MICROBIOLOGY OF THE RHIZOSPHERE

The microbial population is altered both quantitatively and qualitatively by the presence of plant roots. The microorganisms that respond to the presence of root is distinctly different from other soil community, the plant creates a habitat for organisms. In turn the plant is highly affected by the populations it stimulated since the root zone is the site from which inorganic nutrients are obtained and through which pathogens must penetrate. Consequently, interaction between the macro and microorganism in this region has considerable effect on crop production and soil fertility.

Rhizosphere: The rhizosphere is not a well define or uniform part of the soil, instead it represents a poorly defined zone with a microbiological gradient in which the maximal effect of root and microorganism is in the soil nearest to the root. Outer-rhizosphere and inner-rhizosphere had been used to define zones of influence. The inner-rhizosphere is the root surface and its adhering soil and can be refered to as **rhizosplane**. While the outer rhizosphere beyond this zone but still under the influence of the plant root is referred to as **rhizosphere**. The rhizosphere is a region of highly favourable habitat for the proliferation and metabolism of numerous microbial types. A vast microbial community occurs near and on the surface of roots and root hairs. Bacteria are found to be localized in colonies and chains of individual cell, Fungi and actinomycetes are observed but not as frequently as bacteria. The **rhizosphere effect** is measured quantitatively by the **R/S ratio**. This is defined as the ratio of microbial numbers per unit weight of rhizosphere soil R, to the number in a unit weight of adjacent non-rhizosphere soil. The rhizosphere effect is usually greater for bacteria than other soil microorganisms.

Influence of Plant on Microorganism: The microbial population is affected in many ways by the growing plant, and microbial reactions important to fertility are more rapid in the root environment.

1. The most important contribution to the rhizosphere microorganisms is provision of excretion products and sloughed-off tissue to serve as a source of energy, carbon, nitrogen, and growth factors.

2. Plants assimilate inorganic nutrient within the rhizosphere and therefore lower the concentration available for microbial development

3. Microorganisms are affected by root respiration which alters the pH or the availability of certain inorganic nutrients by the evolution of carbon dioxide.

4. Root penetration improves soil structure and brings about aeration for microbial development.

5. In the presence of nitrates denitrification may result in the root region thus increasing release of nitrous oxide and nitrogen gas.

6. The presence of certain plants, reduce the population of nitrifying microorganisms.

7. The cellulolytic organisms increase in number in response to availability of large quantity of cellulosic tissues in the sloughed-off plant materials and the product of their metabolism provide carbonaceous substrates for other organisms.

8. Some root excretions aid the germination of the resting structures of several fungi by providing them with sources of energy. This stimulus to germination is particularly important to plant pathogens that are not vigorous competitors and remain in the resting stage because of nutrient shortage or fungistasis.

9. Roots may liberate antimicrobial agents, in some instances these are antifungal substances. The production of high quantity of carbon dioxide may inhibit germination or affect fungi in other ways.

Influence of Microorganisms: The microorganisms in the rhizosphere may have either favourable or detrimental influence on plant development. Since the microflora is so intimately related to the root system any beneficial or toxic substances produced can cause an immediate and profound response.

1. Microorganisms may favour the growth of higher plant by affecting the availability of various nutrient elements essential for plant growth, most especially carbon, nitrogen and phosphorus.

2. The production of CO_2 in the rhizosphere and the formation of organic or inorganic acids aid in the solubilization of inorganic plant nutrients. This is particularly so with insoluble phosphate containing compounds.

3. At the same time, the vast population of microorganisms demands some anions and cations for their own development leading to immobilization of N and P.

4. Aerobic bacteria remove O_2 from the environment and add CO_2 and either of these processes may reduce root elongation and development or diminish the rate of nutrient and water uptake.

5. Microorganisms may favour plant growth through the production of specific growth stimulating or growth regulating substances such as auxins and phyto hormones eg indole acetic acid, gibberelins and cytokinnins.

6. Some microorganisms are parasitic to plants or produce certain toxic substances which are injurious to plants.

7. Antibiotics may be produced in the rhizosphere and this may affect the growth of root pathogens and even the development of diseases in the above tissues if translocated to stem and intravascular system. Antibiotics are also known to affect the physiology of plant.

8. Some groups of microorganisms form symbiotic relationship with higher plant, eg certain fungi form mycorrhizal association with higher plants and the numbers of free living nitrogen fixing organisms are quite high on the root surface.

MYCORRHIZA

This is the symbiotic association between fungi and root of higher plants, the association is beneficial to most organisms. Mycorrhizas are classified into several groups.

