Legume Inoculation Technology Manual

Putting nitrogen fixation to work for smallholder farmers in Africa

Citation

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Prepared for the N2Africa Project
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Acknowledgements

Much of the materials in this manual have been cited from the following publications:

- FAO. (1984). Legume inoculant and their use. Food and Agriculture Organization. Rome.
- O Hara, G., Howieson J., Drew, E, Ballard, R., Herridge, D., Gemmell, G., Hartley, E., Phillips,L., Deaker, R., Denton, M., Yates, R., Seymour, N., and Ballard, N. (2012). Inoculating Legumes: A Practical Guide. Grains Research and Development Corporation Australia.
- Singleton, P.W, Somasegaran, P., Nakao, P., Keyser, H. H., Hoben, H. J. and Ferguson, P. I. (1990). Applied BNF technology: A Practical Guide for Extension Specialists. University of Hawaii NifTAL Project. USA.

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N2Africa Program Statement

N2Africa is a large scale, research and development project focused on putting nitrogen fixation to work for smallholder farmers growing legume crops in Africa. N2Africa is funded by The Bill & Melinda Gate Foundation through a grant to Plant Production Systems, Wageningen University, in the Netherlands. It is led by Wageningen University together with CIAT-TSBF, IITA and has many partners in the Democratic Republic of Congo, Ghana, Kenya, Malawi, Mozambique, Nigeria, Rwanda and Zimbabwe.

At the end of the four-year project, we will have:

- identified niches for targeting nitrogen fixing legumes;
- tested multi-purpose legumes to provide food, animal feed, and improved soil fertility;
- promoted the adoption and preparation of improved legume varieties;
- supported the development of inoculum production capacity through collaboration with private sector partners;
- developed and strengthened capacity for legumes research and technology dissemination; and
- delivered improved varieties of legumes and inoculant technologies to more than 225,000 smallholder farmers through our Master Farmer Network.

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Background
Why the Call for Inoculants?

Low crop productivity is a general problem facing most farming systems in sub-Saharan Africa (SSA). The problem is more pronounced in grain legumes and are often associated with declining soil fertility and reduced N–fixation due to biological and environmental factors. Unfortunately, the majority of African smallholder farmers are unable to afford the high mineral fertilizer prices. More than 75% of the fertilizers used in Africa are imported, putting pressure on scarce foreign exchange. Low cost and sustainable technical solutions compatible with the socioeconomic conditions of smallholder farmers are needed to solve soil fertility problems. Biological nitrogen fixation (BNF), a key source of N for farmers using little or no fertilizer, constitutes one of the potential solutions and could play a key role in sustainable grain legumes (e.g., soybean) production.

Biological nitrogen fixation is the process whereby atmospheric nitrogen is converted into forms that plants can assimilate. This process occurs when legumes form symbiotic association with compatible rhizobia. The symbiotic association between the rhizobia and the host legume is such that the host legume provides nutrition for the bacteria and the rhizobia fixes nitrogen for the plant. Rhizobia are not universally present in soils and often those present gather little nitrogen.

In order to take advantage of this miraculous association of bacteria and leguminous plants, it is often necessary to provide dependable legume inoculants to assure effective nodulation of leguminous crops. The application of these bacteria to seed or soil is called **inoculation**.

Inoculation with beneficial bacteria can be traced back centuries. Farmers knew that when they mixed soil taken from an area previously grown to legume crops with soil in which non-legumes were to be grown, yields often improved. By the end of the 19th century, the practice of mixing naturally inoculated soil with seeds became a recommended method of legume inoculation in the USA. For almost 100 years, *Rhizobium* inoculants have been produced around the world, primarily by small companies. Today, fewer companies dominate world markets, but this must not inhibit small inoculant manufacturing in Africa.

Root Nodule Bacterial Inoculant: What is It?

A bacterial inoculant is a formulation containing one or more beneficial bacterial strains in an easy-to-use and economical carrier material, which may be organic or inorganic or synthesized from defined molecules. These live bacteria are also known as root nodule bacteria. The bacteria possesses unique features that enable them to enter into symbiotic association with the roots of the legume plant leading to the formation of specialized organs called nodules where atmospheric nitrogen is converted to ammonium and subsequently transported to other parts of the plant. Rhizobia can only fix nitrogen when inside the nodule of a plant. Formulations of these bacteria are prepared by isolation and culturing of the strains in the laboratory.

Legume inoculants are therefore generally referred to as preparations of live rhizobia designed for application to leguminous seeds or soils to ensure effective nodulation of the host resulting in abundant supply of nitrogen for crop growth. It is worth noting that symbiosis can only occur between a legume and rhizobia which are compatible.

