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Biological Nitrogen Fixation to Improve Plant Growth and Productivity

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Abstract – **About 80 % of earth's atmosphere is nitrogen gas. Plants, animals and microorganisms cannot use nitrogen in its inert form. Nitrogen must be converted into its usable form like ammonia which can be used by the plants and other organisms. All organisms use ammonia to form Amino acids, proteins and other nitrogen containing compounds. Biological Nitrogen fixation is a process which converts Nitrogen into Ammonia which can be utilized by the plants. When bacteria die in soil, they release nitrogen containing compounds which can be used by the plants for their growth and development. The production of fixed nitrogen from di-nitrogen for chemical fertilizer accounts for about 25% of the Earth's newly fixed N_2, and biological processes account for about 60%. The requirements for Nitrogen fertilizer is increasing day by day as it is an essential element for the growth and development of plants.It is also a part of chlorophyll, which is very important component of photosynthesis. For more than 100 years, biological nitrogen fixation (BNF) has achieved the attention of scientists concerned with plant mineral nutrition, and it has been used extensively in agricultural practice. The topic of Biological Nitrogen fixation is of great practical importance because the use of chemical nitrogenous fertilizers has resulted in high levels of water pollution and the eutrophication of lakes and rivers. When nitrogen fertilizers are leached in the soil, these cause serious pollution problems, particularly in water supplies. This review focuses on the improvement of the growth and yield of plants having biological nitrogen fixation ability due to certain microorganisms. Symbiotic and free living nitrogen fixing microorganisms have also been discussed. Several different types of associations for biological nitrogen fixation in different plants have been mentioned. These associations include Actinorhizal plants and Frankia, Cycas and Cyanobacteria, Legumes and Rhizobia.**

Keywords – **Free Living Nitrogen Fixation, Symbiotic Nitrogen Fixation, Legume Nitrogen Fixation, Actinorhizal Symbiosis, Rhizobial Symbiosis.**

I. Biological Nitrogen Fixation

Plant growth and productivity is arrested due to the deficiency of certain minerals especially Nitrogen and Phosphorus in soil. Free living as well as symbiotic nitrogen fixing bacteria plays an important role to improve the structure of the soil [1, 2, 3]. Introduction of selected microorganisms improve the conditions for plant development [3]. Inoculation of *Azotobacterchroococcum* and *Streptomyces* sp. on the early growth of black locust (*Robiniapseudoacacia*), Siberian elm (*Ulmuspumila*) and silver-leaf maple (*Acer dasycarpum*) plants improved the growth of these plants in terms of plant height and plant diameter [4]. Similarly another research work indicated that bio-inoculants showed a synergistic effect in increasing crop growth and yield. *Azospirillum* was more effective to improve the growth of plants as compared to the *Azotobacter*. The highest improvement was recorded in the yield and in different growth parameters [5]. Bio-fertilizers are important components of organic farming. These bio-fertilizers are important in sustaining the crop productivity and improving the soil fertility [6]. Biological Nitrogen fixing microorganisms also improve the soil microbiological activities. Hence the plants show improved growth and yield. These microorganisms also produce hormones and some other growth factors which are needed for the plant growth and development. Certain plant pathogens are also removed by these nitrogen fixing microorganisms [7, 8, 9, 10]. We can use suitable environment friendly bio-fertilizers by using suitable microorganisms with substrate to replace the use of chemical fertilizers. Dual application of Azotobactor and Azospirillum along with GV3 and 60kg nitrogen/ha resulted in increasing fruit set, early flowering, improvement of yield and fruit quality of strawberry [11].
The factors which control the amount of fixed nitrogen also include the amount of nitrogen available in the soil to the plants. The ability to fix atmospheric nitrogen is specific in some families. Only 4 genera in Rosaceae out of 122 are capable of fixing nitrogen.

