BIOLOGICAL NITROGEN FIXATION

in grain legumes

Putting nitrogen fixation to work for smallholder farmers in Africa

TABLE OF CONTENTS

1. INTRODUCTION

The N2Africa project aims to put nitrogen fixation to work for smallholder farmers in Africa. With new technologies N2Africa helps farmers to increase yields and benefits of grain legumes (cowpea, groundnut, soybean, common bean, chickpea and faba bean).

Nitrogen is a very important nutrient for plant growth. Non-legume crops, such as cereals, use nitrogen from the soil or from mineral nitrogen fertilizers. Legumes on the other hand can fix nitrogen from the atmosphere. This means that legumes do not deplete the soil nitrogen reserve and do not need nitrogen fertilizer. Instead, legumes add to the nitrogen reserve in the soil and help to maintain soil fertility. Growing legumes in a rotation or in intercrops with non-legumes reduces the need for nitrogen fertilizer for the non-legume crops.

Besides the ability to fix nitrogen from the atmosphere, legume crops have two more advantages for smallholder farmers. In the first place, grain legumes are important food crops. Grain legumes provide energy, protein, minerals and B-vitamins. Secondly, grain legumes often have good market opportunities and can therefore function as a source of income for rural households.

In this manual we first explain what nitrogen is. In the next chapter, we explain what nutrient depletion is and how to maintain sufficient nutrients in the soil. In chapter four we explain how legumes fix nitrogen from the air and how you can use legumes to maintain sufficient nitrogen in the soil. In chapter five we explain how you can increase the amount of nitrogen fixed by the legume. Finally, we explain how you can design and interpret a trial to field-test legume technologies.

2. NITROGEN

What is nitrogen?

Nitrogen is a chemical element. You can find nitrogen almost everywhere. First of all, the earth's atmosphere consists of 80% nitrogen gas. In addition, nitrogen is an important building block of protein. Therefore you find nitrogen in protein rich food sources like meat, fish, eggs, nuts and grain legumes like bean, cowpea, soybean and groundnut.

Nitrogen is a vital component of life. Plants need nitrogen for photosynthesis and the formation of protein and vitamins. Without nitrogen, plants do not grow. The proteins and vitamins formed by plants are in turn important components of the human and animal diets.

Two types of nitrogen

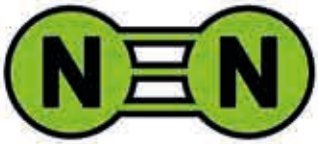
In this guide we distinguish between two types of nitrogen: gaseous nitrogen and mineral nitrogen.

Gaseous nitrogen is the type of nitrogen that occurs in the atmosphere. Nitrogen gas is a molecule with two nitrogen atoms, bound together by a strong bond. Plants are not able to take up this double nitrogen.

Instead, plants use mineral nitrogen. In mineral nitrogen, the nitrogen exists as a single atom. Mineral nitrogen is available for plants in the soil in the form of ammonium or nitrate. Farmers add nitrogen to the soil with manure, compost, or mineral fertilizer.

