rapidly absorbed into organic compounds.



FIG.1. *Rhizobium*

Nitrogen fixation helps in increasing soil productivity and soil fertility. The various behavioural factors such as drought stress, nutrient deficiency, salt stress, fertilizers, pesticides of nitrogen-fixing systems are reviewed. *Rhizobium* can be classified on the basis of the types of the plant they are associated with and also the rate of growth. Few species of *Rhizobium* bacteria include:

- *Rhizobium leguminosarum*
- *Rhizobium alarii*
- *Rhizobium lantis*
- *Rhizobium japonicum*
- *Rhizobium trifolii*
- *Rhizobium phaseolii*
- *Rhizobium smilacinae*

Nitrogen fixation is the essential biological process and the initial stage of the nitrogen cycle. In this process, the free nitrogen available in the atmosphere is converted into ammonia (another form of nitrogen) by certain bacterial species like *Rhizobium*, *Azotobacter*, etc. and the complete process is carried on by natural phenomena.[2]

Rhizobium plural form *rhizobia* are prokaryotes whose main function involves the conversion of stable nitrogen gas in the atmosphere to a biologically useful form. Nitrogenase is an enzyme complex that reduces dinitrogen to ammonia. A huge amount of energy is consumed during the nitrogen fixation and the nitrogenase enzymes are irreversibly inactivated by oxygen. Acetylene reduction assay is used to measure the nitrogenase activity. A very less portion of species is capable of carrying out nitrogen fixation. That is around two genera of archaea, twenty genera of cyanobacteria and much more. A symbolic amount of nitrogen remains after the harvesting of grains even though a large amount of nitrogen is removed at the time of harvesting. It is mainly taken into account when there is no usage of nitrogen fertilizers. It is usually seen in less industrialized countries.[3]

Nitrogen is one of the most supplied plant nutrients as it is one of the common deficiency found in soils. Several environmental concerns are raised regarding the supply of nitrogen to the soil.

Rhizobium infects the roots of leguminous plants. They are usually found in the soil and produce nodules after infecting the roots of the leguminous plants. As a result, nitrogen gas is fixed from the atmosphere. This nitrogen is made available to the plants that help in their growth and development. When the legume dies there will be a breakdown of nodules. As a result, *Rhizobium* is released back to the cell where it can infect a new host.

Specific strains of *Rhizobium* are required to make the nodules functional in order to carry out the process. This increases the yield of the crops. Legume inoculation has been an agricultural practice for several years and has constantly improved over time.[4]

Rhizobia are genetically diverse and physiologically heterogeneous group of symbiotic nitrogen fixing bacteria that form nodules on the roots or rarely on the stem of legume hosts, within which the bacteria fix atmospheric nitrogen into ammonia. A fully functional symbiosis requires successful survival ability of bacteria even under adverse environmental conditions. Within the soil, *rhizobia* frequently encounter various stresses that affect their growth, their initial steps of symbiosis and the capability of nitrogen fixation. Inoculation of stress tolerant strains of *rhizobia* may enhance the nodulation and nitrogen fixation ability of plants under stress conditions. The ability of legume hosts to grow and survive in saline conditions is improved when they are inoculated with salt tolerant strains of *rhizobia*. About 60% of the Rajasthan state falls within the desert region (34°35'N to 30°10'N latitude and 69°31'E to 76°55'E longitude) and covers the largest area (61%) of hot arid regions of the Indian Desert (DST, 1994). The natural *rhizobia* of wild legumes growing in arid zones exhibit higher tolerance to prevailing adverse conditions like salt stress, elevated temperature and drought. Selection of effective, efficient and compatible stress tolerant *rhizobial* strains could help in ecological rehabilitation of degraded soils and increases soil fertility thereby improving the growth of associated plants of this region. However, only few studies on this aspect have been carried out in dry regions of Rajasthan.[5,6]

III.METHODOLOGY

To collect nodules and isolate *rhizobium* . .