Legumes can play an important role in the sustainable agricultural development without harming the environment. Legume Nitrogen fixation recovers the losses of Nitrogen from the soil, hence soil become more productive and fertile. The main source of fixed nitrogen addition in the soil is due to Rhizobium-legume symbiosis. This Rhizobium-Legume symbiosis also shows a renewable source of nitrogen for plants. Different legume crops and pasture species add Nitrogen in the range of 200 to 300 kg of N ha/year. BNF adds Nitrogen in the range of 139 to 175 million tons of Nitrogen, while symbiotic associations add about 35 million to 44 million of Nitrogen. Nitrogenases are enzymes used by some organisms to fix atmospheric nitrogen gas (N_2). Typically nitrogenases are coded by Nif genes. Biological Nitrogen fixation can also help to produce a fertile land. Rhizobia play a very important role in symbiotic nitrogen fixation. This system is found in leguminous plants. Approximately there are about 700 genera and 1300 species of leguminous plants which could develop root nodules and fix atmospheric Nitrogen. Rhizobial symbiosis adds much of the fixed nitrogen in the soil ecosystem as compared to the other free living symbiosis. The fixed forms of Nitrogen such as ammonia, nitrate, and organic nitrogen can be used by the plants. The nitrogen deficient pasture lands can be made more productive by growing legumes with the grasses. The introduction of *Stylosanthesguianensis* in a pasture land resulted in the improvements of fixed nitrogen for the other plants.Free-living diazotrophs, e.g. Green sulfur bacteria, Cyanobacteria, Azotobacteraceae, Symbiotic diazotrophs, e.g. Rhizobia, Frankia andActinorhizal interactions also result in the increase of Annual Nitrogen Fixation. These Actinorhizal symbiotic interactions are found in forests, wetlands, temperate and tropical regions and fields [12]. BNF can also be improved by using some naturally occuryingBradyrhizobia. Efforts should be made to improve the BNF in legumes. These efforts include the selection and engineering of suitable Nitrogen fixing strains of Brady-rhizobia and symbiotic effectiveness and function of these strains should be tested. Different Mutagenetic approaches can be utilized to improve the nitrogen fixing efficiency of these Nitrogen fixing symbiotic bacteria [13, 14].Inoculation of leguminous plants by suitable rhizobial and Brady-rhizobial strains can improve the Biological Nitrogen fixation efficiency of leguminous plants. Nitrogen fixation requires sufficient quantity of carbohydrate/kg of nitrogen fixed and of equivalent of 25-28 molecules of ATP for each molecule of nitrogen fixed. A legume-Rhizobium symbiosis is well established in a loamy soil.Barriuso and Solano (2008) demonstrated that soil microorganisms provide nutrients to the plants by decomposing organic matter [15]. There are several other advantages of soil microorganisms to the plants. Hayatsu*et al.*, (2008) also indicated that plant growth promoting microorganisms benefit plants by several ways [16]. Plant productivity can be improved by the introduction of useful microorganisms in soil in the form of bio-fertilizers. If Nitrogen fixing microorganisms are actively performing their function in soil then the use of mineral fertilizers can be replaced by using suitable microorganisms. Hence Biological Nitrogen fixation is one of the most important processes performed by soil microorganisms to convert nitrogen into the available form for the plants.

Leguminous plants develop nodules on their roots due to their association with specific rhizobial strains.Under nitrogen limiting condition, capable plants develop association with a host specific strain of bacteria called Rhizobia. Nitrogen gas from the atmosphere is converted to ammonia within the legume, which is then assimilated to amino acids, nucleic acids etc. Plants that contribute to Nitrogen fixation include the legume family Fabaceae. They contain symbiotic bacteria called Rhizobia within the nodules that produce nitrogen compounds within the rhizobia.