NITROGEN

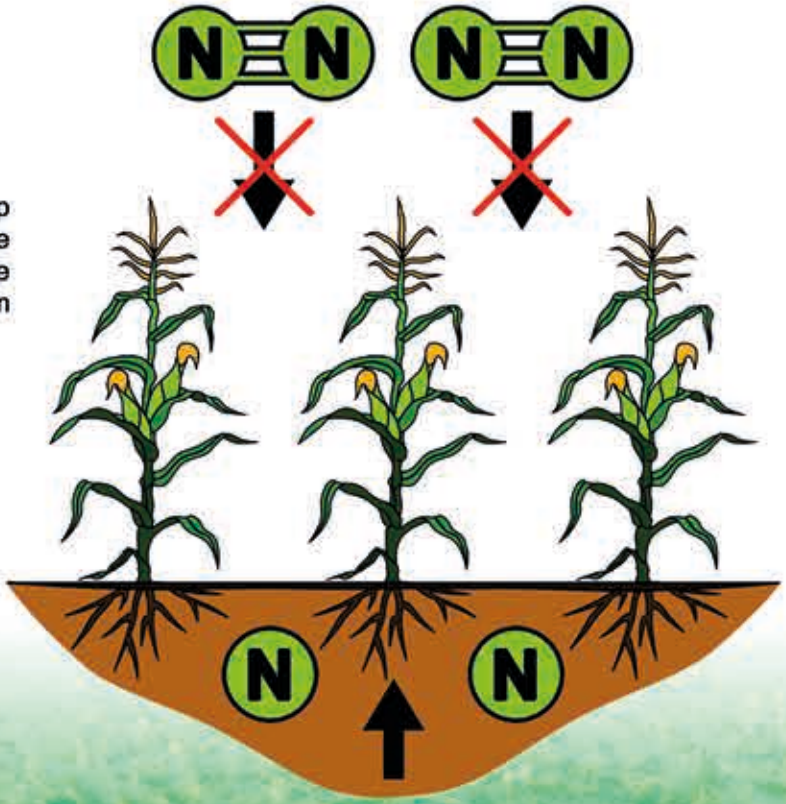
N IN ITS DIFFERENT FORMS



[1] Gaseous nitrogen from the air exists of two N atoms. A really strong bond keeps the two N atoms together. Gaseous nitrogen has the symbol N_2.

[2] Mineral nitrogen from the soil has only one N atom. Mineral nitrogen has the symbol N.

[3] Plants take up mineral N from the soil, but cannot take up gaseous N_2 from the air.



3. NUTRIENT CYCLES ON THE FARM

Nutrient depletion

Crops need water, sunlight and sufficient nutrients to produce a good harvest. The most important nutrients for plant growth are nitrogen (N), phosphorus (P) and potassium (K). Crops take these nutrients from the soil and use them to produce stems, leaves, and grains, tubers or fruits.

If we harvest the crop for consumption or sale, the nutrients that were contained in the soil are taken away. Cultivating a piece of land for a long time without returning nutrients in the form of fertilizer, compost or manure eventually leaves few nutrients in the soil. The soil becomes infertile and crops do not grow well anymore. To maintain good crop production, it is therefore very important to maintain nutrient stocks in the soil.

Maintaining nutrient stocks

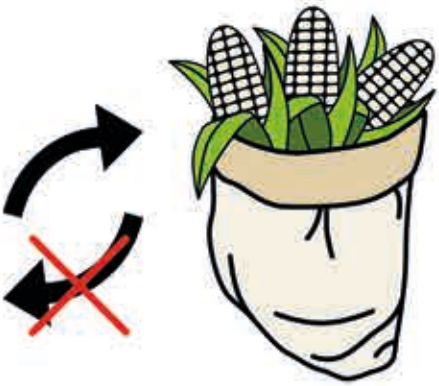
There are different ways that help to maintain nutrient levels in the soil:

- Recycling nutrients within the farm
  - Return crop residues and manure from animals to the field as much as possible.
- Adding nutrients to the soil in the form of mineral fertilizer
  - A mineral fertilizer can contain just one nutrient (ammonium nitrate for example only contains N) or several (NPK contains three).
- Using legumes for Biological Nitrogen Fixation (BNF)
  - Legumes fix nitrogen from the atmosphere and make the nitrogen available to other crops. Incorporate the nitrogen rich residues in the soil, or return the manure of the animals fed with the legume residues to the field. The legume roots and nodules also contribute nitrogen to the soil.