Ectomycorrhiza: This association consists of septate fungal cells infecting roots of trees and shrubs. The fungi form compact mantle or sheath over the root surface and penetrate between the cells of the root cortex to form a complex intercellular system called the Hartig net. The ectomycorrhizal fungi produce auxins responsible for some morphological differences between mycorrhizal and nonmycorrhizal roots. Many fungi from classes Basiodiomycetes, Ascomycetes, Zygomycetes and Mushrooms form ectomycorrhiza. It is usually difficult and sometimes impossible to plant seedlings of trees in grassland soils or other new areas, particularly in forest establishment without introduction of the fungi partner.

Ectendomycorrhiza: This is similar to ectomycorrhiza except that the external mantle of fungi sheath may be much reduced or absent, the Hartig net is well developed and the hyphae also penetrate the host cells.

Ericaceous mycorrhizas: These include the mycorrhizas associated with Arbutus, Ericales and Monotropaceae.

Arbutoid mycorrhizas are formed as mantle which serve as storage organ, when Hartig net is present it penetrates the outer layer of the cortical cells. The septate hyphae form intracellular coils that eventually disintegrate within the cell.

Ericoid mycorrhiza occurs between the ericaceous plants and a fungus called *Pezizella*. The fungus forms multiple coils within the cells and up to 42 % of the root cells can be occupied by the fungal hyphae.

Monotropoid Mycorrhiza: A member of the Basidiomycetes called *Boletus* infects both the monotropa and the roots of neighboring trees. The seed of monotropa is very small it will initiate germination but will not develop further until infected with *Boletus*. Phosphate and glucose can be translocated by the fungi over a distance of 1 to 2 m to the tissue of monotropa.

Orchidoid mycorrhizas: Orchid seedlings are very small and the seedlings pass through a seedling stage during which they are unable to photosynthesize. Since the seeds are too small to contain reasonable reserves, a germinating embryo does not develop further unless it receives an outside supply of carbohydrates or is infected by a compatible mycorrhizal fungus. The infection in orchids spreads from cell to cell, with hyphae coils taking up a large portion of the volume of infected cells. The intracellular hyphae have a limited live and degeneration can occur as early as 30 to 40 hours after initiation of infection and is usually complete within 11 days. Fungi genera involve include *Rhizoctonia, Marasmius, Armillaria* and Fomes. Parasitism of host plant can occur as in monotropoid mycorrhizas. However unlike monotropoid mycorrhizas, a Hartig net, well developed fungal sheath and specialized haustoria are absent.

Arbuscular mycorrhizal fungi (AMF): These are the most common forms of mycorrhizas it involves fungi classified as Zygomycetes. The aseptate hyphae infect the root cells of nearly all cultivated plants, forest trees, shrubs and herbaceous species. No visible structural change is observed on the root except on onion where a sight yellowing of the root is observed. Internal vesicles constituted by fungal hyphae expansion filled with lipids are used for storage. There is another internal structure in the root cortex called arbuscule, this consist of finely branched hyphae and persist within the plant cell for 4 to 10 days. After which they are digested by the plant cell and new ones are formed in other cells. Nutrient transfer occurs between the finely branched fungal mycelium and plant cell membranes. Some fungi hyphae and resting spores extend externally in the soil. Five major genera of the family Endogonaceae form AMF, these are; *Glomus, Gigaspora, Acaulospora, Sclerocystis* and *Scutellospora*. They are distinguished by the morphology of their resting spore.

FUNCTIONS OF MYCORRHIZAS

1. The major contribution of the fungus is in nutrient uptake and translocation, especially of phosphorus and sometimes nitrogen.

2. Mycorrhizal plants have the capacity to withstand or tolerate drought better than non-mycorrhizal plants.

3. The plants are protected from pathogens, nematodes and heavy metal concentrations in the rooting zone.

4. The fungi partners obtain carbon from the plant.

5. Mycorrhizal fungi have aggregating effect especially on coarse soil.

6. The production of phyto-hormones such as cytokinins may have positive effect on plant growth.

BIOLOGICAL NITROGEN FIXATION

Nitrogen fixation is the conversion of atmospheric nitrogen to plant available nitrogen. It can be brought about by electrical discharge (lightning) or other ionizing phenomena of the upper atmosphere (Haber process). The fixed nitrogen is added to the soil as a component of precipitation.

$$N_2 + 3H_2 \xrightarrow[450^oC,\ Catalyst]{200\ atms} 2NH_3 \text{--------} NH_4OH$$

Most of the nitrogen fixed into the soil is however accomplished by biological nitrogen fixation. There are two types of biological nitrogen fixation; Symbiotic nitrogen fixation and Non symbiotic nitrogen fixation.