II. Factors affecting Biological Nitrogen Fixation

Several factors limit Biological Nitrogen Fixation. These factors include excessive soil moisture, drought, Phosphorus deficiency, excessive minerals, deficiency of certain minerals and soil acidity etc. Nitrogen fixation is also affected by extreme temperature and light conditions. Very high temperaturealso results in the failure of BNF. Among the Biotic factors if rhizobial species are decreased in number then BNF is also reduced. The reduction of the photosynthetic ability of legumes results in no BNF. Root nodules are decayed by the reduction of photosynthetic efficiency. Insects and Nematodes also affect the Nodule formation, development and function is affected. Intercropping of leguminous plants with non-legumes also results in the competition for nutrients and water. This competition can affect Nitrogen fixation efficiency.

If there are less number of nodules on legumes or root nodules are absent, then we can inoculate Rhizobia in the soil. Addition of Rhizobial strains in a soil gives better BNF efficiency as compared to the native rhizobial strains present in the soil. Different nodulating species of Rhizobia are specific, for example a rhizobial species that nodulate cowpea may not nodulate any other legume. Hence particular group of Rhizobia associate with particular leguminous plants. The nitrogen fixing systems require more phosphorus and some other nutrients. These nutrients are needed for plant growth and nodule development.

Different methods are used to improve the Biological Nitrogen fixation efficiency. One of the method is screening of different Rhizobial strains to select an effective strain. For some crops duel inoculation of Rhizobia and Mycorrhizal Fungi are effective. Genetic engineering techniques have been applied to develop legumes to make an association with effective rhizobial strains. Different cultural practices like removal of weeds from crops and introduction of Nitrogen fixing components in crops can improve the BNF efficiency, hence plant yield can improve. The actinorhizal plants and Legumes, the two major nitrogen-fixing groups of plants share a relatively close ancestor, as they all part of a clade within the rosids which is often called the *nitrogen-fixing clade*.

III. Actinorhizal Symbiosis

Actinorhizal plants are a group of angiosperms which have the ability to form a symbiosis with the nitrogen fixing actinobacteria *Frankia*. There are about 220 species which represent Actinorhizal plants Thesespecies are distributed three orders, Fagales, Rosales, and Cucurbitales [17,18]. There are about 8 families and 24 generashowing Actinorhizal symbiosis (Betulaceae: *Alnus*; Casuarinaceae: *Gymnostoma*, *Casuarina*, *Allocasuarina*, *Ceuthostoma*; Coriariaceae: *Coriaria*; Datiscaceae: *Datisca*, Elaeagnaceae: *Elaeagnus*, *Hippophae*, *Shepherdia*; Myricaceae: *Myrica*, *Comptonia*; Rhamnaceae: *Colletia*, *Discaria*, *Kenthrothammus*, *Retanilla*, *Talguenea*, *Trevoa*, *Ceanothus*; Rosaceae: *Dryas*, *Purshia*, *Cowania*, *Cercocarpus*, *Chamaebatia*). Actinorhizal plants are found on all continents except for Antarctica. They provide benefit of nitrogen fixation to the plants which are present in poor soil [19, 20].Nodules develop in the roots under nitrogen limiting conditions. Frankia is nitrogen fixing filamentous bacteria which cause an association with the Actinorhizal plants. This association is similar to the *Rhizobia* bacteria that are found in the root nodules of legumes in the Fabaceae family. Bacteria of this genus also form root nodules [20].

IV. Cycads and Cyanobacteria

Cycads form symbiotic association with nitrogen fixing cyanobacteria.Cycads provide food in the form of fixed carbon to the Cyanobacteria in exchange for fixed nitrogen which is provided by Cyanobacteria. These cyanobacteria live within the roots of Cycads [21].

Cycads also developpre-coralloid roots [Fig. 1] which are specialized symbiotic organs at a young age. These rootsgrow upwards towards the surface of oil instead of downward into its depths. The pre-coralloid roots convert into corraloid roots upon successful colonization. so named for their resemblance to coral. Within the coralloid root is the cyano bacterial zone, which is the region inhabited by cyanobacteria [22]. This has unique characteristics which facilitate a close relationship between cycad and cyanobacteria. The process of Cyanobacterial colonization of Cycad coralloid roots involves themutual coordination of action and reaction by both organisms.