NUTRIENT CYCLES ON THE FARM

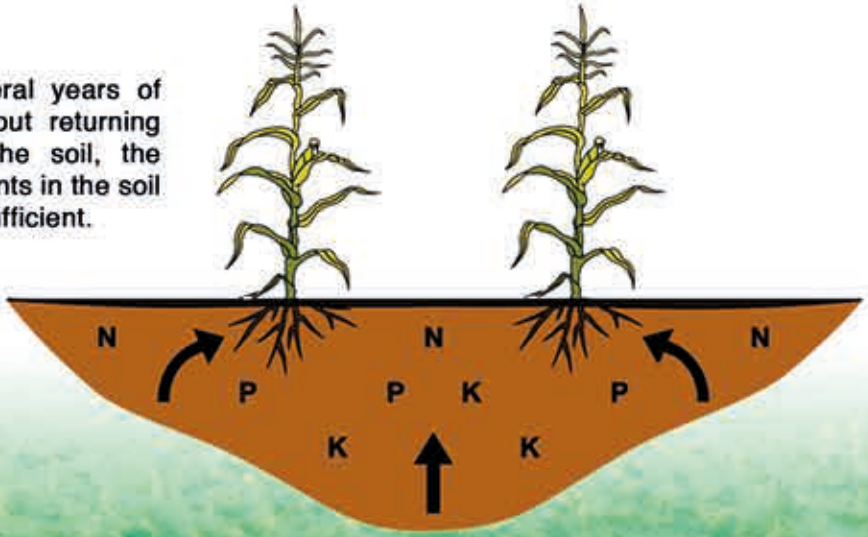
NUTRIENT DEPLETION THROUGH CONTINUOUS CROPPING

[1] Crops need sunlight, water and nutrients. The crops take up nutrients from the soil and accumulate them in their roots, leaves and grains.



[2] With the harvest, we take away the nutrients contained in the grains and biomass. This means that nutrients are removed from the soil.

[3] After several years of cropping without returning nutrients to the soil, the stock of nutrients in the soil is no longer sufficient.



4. BIOLOGICAL NITROGEN FIXATION

As explained before, nitrogen comes in different forms. It can come in the gaseous nitrogen from the atmosphere (N_2), and it can come in the mineral form of ammonium or nitrate which plants can use (N).

Legumes form a symbiosis with bacteria. A symbiosis literally means: living together. Through this symbiosis, legumes are the only crops which can use the gaseous double-nitrogen from the air. The bacteria change the unusable double-nitrogen into useable mineral single-nitrogen. We call this process biological nitrogen fixation.

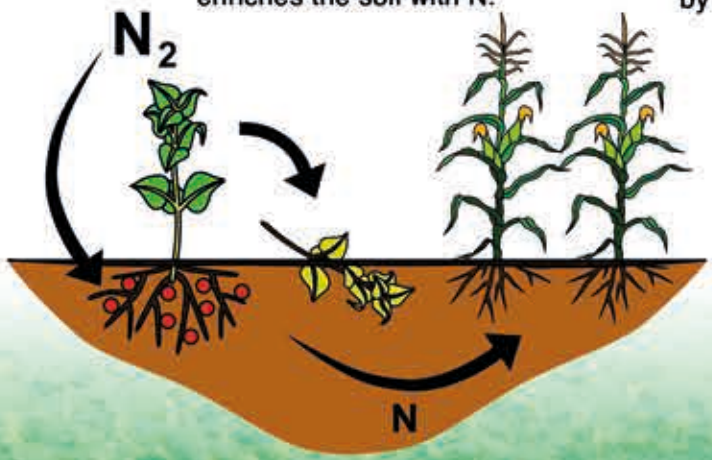
When legumes 'fix' the unusable nitrogen from the atmosphere, they also make it available to other plants in the form of nitrogen rich crop residues, roots and nodules. In addition, legumes make gaseous nitrogen available to humans in the form of protein rich grain and leaves.

BIOLOGICAL NITROGEN FIXATION BY LEGUMES

[1] Legumes fix nitrogen from the air through Biological Nitrogen Fixation (BNF).

[2] Crop residues from legumes are rich in N. Returning the crop residues to the field after harvest enriches the soil with N.

