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Rhizobium and Nitrogen Fixation

Nitrogen is an essential constituent in many of the compounds in living cells. It is found in amino acids that form proteins and in the nucleoside phosphates that are in nucleic acids. Nitrogen can be found in many forms in the biosphere. Molecular nitrogen (N_2) forms about 78% of the atmosphere by volume, however, this reservoir of nitrogen is not directly available to plants. N_2 has a strong triple covalent bond, and higher plants do not have the ability to break this bond directly. Plants instead assimilate nitrogen in the form of nitrate (NO_3^-) or ammonia (NH_4^+).

The availability of nitrogen to plants can be a limiting factor in agricultural production. As a result, producers will often add nitrogen to the soil to meet nitrogen requirements for crops. The process where molecular nitrogen is changed into ammonia or nitrate is known as nitrogen fixation. This can be done through industrial processes. Producers can then add a chemical fertilizer to soils to provide a source of nitrogen. However this can be costly and over-application can lead to waste and pollution.

There are also natural processes which result in nitrogen fixation. You may be interested to know that 10% of nitrogen that is fixed through natural process comes from lightning! Certain microorganisms can also fix nitrogen. Approximately 90% of nitrogen that is fixed through natural processes is carried out by free-living bacteria including cyanobacteria and by bacteria, in symbiotic association with plants. These microorganisms contain enzyme systems that can catalyze chemical reactions to break the N_2 triple bond.

The relationship between the plant and the bacteria is known as a symbiotic relationship since both the plant and the bacteria benefit from their relationship. Legumes including peas, lentils and alfalfa can form symbiotic associations for nitrogen fixation with a soil bacterium called *Rhizobium*. The *Rhizobium* enters into the roots and forms nodules, which the bacteria then use as their home. In the nodules, the *Rhizobium* fixes N_2 into a form that the plant can use. Nitrogen is fixed by binding it to hydrogen and making it into ammonia which the legume plant can use. Rhizobia benefit as the plant provides carbohydrates to the rhizobia. Carbohydrates are required by rhizobia as a source of energy. Also, the carbohydrates produced by the legume plant are transported to the nodules where they are used by the rhizobia as a source of hydrogen in the conversion of nitrogen to ammonia.

This interaction is quite specific. Specific *Rhizobium* species fix nitrogen only with a specific type of legume. Soil may lack the number of the specific rhizobia that is required for nitrogen fixation

Producers can add *Rhizobium* to the soil to ensure that their legume crops have a source of nitrogen by adding an appropriate *Rhizobium* species to the seeds of the crop. Rhizobia can be introduced to soils through a variety of different methods of inoculation. You could add an in-furrow granule mix or coat the seed with the bacterium. It is important that the inoculant remains in the correct proximity of the germinating seed so that the bacteria can infect the root hairs. After the crop germinates, the rhizobia enter into the root hair and spread towards the root. When the plant grows, *Rhizobium* will be available to convert nitrogen for the plants.

Rhizobium typically occurs free-living in the soil. To form a symbiotic relationship, the bacteria must first migrate towards the rhizosphere, the area around the roots. This specificity requires that there be a mechanism for host recognition. This is mediated by plant compounds, usually flavonoids, which are produced by the roots of the host plant. These flavonoids influence a series of genes in *Rhizobium* known as the *nod* genes, the genes involved in infection and nodule formation. The rhizobia then bind to the root surface. In response to factors produced by the rhizobia, the root hair begins a curling growth, and the rhizobia continue to divide within the coils. An infection thread, which allows the rhizobia to move into the root hair and eventually to other plant root cells, is subsequently formed. Eventually the bacteria stop dividing and form bacteroids, which fix nitrogen.

The *nod* genes are expressed at specific stages of development. The *nod* genes code for proteins called nodulins. Nodulins, including leghemoglobin, are expressed at specific stages of nodule development.

There are a series of genes involved in nitrogen fixation, referred to as the *nif* genes or *fix* genes. The *nif* genes are found in both free-living and symbiotic nitrogen fixing bacteria and include the structural genes for nitrogenase and other regulatory genes.

The greater study of the infectious process and the genes involved and the physiological aspects will lead to better nitrogen use by plants grown on nitrogen-poor soils in agriculture and can reduce the requirement for chemical fertilizers.

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