- a. Recognizing legumes and identifying them in the field
- b. Recovering nodules in the field
- c. Preserving nodules
- d. Examining nodules and bacteroids
- e. Isolating *Rhizobium* from nodule
- f. Performing the presumptive test
- g. Authenticating the isolates as *Rhizobium*
- h. Preserving *Rhizobium* cultures Requirements

To observe the infection process.

- a. Culturing strains of *rhizobia* in YM
- b. Germinating seeds
- c. Preparing a Fahraeus-slide
- d. Inoculating the seedlings
- e. Observing the root-hairs under the microscope
- f. Comparing root hair deformations Requirements

To study cultural properties, cell morphological characteristics and some nutritional requirements of *rhizobium*.

- a. Preliminary subculturing of different bacterial cultures
- b. Comparing cell morphology and gram stain reactions of *Rhizobium* with those of other microorganisms
- c. Determining gram stain reactions of various bacteria
- d. Characterizing growth of *rhizobia* using a range of media
- e. Observing growth reactions on modified media Requirements

To quantify the growth of *rhizobium*.

- a. Preliminary culturing of fast and slow- growing *rhizobia*
- b. Determining the total count with a Petroff- Hausser chamber
- c. Using the Petroff-Hausser counting chamber
- d. Estimating cell concentration by optical density
- e. Determining the number of viable cells in a culture by plating methods

f. Determining the mean-generation (doubling) time of *rhizobia* Requirements

To count *rhizobia* by a plant infection method

- a. Preparing inoculants
- b. Setting up the plant dilution count in plastic growth pouches
- c. Planting seeds in growth pouches
- d. Inoculating for MPN count
- e. Determining the most probable number Requirements