[3] Other crops can use the nitrogen fixed by legumes.



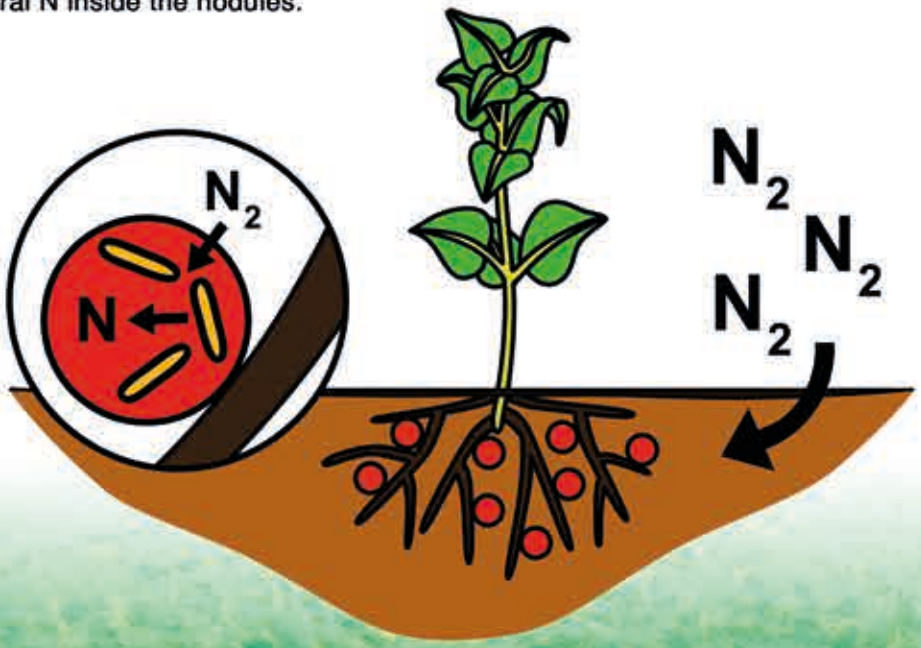
How BNF works

Legumes fix nitrogen through a symbiosis with bacteria. These bacteria are called rhizobia. The rhizobia live in the soil. When rhizobia encounter the root of a legume, the root forms a nodule. The rhizobia live and multiply in this nodule. In the nodule, the rhizobia take N_2 from the air and break the bond. The plant then uses the single N atoms for its growth and forms protein-rich grains and leaves.

BIOLOGICAL NITROGEN FIXATION: THE RHIZOBIUM-LEGUME SYMBIOSIS

[1] Rhizobia interact with the legume roots and form root nodules. Rhizobia transform N_2 gas from the air into mineral N inside the nodules.

[2] The legume uses the mineral N fixed by the rhizobia for its growth.



Promiscuous and specific legumes

Naturally, many different rhizobia species occur in the soil. But not all rhizobia are able to fix nitrogen with each legume species. For effective nitrogen fixation, it is essential that the rhizobia from the soil are of the right type.

Some legumes can form nodules and fix nitrogen with many different types of rhizobia. Groundnut and cowpea are among those legume species which can always easily find the right rhizobia species in the soil they grow in. These legumes are said to be **promiscuous**.

Soybean, faba bean and chickpea on the other hand can only form nitrogen fixing nodules with some specific rhizobia. These rhizobia do not occur naturally in most African soils. This means that soybean, faba bean and chickpea often do not find the right rhizobia in the soil and do not always fix nitrogen. Legumes that need **specific** rhizobia are said to be specific.

To ensure that specific legumes fix nitrogen, you can treat the seed with rhizobia from a packet. This is called **inoculation**. We will explain more about inoculation in the next chapter.