Normally, inoculants are made to be applied to seeds because this is an easy and convenient way of putting the rhizobia in the root zone of the developing seedling where infection of the root hairs can occur and nodules develop. The process of rhizobia fixing nitrogen for legumes is undoubtedly one of the success stories of Agriculture as it is environmentally friendly and also cost effective.

Desirable Qualities of a Legume Inoculant

We need to be aware that, not all inoculants produced are of good quality, and it is not easy to distinguish good inoculants from poor ones. Inoculants have expiration dates that should not be exceeded when purchased. Check the reputations of the manufacturers and ask other users about their experience. Read the label carefully to select the right type of inoculant and to make sure that the inoculant is fresh. Also be sure that the inoculant has been stored properly. Finally, you can conduct a grow-out test to compare the quality of the various inoculants.

The ideal inoculant should have the following qualities:

- ability to form N-fixing nodules on the species and cultivars of legumes for which the rhizobia is recommended.
- competitiveness in nodule formation and survival in the presence of other infective rhizobia in the soil.
- prompt nodulation and good N2-fixation over a wide range of soil types.
- good growth ability in the carrier and in the soil.
- persistence in the soil.
- effectiveness on a wide range of host genotypes or cultivars.
- tolerance to soil stress factors including acidity, alkalinity, salinity, high concentration of aluminum and manganese.

It is almost impossible to find an inoculant with all these qualities. However, any inoculant must contain superior rhizobia capable of producing nodules and fixing nitrogen on the plants for which it is designated.

Forms of Inoculants

i) Powdered Solid Inoculant

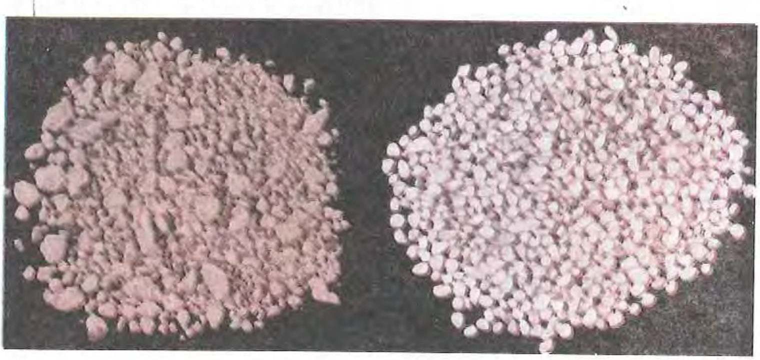
This form is used as seed coating before planting. This type of inoculant consists of finely ground peat with a single strain of rhizobia added in a nutrient suspension. Seeds inoculated with peat inoculant are best sown on the day of inoculation to maximize the number of live rhizobia delivered with the seeds to the soil. The smaller the particle size, the better the inoculant will adhere to the seeds. Coverage of powdered inoculants is enhanced by adhesives that increase amounts of inoculant on seed.

ii) Liquid Formulations

These inoculants use broth cultures or liquid formulations, mainly in water, but also in mineral or organic oils. The seeds are either dipped into the inoculant before sowing, or an applicator evenly sprays the liquid inoculant on the seeds. After drying, the seeds are sown. This method ensures even coverage of the seeds without interference with the seed monitoring system of the planters or inoculum loss when dried. Alternatively, the suspension can be sprayed directly into the furrow or on the seeds before sowing. The in furrow inoculant provides a larger amount of bacteria to the plant than seed inoculation. In rhizobia, this improves plant nodulation. These inoculants are currently popular in the USA, Canada, Argentina, and Brazil, mainly for soybeans for use in mechanized agriculture.

iii) Granular Inoculants

These consist of formulations of clay or peat granules impregnated with *rhizobia.* They are applied directly to the furrow together with the seeds. Granular inoculants are not widely available in Africa and are useful when, seeds are heavily treated with pesticides or when larger doses are needed. Their use with groundnuts is widely practised. These inoculants are new, as yet unproven in many environments.



Is Inoculation Worth the Extra Cost?

The knowledge behind inoculation is really quite captivating and deserves to be studied further because, often, advice is given for inoculant application without an understanding of what it really does. Legume plants continually benefit from their symbiotic association with the rhizobia in the inoculants throughout their life cycle and as a result the health and productivity of the inoculated plants are improved. There is also a yield increase that more than pays for the input cost of the inoculant itself.

Do I Need Inoculation?

Most soils do not contain very many, if any, of these bacteria. In order to insure good nitrogen fixation by the legume, it is necessary to inoculate the legume with the proper strain of bacteria prior to planting the seeds. Inoculating the seed is a good insurance that the plant will be properly equipped to grow to its maximum potential and compete. Inoculated legume can increase the amount of soil nitrogen from residues left after harvesting and this can considerably reduce the cost of mineral nitrogen fertilizer.

