Biological nitrogen fixation in legumes

Understanding the process

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Legumes Translated

In nature, biological nitrogen fixation (BNF) provides most of the reactive nitrogen that is required for protein formation and plant growth. Legumes host BNF, so understanding BNF provides a foundation for many decisions made in legume cropping.

Legumes are the most important hosts of BNF in terrestrial ecosystems, especially agricultural ecosystems. Nitrogen fixed by legumes is an alternative to synthetically-fixed nitrogen in fertilisers. Because of BNF, introducing legumes into cropping systems reduces some of the damaging emissions from the agricultural nitrogen cycle, especially nitrous oxide (N_2O) which is a potent greenhouse gas.

Applicability

Theme: Nitrogen nutrition of crops

For: Farmers

Where: All arable farms

Timing: Throughout the cropping cycle

Impact: Better legume crops

Outcome

The direct effect of improved BNF is higher yielding crops, often associated with higher protein content. About 800,000 tons of di-nitrogen (N_2) from the air is fixed each year by BNF in cultivated grain and forage legumes in the European Union. The main grain legume crops (soybean, pea and faba bean) account for about one third of this. A high rate of BNF is the foundation of successful and sustainable production. The agronomic success of grain legume crops depends to a great extent on the amount of nitrogen fixed in the nodules of their root systems. This means paying attention to establishing and maintaining the symbiosis between the host plant and the bacteria of the genus *Rhizobium* and *Bradyrhizobium*. The total amount of nitrogen fixed usually ranges from 100 to 300 kg N/ha depending on factors such as legume species (and cultivar), length of growing season and environmental conditions.

The symbiosis between soil bacteria and legumes promotes nitrogen uptake by the plants themselves and enriches the soil with nitrogen through root exudates and residues, making legumes a preferred precursor to many crops. Growing legumes is a cheap and affordable way to enrich soils with nitrogen. Including them in crop rotations creates favorable conditions for growing subsequent crops with reduced use of artificial nitrogen fertilisers.

Role of leghemoglobin and practical consequence

Biological nitrogen fixation is a fascinating process. The rhizobium invades the roots of compatible host legume plants, leading to the development of specialized root structures that we know as nodules. In the nodule, the bacteria reduce N_2 to ammonia using the nitrogenase enzyme complex, which is produced within the bacterium. For BNF to progress, the nitrogenase needs to be protected from oxygen. The root nodules protect the nitrogenase-based process from oxygen using an iron-linked protein called leghemoglobin. Leghemoglobin controls the concentration of free oxygen in the cytoplasm of infected plant cells, protecting nitrogenase from oxygen while at the same time enabling the provision of oxygen for respiration in root tissue to supply the energy required. A fascinating part of this is leghemoglobin is closely related to the hemoglobin in blood with an analogous function in transporting oxygen. Like hemoglobin, leghemoglobin is red when

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charged with oxygen. This explains why healthy root nodules are pink. The presence of a large number of nodules that are pink when split open is a reliable indicator of successful establishment of BNF in legumes crops (Figure 1).

Figure 1. A close-up photograph of a split nodule showing the characteristic pink colour. This is indicative of a successful establishment of the rhizobium and active biological nitrogen fixation. Photo: www.gartensoja.de

Biological nitrogen fixation requires energy

For BNF, the conversion of each molecule of N_2 to two ions of ammonium NH_4^+ requires 16 molecules of ATP. The end result is this conversion requires energy from the host legume plant. Symbiotic nitrogen fixation uses about 4-16 % of host plant photosynthate in faba bean and soybean plants. This energy cost is one of the reasons why grain legumes crops are lower yielding than comparable cereal crops. However, under good growing conditions, faba bean and soybean compensate for the energy demanding BNF by boosting growth further.

Establishing the symbiosis

Establishing the symbiosis begins with the removal of flavonoids by the bacterium from the host legume plant. This stimulates the synthesis of specific signaling molecules in the bacteria called „nod factors". Nod factors are required for both bacterial invasion and nodule formation. The molecular structure of nod factors is specific to the different species of *Rhizobium*. The rhizobial bacteria attach to the tips of the root hairs, causing them to twist forming an 'infection thread' structure that allows the bacteria to reach the root cells of the host plant. The infection thread grows towards the centre of the root and the bacteria are released into the cells of the newly formed root nodule where the nitrogen fixation takes place. The bacteria stimulate the host plant cells to produce the leghemoglobin. The nitrogen that is fixed is then available to the whole of the host plant with the result that high yielding legume crops do not require fertiliser nitrogen.