Effective and ineffective nodules

Sometimes a specific legume forms nodules with a rhizobium species that is not the right species for this legume. In this case, no nitrogen is fixed in the nodule. You can check if a nodule is fixing nitrogen by cutting it open. Effective nodules that fix nitrogen are pink or red inside. Ineffective nodules that cannot fix nitrogen usually are green or white inside.

BIOLOGICAL NITROGEN FIXATION

PROMISCUOUS AND SPECIFIC LEGUMES

[1] Promiscuous legumes form effective nodules with many rhizobia species.

[2] Specific legumes only form effective nodules with a few specific rhizobia species.

[3] Specific legumes usually do not form nodules with other rhizobia species than the ones they need.

[4] Specific legumes sometimes form ineffective nodules with other rhizobia than the ones they need. Ineffective nodules do not fix nitrogen.

5. MANAGEMENT OF BNF

If a legume grows well and achieves a high yield, it is also likely to fix a lot of nitrogen. However, the legume yields well and fixes much nitrogen only if it is managed well.

Good management includes amongst others adoption of improved crop varieties. Special varieties of cowpea, groundnut, common bean, soybean, chickpea and faba bean have been developed that are able to fix nitrogen better than some local varieties. Proper use of mineral or organic fertilizers, adjusting plant density and timely planting and weeding are also very important. In the case of specific legumes such as soybean or chickpea, often the soil does not contain enough rhizobia of the right species. It is therefore best to inoculate seeds with rhizobia from a package before planting the seeds. Below we first explain the proper use of fertilizers, and then inoculation.

Fertilizers

Because legumes can fix their own nitrogen, it is not necessary to use N-fertilizers such as urea or ammonium nitrate on legumes. When nitrogen is plentiful in the soil, legumes will not invest much energy to form nodules. Instead, they will use more nitrogen from the soil. Legumes thus fix less nitrogen from the air when nitrogen is plentiful in the soil.

However, it is important that legumes have enough phosphorus (P). Without enough phosphorus in the soil, a legume cannot fix nitrogen. You can recognize P deficiency by reduced plant growth and a reddish-purple colouring on the lower leaves.

Applying phosphate fertilizers helps when there is not enough P in the soil. There are different sources of P-fertilizer, for example single-super phosphate (SSP), triple super phosphate (TSP) or ground rock phosphate.

MANAGEMENT OF BNF

The compound fertilizers DAP, NPK and compound L also contain P.

Which source of P is best depends upon soil properties, local availability and prices. If you have the choice between SSP or TSP and NPK (or any other compound fertilizer containing N), choose SSP or TSP. The nitrogen contained in NPK, DAP or compound L fertilizer inhibits nitrogen fixation by the legume.

Legume crops also need several other nutrients including potassium (K), sulphur (S), calcium (Ca) and magnesium (Mg) and micro-nutrients such as zinc (Zn), molybdenum (Mo), copper (Cu) and boron (B) for good growth and nitrogen fixation. Special fertilizer blends for legumes such as Sympal in Kenya contain several of these nutrients. Manure contains many different nutrients including micronutrients.

To apply fertilizer, make furrows of 5 cm deep next to the planting rows. Place the fertilizer in the furrows and cover with soil. You can also place fertilizer directly in planting holes or furrows made for planting. Always cover fertilizer with 3 cm soil before planting the seed. If you place the seed directly on the fertilizer, the fertilizer will 'burn' the seed. If using TSP, DAP or compound L, use 100 kg/ha. If using SSP, use 225 kg/ha. If using Sympal, use 200 kg/ha.

K deficiency symptoms in soybean.

K deficiency symptoms in common bean.

Soybean plants without P applied are stunted (front) compared to soybean plants with P applied (back).

Inoculation

For most varieties of soybean and chickpea, there are not enough rhizobia in the soil naturally for good BNF. To ensure that soybean finds the right rhizobia and forms effective nodules, we can directly apply rhizobia from a package on the seed before planting. These rhizobia are raised in a laboratory. The rhizobia are then combined with a carrier material, such as peat, and put into a package. This is called **inoculant**. Applying inoculants on the seeds is called **inoculation**.