Legume Inoculant Application Techniques

Commonly, two application methods are used during inoculation of rhizobia to legumes. This is the direct inoculation, where the inoculant is placed in direct contact with the seed (seed applied inoculant), and indirect inoculation, where by the inoculant is placed alongside or beneath the seed (soil applied inoculant). By analyzing the advantages and disadvantages of the two methods, it can be concluded that while there is currently room for both methods, the future probably lies with seed inoculation. Seed inoculation is cost effective and also gives assurance of the availability of rhizobia to colonize the roots of legumes.

Seed-applied inoculants may be used in the following ways:

a) **Dusting:** In this method, the inoculant powder is mixed directly with seed without using any water or other liquid. The dry inoculation method is often considered disadvantageous because the attachment of the inoculum to the seed is poor and much of it is lost prior to and during sowing. The rhizobia also have little protection when this method is used and therefore their survival is poor compared to the other methods. Some inoculants contain dry adhesives and are intended for dusting.

b) **Slurry:** Applying inoculants using this method involves initial mixing of the inoculant with water to form a uniform, pourable suspension. In some instances, gum arabic or sucrose of methyl ethyl cellulose may be added to the water to improve adhesion of the inoculant to the seed. The slurry obtained is then added to the seeds and mixed.

c) **Seed Pelleting:** Inoculants can be made into slurry and mixed with the seeds. The seeds are then coated with finely ground lime, clay, rock phosphate, charcoal, dolomite, calcium carbonate or talc depending on soil conditions and plant needs. The method has several advantages, such as protection of rhizobia against low soil pH, desiccation, acidic fertilizers, fungicides or insecticides.

On the other hand, seed coating is not always the best way to inoculate. Some inoculants are designed to be placed directly into the soil. This practice is recommended under the following conditions:

1. When seeds are heavily precoated with pesticides or herbicides.
2. When planting in hot, dry soil. If legume seeds are planted in hot, dry soil and must wait for rain before they germinate, the rhizobia used to coat them are likely to die. Under these conditions, the rhizobia will survive better if the inoculant is placed in the soil below the seeds. Dry granules are recommended for dry soils.
3. When seed inoculation has failed.
4. When large numbers of rhizobia are needed or where fungicides/ pesticides are coated.

Materials Needed for Seed Inoculation

- A medium sized container with (airtight) lid
- Water not hot or containing heavy metals
- Tablespoon or coca cola lid
- W ooden stirrer / Spatula
- Certified seeds
- Sticker dissolved previously in hot water
- Inoculant

Plate 1. *Materials needed for seed inoculation*

Practical Steps to Inoculant Application – Slurry Method

The inoculant supplied to farmers by N2Africa may contain stickers and as such there is no need to apply any more before adding the inoculant.

Pour 1 kg of certified seeds into the medium sized container.

Sprinkle **small amount of water** onto the seeds; this is to ensure adhesion of the inoculant on the seeds. Do not wet the seeds!

Gently stir to ensure uniform mixing of the seeds and water.

Using a tablespoon or a coca cola lid measure and pour the inoculant onto the seeds. A half-filled tablespoon of inoculant weighs approximately 4 g.

5

Cover the plastic container with the lid.

6

Swirl **gently** to ensure proper adhesion of inoculant to seeds. Occasionally remove the lid and check for proper mixing. Seeds turn **black** when properly coated with the inoculant.

Well coated inoculated seeds will appear as shown.

7

Air dry coated seeds **under a shade** for 15 to 20 minutes.

How do I Know the Inoculant Worked?

The leaves of the inoculated plants will be deep green whereas the lower leaves of the uninoculated plants will become yellow. For highly effective inoculant, the inoculated plants will have large nodules; red on the inside. The uninoculated plants tend to be stunted.