IV.OBSERVATIONS

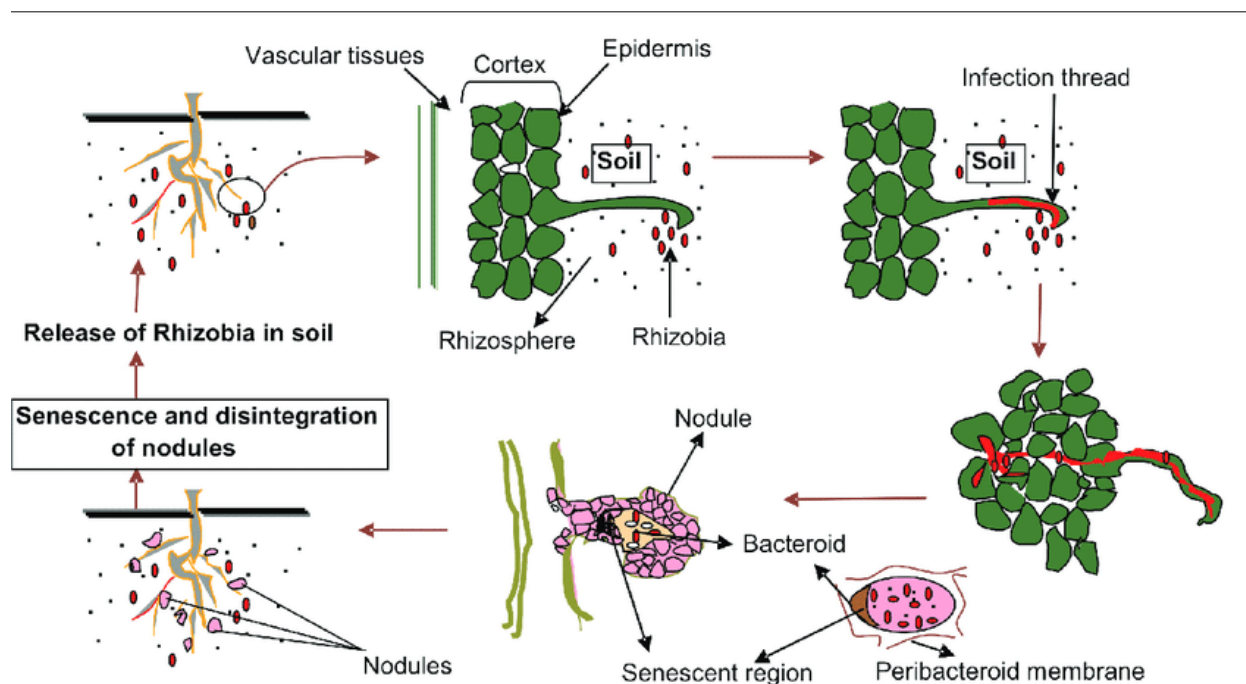


FIG.2. Penetration of *Rhizobium* in root hair and formation of nodule

Bacterial accessory genes are genomic symbionts with an evolutionary history and future that is different from that of their hosts. Packages of accessory genes move from strain to strain and confer important adaptations, such as interaction with eukaryotes. The ability to fix nitrogen with legumes is a remarkable example of a complex trait spread by horizontal transfer of a few key symbiotic genes, converting soil bacteria into legume symbionts. *Rhizobia* belong to hundreds of species restricted to a dozen genera of the Alphaproteobacteria and Betaproteobacteria, suggesting infrequent successful transfer between genera but frequent successful transfer within genera. Biological nitrogen fixation gives legumes a pronounced growth advantage in nitrogen-depleted soils and is of considerable ecological and economic interest. In exchange for reduced atmospheric nitrogen, typically given to the plant in the form of amides or ureides, the legume provides nitrogen-fixing *rhizobia* with nutrients and highly specialised root structures called nodules. The different functional genomics approaches that have been performed on *rhizobial* symbiosis, with a focus on studies investigating the molecular mechanisms used by the bacterial partner to interact with the legume. The main challenges faced in the reclamation of severely degraded lands are in the management of the systems and finding plant species that will grow under the harsh conditions common in degraded soils. This is especially important in extremely adverse situations found in some substrates from mining activities or soils that have lost their upper horizons. Under these conditions,



recolonization of the area by native vegetation through natural succession processes may be extremely limited. Once the main physical and chemical factors restrictive to plant growth are corrected or attenuated, the introduction of leguminous trees able to form symbioses with nodulating *Nâ*,,-fixing bacteria and arbuscular mycorrhizal fungi constitutes an efficient strategy to accelerate soil reclamation and initiate natural succession.[7,8] These symbioses give the legume species a superior capacity to grow quickly in poor substrates and to withstand the harsh conditions presented in degraded soils. *Ensifer* sp. PC2 is an aerobic, motile, Gram-negative, non-spore-forming rod that was isolated from a nitrogen-fixing nodule of the tree legume *P. cineraria* (L.) Druce (Khejri), which is a keystone species that grows in arid and semi-arid regions of the Indian Thar desert. Strain PC2 exists as a dominant saprophyte in alkaline soils of Western Rajasthan. It is fast growing, well-adapted to arid conditions and is able to form an effective symbiosis with several annual crop legumes as well as species of mimosoid trees and shrubs. Strain PC2 exists as a dominant saprophyte in alkaline soils of Western Rajasthan. It is fast growing, well-adapted to arid conditions and is able to form an effective symbiosis with several annual crop legumes as well as species of mimosoid trees and shrubs.[9]