Benefits of inoculation

- Inoculation ensures good nodulation.
- With good nodulation, the legume can fix its own nitrogen.
- When more nitrogen is fixed, legume yields increase. Following crops or intercrops also benefit from the fixed nitrogen.
- Inoculants are much cheaper than nitrogen fertilizers.

MANAGEMENT OF BNF

Inoculation is easy, but you have to be very careful about one thing: inoculants contain living organisms which will die when exposed to sun or heat. The organisms will also die when the inoculant is stored in an open package. Therefore always store inoculants in their original package in a clay pot in the coolest place of the house. It is best to use the inoculants the same season you bought or received them, and use them quickly after opening the bag. Do not keep the inoculants for next year.

Different brands of inoculants need different inoculation methods. For some types of inoculants you need to prepare a sticker solution first, and for others you don't. It is therefore important to always carefully read the instructions on the package.

Different inoculant products have different application procedures.

Important points about inoculants

- The right inoculant must be used with the right legume. You should not apply, for instance, a bean inoculant on soybean seed. Always check the label on the package.
- Inoculants contain living organisms that must be protected from heat and sun. Therefore always store the package in a cool place away from direct sunlight.
- Inoculants can become contaminated and lose their effectiveness when stored in an open package. Always store inoculants in their original package and use them quickly after opening the bag.
- Inoculate seeds just before planting.
- Follow the instructions on the package for the inoculation procedure.
- Protect inoculated seeds from direct sunlight by covering the container with paper, cloth or gunny bag and keep in the shade until planted. Plant in the morning or evening, to avoid direct sunlight.
- Do not use inoculants after their sell-by date because then the inoculant may no longer be effective. Check the product's expiration date.

6. FIELD TESTING

The main goal of N2Africa is to increase the benefits from nitrogen fixation for farmers. In chapter five we discussed several management options to increase nitrogen fixation. Important options that can make a difference in yield and nitrogen fixation are the choice of legume variety, use of fertilizer and inoculation.

In N2Africa we test which legume technologies work best and where. Which technology works best depends on the climate, but also on the field the legume grows in. Differences in inherent fertility and past nutrient management for example result in different responses to nutrient inputs. Rhizobia populations also vary across fields. Differences in soil fertility can occur on a small scale, and therefore the results of field-tests can differ from place to place.

The importance of a control treatment

Designing a field-test is easy, but it is very important that you always have a control treatment. A control treatment is a treatment without any inputs, or a treatment comparable to farmer's common practice in an area. If you for example test the need for inoculation and the need for P-fertilizer in soybean, the control treatment would be soybean without inoculation and without P-fertilizer. A control treatment allows you to evaluate the effects of the new technologies and helps you decide whether the investment in a technology is economic or not.

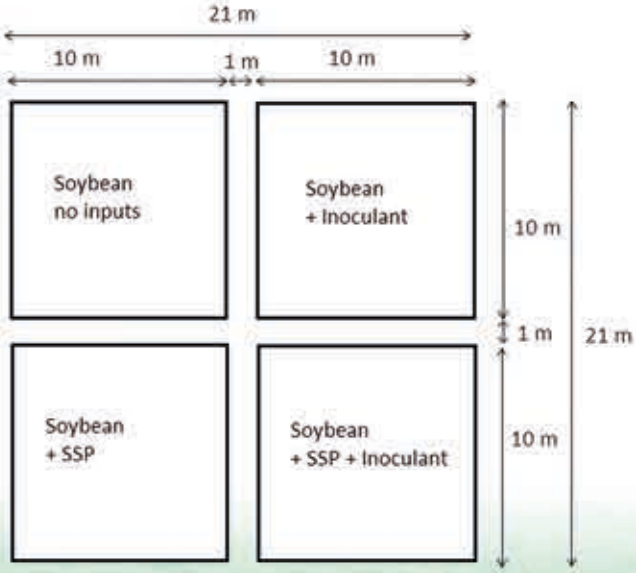
A typical test for specific legumes

For soybean and chickpea, but sometimes also common bean, a key test is to determine whether the rhizobia that occur in the soil naturally are of the right species or that inoculation with rhizobia is necessary. In addition it is important to test whether the soil has sufficient P or that you need to apply P-fertilizer.