Legumes plants form two types of nodules: indeterminate ovoid shaped and determinate round shaped (Figure 2).

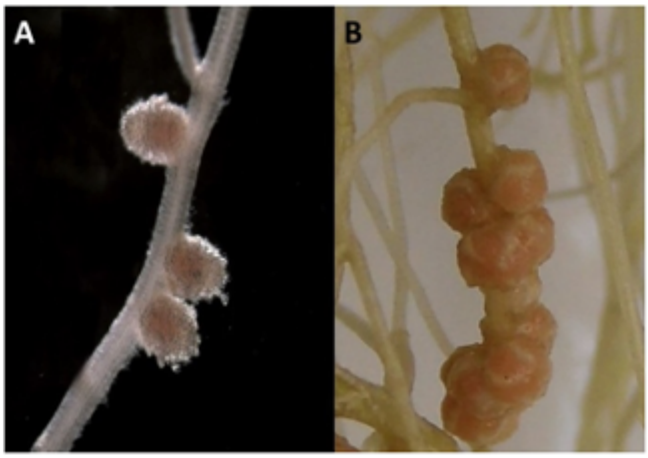

Figure 2. Shape of nodules in legume plants. A indeterminate; B-determinate.

The nodules are rich in iron and protein providing a rich source of food for larvae of certain weevils (*Sitona* lineatus and other *Sitona* species). The leghemoglobin is also so similar to mammalian blood that it is used in substitute meat products.

It is important to have the right bacteria

The specificity of nod factors means that each legume has a specific type of symbiotic bacteria in the family *Rhizobiaceae*: *Rhizobium leguminosarum* for pea, broad bean, vetchling and lentil; *Rhizobium phaseoli* for common bean; *Rhizobium ciceri* for chickpea; *Sinorhizobium meliloti* for alfalfa and other medics, yellow melilot and fenugreek; *Rhizobium trifolii* for clover;

Figure 3. Nodulated roots of soybean plants from the field. Photo: Leopold Rittler (Donau Soja).

Bradyrhizobium lupini for lupins; *Mesorhizobium loti* for sulla and trefoil; *Rhizobium vigna* for cowpea and other Vigna species, peanut; *Rhizobium simplex* for sainfoin; *Bradyrhizobium japonicum* for soybean (Figure 3).

Key practice points

Establishing the symbiosis between the nitrogen-fixing bacteria and the host legume plant is a key objective for every farmer growing legume crops. In addition to the natural route, significant BNF can be obtained by inoculating the seeds with an appropriate strain of the nitrogen-fixing bacteria. Such inoculation is essential for soybean because European soils do not contain the required species. In contrast, European soils contain strains that infect pea, faba bean, common bean and clover, so the response to inoculation is very variable. In some situations, naturally occurring local strains of nitrogen fixing bacteria in the soil are lacking or have low nitrogen-fixing activity. This necessitates the introduction into the soil of selected strains of nitrogen fixing bacteria characterized by high nitrogen-fixing activity. How this is done for soybean is described in detail in the Legumes Translated Practice Note 1.

The other practice points arising from these biological processes include the need to protect the root nodules. Pea and bean weevil (*Sitona spp.*) adults eat the leaves but this has little effect of the crop yield. The more significant damage is done by the larvae feeding on the nodules. Their control is important where infestation is high. Integrated pest management of *Sitona spp.* including the use of biocontrol and pheromone-baited traps is required is some situations. This must be done according to local best practice and regulations.

Due to the energy demands of the process, ensuring that the crop grows well is fundamental to high rates of BNF, which in turn supports further crop growth. This positive cycle explains how high yielding legumes crops are produced under good growing conditions without any other nitrogen source.

Further information

AgroBioInstitute, Agricultural Academy, Bulgaria supplies inoculants for soybean, alfalfa and bird's-foot trefoil. Other parts the Agriculture Academy supply basic seed of Bulgarian cultivars of soybean, alfalfa, beans, lentils and garden and fodder peas.

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