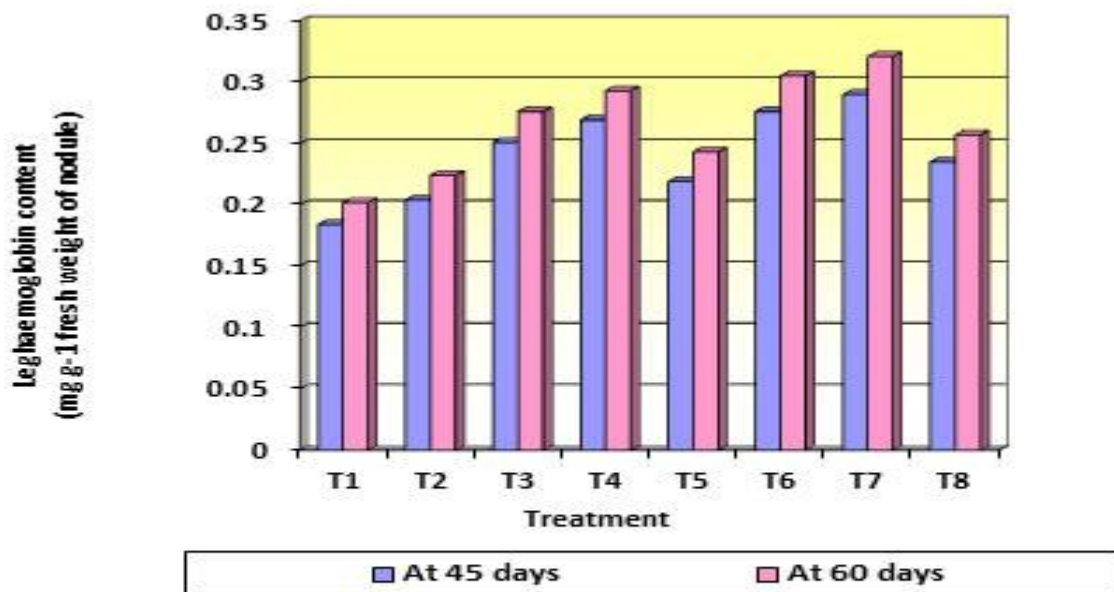


Fig. 3. Effect of liquid consortium of salt tolerant *Rhizobium* on fresh weight of shoot and root of soybean at flowering and harvesting stages

Fig.3. Effect of Inoculation of Salt Tolerant *Rhizobium* on Nodulation and Leghaemoglobin Content of Soybean

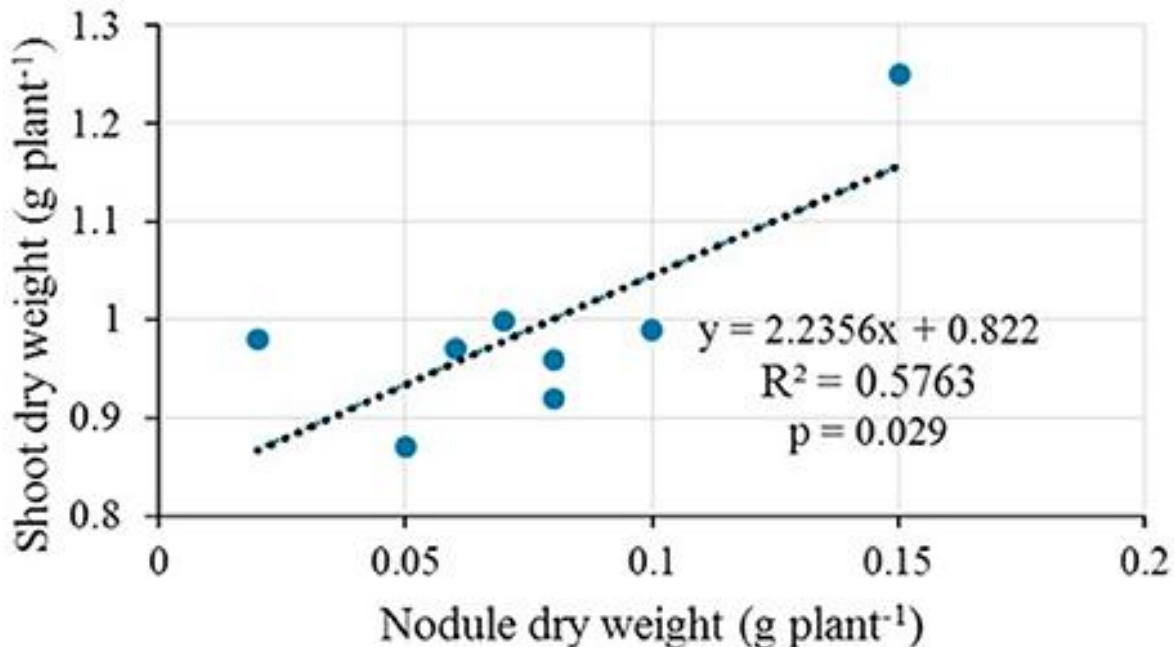


Fig.4: Potential of Native *Rhizobia* in Enhancing Nitrogen Fixation and Yields of Climbing Beans *Phaseolus vulgaris* L.