FIELD TESTING

When you test the need for inoculation and the need for P-fertilizer you need four treatments. First, you need a control treatment. The control treatment does not get any inputs apart from legume seed. Second, you need to test the effect of P-fertilizer alone. Third, you need to test the effect of inoculation alone. In the fourth treatment, you combine inoculation and P-fertilizer to see the combined effect of the two technologies. In your experimental design, each treatment has its own plot.

When you are planting your experiment, you have to make sure you first plant the plots without inoculation, and then your plots with inoculation. If you do it the other way around, you might by accident put some inoculant on the seeds for the plots without inoculants. In that case the results of your test will not be valid.

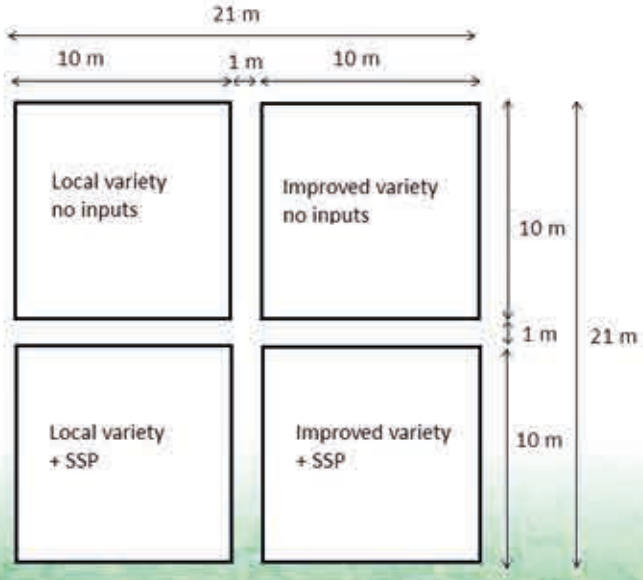


Design to field-test the need for inoculation and P-fertilizer in soybean

A typical test for promiscuous legumes

In case of promiscuous legumes such as cowpea and groundnut you do not have to test the need for inoculation. Instead, you can test different varieties and the need for P-fertilizer or manure.

In the example below we illustrate how to compare a local variety with an improved variety combined with the need for P-fertilizer. In this experiment you also have four treatments. In the first plot you plant the local variety without applying P-fertilizer. In the second plot you plant the improved variety without P-fertilizer. In the two remaining plots you plant both varieties with P-fertilizer. In this way you can separate the effects of the variety and of the fertilizer. This allows you to decide which technology gives the highest yield or is the most economic.



Design to field-test two different varieties of the same legume, and the need for P-fertilizer.

Evaluating a field test

Evaluate your field test to determine the best option for a specific area or a specific field. Important factors to evaluate are the amount of biomass, the grain yield, root nodulation and susceptibility to pests and diseases. Also keep in mind the costs for the various inputs used to determine whether a technology is economically sound or not.

In the case of the need for inoculation and need for P-fertilizer trial, there is no response to either inoculation or P-fertilizer if all plots perform similarly. If the plants in the inoculated plot grow best, it is advisable to inoculate. Similarly, if the plants in the plot with P-fertilizer grow best, it is advisable to use P-fertilizer. If the plants in the plot with both P-fertilizer and rhizobia grow best, this means that the two management strategies together are even better, and it is advisable to use both management strategies.

For more information see www.n2africa.org

Putting nitrogen fixation to work for smallholder farmers in Africa