When Cyanobacteria come into contact with a pre-coralloidroot, the cyanobacteria enter inside it and proceed to the cyano-bacterial zone. There are some permanent changes in the symbiotic tissue and at this stage final transformation occur from pre-coralloids nto symbiotic coralloid roots.

Then these colonies of Cyanobacteria grow And develop under the direction in the roots of Cycas With the passage of time, the Cycad coralloidmasses grow and degenerate according to environmental conditions and the needs of the plant [23].

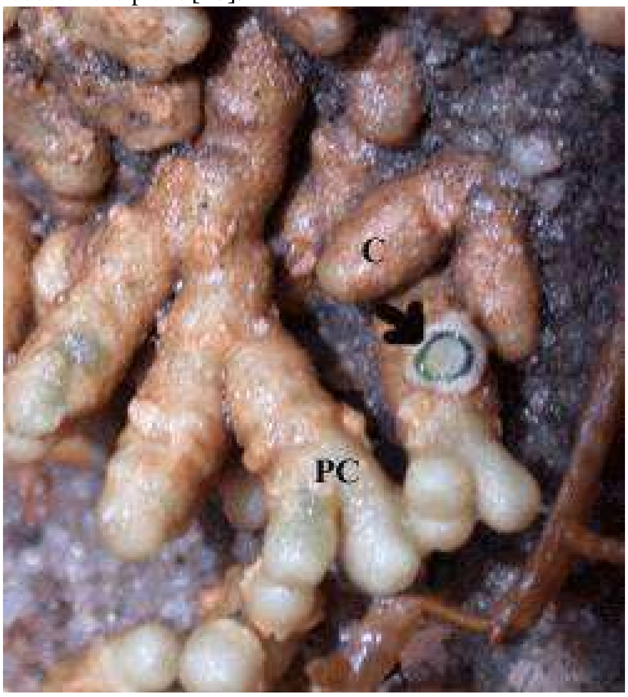

Fig. 1. Coralloid and Pre-coralloid roots growing on a *CycasCircinalis* at the New York Botanical Garden. The arrow head pointing the Green Cyano-bacterial Zone in a broken root. Fig. from wikkipedia.

V. Legumes and Rhizobium Symbiosis

The association between *Rhizobium* or *Bradyrhizobium* and legumes provide a huge supply of N for legumebased crop and pasture production than the use of fertilizer-N. The nitrogen contents of the grasses of pasture lands is also improved by growing legumes with these grasses. Rhizobia and Brady-rhizobium species fix Atmospheric N_2 symbiotically by associating with legumes[24]. Various legume crops and pasture species add Nitrogen in the range of 200 to 300 kg per hectare [25]. Nitrogenis added into terrestrial ecosystems from the symbiotic relationship between legumes and their rhizobia amount to at least 70 million tons of N per year [26]. The development of better legume varieties, development of efficient agronomic practices, and increased efficiency of the nitrogen-fixing process itself by better management of the symbiotic relationship between plant and bacteria can fulfill the requirements of Nitrogen for plants.

Another association is between Algae and a Cyanobacteria. A diatom algae, *Rhopalodiagibba*, form as association witha cyano-bacteria. The spheroid bodies of Cyanobacteria live in the cytoplasm of the diatoms and are inseparable from their hosts. The associations of Nitrogen fixing microorganisms with plants result in the fixation of nitrogen hence plants fulfill their requirements of Nitrogen which is needed for their growth and development.

VI. Conclusion

Plant growth is improved due to THEIR association with Nitrogen fixing micro-organisms, hence yield of these plants is enhanced. Leguminous plants are in advantage of having association with Nitrogen fixing bacteria, similarly the Actinorhizal plants benefit by association with Frankia. Cycas takes its nitrogen requirements by nitrogen fixing Cyanobacteria. Further research is needed in this area for the transformation of genes responsible for Nitrogen fixation in non-leguminous and plants of Nitrogen deficient soils.

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