V.DISCUSSION

Nitrogen is an important element for all organisms; it is derived from dinitrogen (N₂), which comprises up to 78% of the earth's atmosphere, via nitrogen fixation. Nitrogen fixation converts N₂, a metabolically useless form of nitrogen for most organisms, into ammonia, which can be metabolized by most organisms. Thus, nitrogen fixation is essential for life. Nitrogen fixation is carried out mainly in soil by nitrogen-fixing bacteria and archaea. Among nitrogen-fixing bacteria, *rhizobia* can live in soil as saprophytes or in the root nodules of their host legumes as symbionts. In these nodules, *rhizobia* fix atmospheric nitrogen for use by their host, while the host supplies the *rhizobia* with carbon from photosynthesis. This symbiosis between *rhizobia* and legumes is a supreme example of plant-microbe mutualism, and it is beneficial not only for leguminous plants (e.g., soybean, chickpea, pea, common bean, and alfalfa) but also for the global nitrogen cycle.[10,11]

In the *Rhizobium*-legume symbiosis, which is a N₂-fixing system, the process of N₂ fixation is strongly related to the physiological state of the host plant. Therefore, a competitive and persistent *rhizobial* strain is not expected to express its full capacity for nitrogen fixation if limiting factors (e.g., salinity, unfavorable soil pH, nutrient deficiency, mineral toxicity, temperature extremes, insufficient or excessive soil moisture, inadequate photosynthesis, plant diseases, and grazing) impose limitations on the vigor of the host legume. Increasing salt concentrations may have a detrimental effect on soil microbial populations as a result of direct toxicity as well as through osmotic stress. Some legumes, e.g., *Vicia faba*, *Phaseolus vulgaris*, and *Glycine max*, are more salt tolerant than others, e.g., *Pisum sativum*. It has been reported that some *V. faba* tolerant lines sustained nitrogen fixation under saline conditions. Other legumes, such as *Prosopis*, *Acacia*, and *Medicago sativa* are salt tolerant, but these legume hosts are less tolerant to salt than are their *rhizobia*. Although the root nodule-colonizing bacteria of the genera *Rhizobium* and *Bradyrhizobium* are more salt tolerant than their legume hosts, they show marked variation in salt tolerance. *Rhizobium* strains from *Vigna unguiculata* were tolerant to NaCl up to 5.5%. *Rhizobia* from woody legumes also showed substantial salt tolerance: strains from *Acacia*, *Prosopis*, and *Leucaena* are tolerant to 500 to 850 mM NaCl. The content of polyamines, e.g., homospermidine, increases in salt-tolerant cells and acid-tolerant strains of *R. fredii*. This polyamine may function to maintain the intracellular pH and repair the ionic



imbalance caused by osmotic stress. Osmotic stress (shock) results in the formation of specific proteins in bacteria. The Na^+ , K^+ , and Mg^{2+} concentrations are increased in cells of cowpea *Rhizobium* under salt stress. These organic osmolytes (amino acids) and the inorganic minerals (cations) may play a role in osmoregulation for this *Rhizobium* strain. The occurrence of *rhizobial* populations in desert soils and the effective nodulation of legumes growing therein emphasize the fact that *rhizobia* can exist in soils with limiting moisture levels; however, population densities tend to be lowest under the most desiccated conditions and to increase as the moisture stress is relieved.[12,13]