Non Symbiotic nitrogen fixation

This biological nitrogen fixation is carried out by organisms which live freely in soil as represented by two groups of microorganisms; bacteria and blue-green algae (cyanobacteria).

Bacteria genera involve in nitrogen fixation could be aerobes, facultative anaerobes and anaerobes

Examples of aerobic bacteria include, species of *Azotobacter* and *Beijerickia*

Examples of facultative anaerobe include species of *Bacillus.*

Examples of anaerobe include species of *Clostridium*

For the blue-green algae, the examples include species of Nostoc, Plectonema and Anabaena.

These nitrogen fixers add between 0.2 and 5 kg N/ha/annum, whereas most crop plants require 50 – 200 kg N. This process is therefore thought to be of little significance agronomically. However, the process is of value in rice paddies where blue - green algae, which depend on photosynthesis freely, fix nitrogen. The process is highly influenced by the population of nitrogen fixers in the soil and this in turn is dependent on soil pH.

Symbiotic nitrogen fixation

This is the production of plant available N from atmospheric nitrogen by organisms that live in association with plant roots. These bacteria reside in the roots of legumes causing swellings known as nodules in legume roots. They derive their food and nutrients from the legumes and in turn supply the legumes with nitrogen fixed from the atmosphere. This symbiotic relationship is most common between legumes and *Rhizobium* species. Examples of legumes include trees such as *Acasia, Mimosa and Cassia,* shrubs such as *Ceasalpina* and *Indigofera* as well as crops such as cowpea, soybean, groundnut, pigeon pea, etc. The Rhizobium species are host specific and when the right species are not present in the soil, where the legume is grown, nodulation may not occur and even when some nodules are formed nitrogen fixation may not occur at all or at significant level. Therefore, under certain conditions Rhizobium inoculation is recommended.

Grain legumes are capable of fixing about 150 kg N/ha/annum, an amount that meets the nitrogen requirement of many crop plants.

Mechanism of nitrogen fixation

The central reaction according to which biological nitrogen fixation proceeds and the enzyme responsible for the reaction are the same in all organisms (Symbiotic and non-symbiotic). $N_2 + 6e^- + 8H^+ + 12ATP$ ---------- $2NH_4 + 12ADP + 12pi$

A molecule of nitrogen gas reduced to ammonia requires energy to proceed. It is estimated that 12 moles of ATP are required for 1 molecule of N_2, which is reduced to 2 molecules of ammonium.

The enzyme responsible for nitrogen fixation is called **Nitrogenase**. It consists of two proteins (i) Iron protein also called Azoferredoxin or Component II or Azofer (ii) Molybdenum iron protein also called molybdo-ferredoxin or Component I or Azofermo. The individual component does not catalyse any reaction only the combined system of both proteins is regarded as an enzyme.

Both of the nitrogenase proteins are Iron-sulphur components. The iron protein is the smaller of the two with molecular weight ranging from 50,000 – 70,000. It is rapidly but reversibly inactivated by oxygen.

The molecular weight of molybdenum iron protein range from 100,000 – 300,000 and also reversibly inactivated by oxygen

Factors influencing nitrogen fixation

Biological factors

1. There has to be appropriate organism-host relationship or compatibility, because not all rhizobia can form nodules in all legume roots.

2. The plant should be nutritionally healthy to carry out optimal photosynthesis so as to obtain enough carbon for itself and the organism.

3. Infection by other organisms should be minimal, eg infection of roots by nematodes.

Chemical factors

1. High soil nitrogen content discourages nitrogen fixation.

2. Soil pH has a profound influence on the process, soil pH of 6 – 7.5 favour nitrogen fixation.

Physical factors

Aeration is important as most of the organisms involved are heterotrophic and aerobes. However, the presence of oxygen could retard the process, since it is a reduction process.

The presence of a pigment called **Legume haemoglobin** salvages the problem by binding O_2 thereby rendering it inactive, to be released only when needed. A cross section of an active nodule is reddish brown due to the presence of legume haemoglobin.

Agronomic importance of nitrogen fixation

1. In symbiotic nitrogen fixation the organisms live inside plant root, nitrogen fixed is immediately assimilated into products needed by plants such as amino acids, amines etc. In non-symbiotic fixation, the nitrogen is fixed into soil and absorbed by plants.

2. When ploughed in as green manure or incorporated as crop residues, the nitrogen synthesized into organic N can be mineralized as nitrates and be of benefit to crops that will be subsequently grown in the soil.

3. In mixed cropping, when legumes and non-legumes are grown in mixture, the non-legume benefit from nitrogen fixed by legumes.

Nitrogen fixation is so important that where it is suspected that the appropriate strains of rhizobia are not present in the soil, legume seeds are inoculated with such strains.