A strain of *Prosopis* (mesquite) *rhizobia* isolated from the desert soil survived in desert soil for 1 month, whereas a commercial strain was unable to survive under these conditions. The survival of a strain of *Bradyrhizobium* from *Cajanus* in a sandy loam soil was very poor; this strain did not persist to the next cropping season, when the moisture content was about 2.0 to 15.5%. Soil salinity and acidity are usually accompanied by mineral toxicity (specific ion toxicity), nutrient deficiency, and nutrient disorder. Salt damage to nonhalophytic plants grown in nutrient solution is often due to the effect of ion imbalance (disorder) rather than the osmotic potential. This disorder might occur by specific toxicity of ions such as Na^+ and Cl^- and might be balanced by increasing the concentration of counterions, like K^+ and Ca^{2+} , against Cl^- . It has been suggested that K^+ and NO_3^- inhibited Na^+ and Cl^- translocation from the roots to the shoots of *Arachis hypogaea*, so that leaf growth was protected against salt damage. The dominant ions in saline waters and saline soils which are available in arid zones are Na^+ and Cl^- . The *rhizobia* of wild legumes in arid zones, exhibit higher tolerance to the prevailing adverse conditions, e.g. salt stress, elevated temperatures and desiccation. These *rhizobia* may be used to inoculate wild, as well as, crop legumes, cultivated in reclaimed desert lands. Recent reports indicated that the wild-legume *rhizobia* formed successful symbioses with some grain legumes. Moreover, intercropping of some N_2 -fixing tree legumes (e.g. *Lablab*, *Leucaena*, *Sesbania*, etc.) to pasture grasses improved biomass yield and herb quality. [14]

VI.RESULTS

Thar Desert strains from closely related strains within genera *Ensifer*, *Rhizobium* and *Bradyrhizobium*. Some of the strains showing phosphate solubilization as well as phytase activity suggest that the root nodule bacteria associated with native legumes of the Indian Thar Desert in alkaline soil are useful as their rhizospheric presence could help in reclamation of the fragile soil by increasing available phosphate for the plants. The Aravalli region harbor germplasm of a number of species of legumes. Some of these are unique for several reasons as they are endemic. There is scope for introduction of certain legumes which can be cultivated for increasing productivity and income and for increasing productivity of other legumes through introduction of endophytic *rhizobia* associated with these plants. The two important legumes namely *Mucuna pruriens* and *Pueraria tuberosa* are native to this region. These lianas are also medicinally important. There is very less literature available as per nitrogen fixation studies on these plant species. Plants growing in Indian Thar Desert are important bio-resources possessing many useful characteristics that make them to survive in extreme environmental conditions. Plants thriving in arid and semi-arid regions of non-agricultural lands are like supporting commodity for poor, laborious and innocent local peoples. These plants give them food, fodder, shelter as well as provide medical/health support in famine and uncongenial conditions. Therefore such plants should be given priorities to work. The current investigation will be useful in improving plant performance. Depending on the results of cross inoculation studies it might be possible to find out broad host range *rhizobia* applicable to agricultural/industrial crop and large scale inoculants preparation.[15]

VII.CONCLUSION

The documentation of nodulating native legume species and the *rhizobial* diversity associated with them in various parts of world has gained attention as this symbiotic association provides fixed nitrogen, improves productivity of plants in an ecofriendly manner. Before field-based applications the symbiotic bacteria need to be assessed for their N fixing ability as well as characterized at molecular level. The phylogeny based on symbiosis-essential genes supplemented with the host-range studies helps in better understanding of the symbiotaxonomy of *rhizobia*. More efficient symbiotic couples need to be screened by cross-nodulation studies for their application in agricultural practices. The most appropriate species should be chosen and cultivar for prevailing conditions in accordance with the priorities of the grower, e.g. high grain yield of a particular legume, high level of fixed N in stover for animal feed, or high level of fixed N in stover to be incorporated into the soil for the benefit of a subsequent cereal crop in Rajasthan.



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