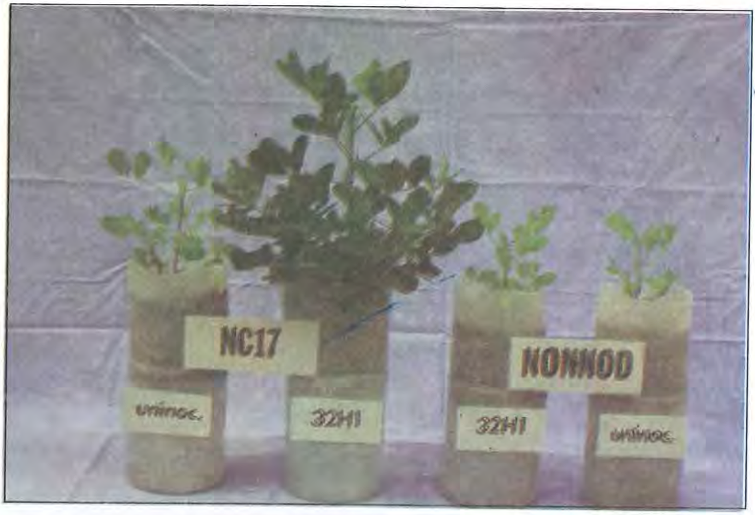


Plate 2. *The effects of inoculation on legumes*

Guidelines for Handling Inoculants

- Purchase and use fresh inoculants to ensure high populations of active bacteria in the package.
- Read the labels on the inoculants carefully and adhere closely to the instructions. The extension agent needs to read the label on the inoculant package carefully when recommending an inoculant to farmers. The label contains important information, such as the proper legume species for the inoculant and its expiration.

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LEGUME SEED INOCULUM FOR : Soy Bean

Rhizobia : Tal 102, 377, 379

Expiration Date: August 1990

Perishable: Inoculant contains LIVE nitrogen-fixing bacteria!

DO NOT * Keep in direct sunlight
DO NOT * Use inoculant past expiration date
DO NOT * Store inoculated seed
DO NOT * Store at temperatures above 28 or below 4 degrees C
DO NOT * Freeze

INOCULATING SEEDS:

Sticker : To improve adherence of inoculum to seed, use either a sugar solution or water.

1. Dissolve 1 part sugar to 9 parts water. Add 20 ml of sugar solution to 1 kilogram of seed. – OR – Add 20 ml of water to 1 kilogram seed if sugar is not available.
2. Add sticker to seeds and mix evenly in a plastic bag or bucket.
3. Add inoculum to that amount of seeds that will be planted immediately.
4. Mix inoculum and seed thoroughly. Allow about a 30 minute period for air drying of seeds.
5. Protect inoculated seeds from direct sunlight, heat, and excessive drying.

Net Weight : 300 grams

300 grams INOCULATES 30 kilograms of seed

- Make sure that you have the proper inoculant. Each species of legume requires a specific type of rhizobacteria for nitrogen production. The package will be labeled with the plant species for which the inoculant is intended.
- You cannot use too much inoculant; but you can use too little. It is OK to use 2 or 3 times the recommended rate on the package. This is especially useful if planting in soils which have never had this type of legume before or which are not going to be irrigated, as some of the *rhizobium* will die if rains are not timely.

- The inoculant comes pre-mixed in a peat powder to allow for ease of handling. The peat and bacteria must stick to every seed to be effective. The best way to make the powder stick is to slightly wet the seeds prior to applying the inoculant. Non-chlorinated water can be used, but much better results are obtained by using a combination of whole milk and molasses.
- Plant seeds immediately after inoculation. If the seed is too wet to plant, allow the seed to dry in the shade.
- Cover the seed in the ground immediately. The longer the seeds stay exposed to sunlight, the less effective the inoculation will be. The bacteria die when exposed to sunlight.
- Irrigate immediately. This will increase the survival and germination of seeds and inoculant. But under some conditions, elite rhizobia can colonize soil after repeated healthy inoculation, and they become part of soil biota.
- The inoculant is non-toxic and has no adverse effect when it gets to your body, on other seeds, plants or soil.

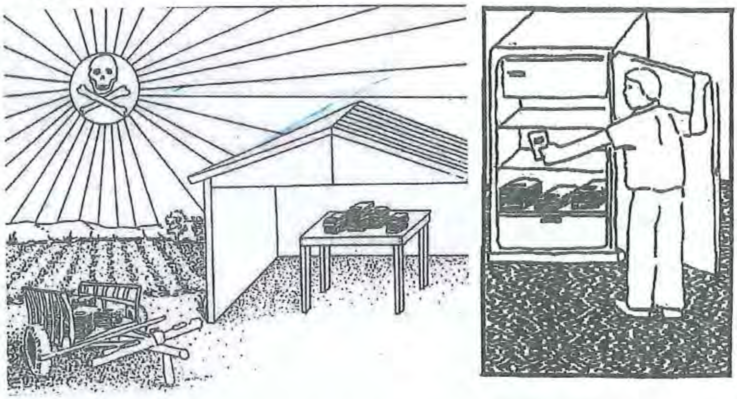
Do I Need to Inoculate all the Time?

Inoculation is always recommended unless there is convincing evidence that inoculation is not necessary. The persistence of rhizobia in the soil is affected by the soil type, pH, moisture and excessive temperature. Inoculation can be used as insurance, when one is uncertain about the nature and type of native rhizobia present in the soil.

Guidelines for Storing Inoculants

The bacteria in the inoculant are perishable and as such it needs to be handled properly. One cannot visibly tell whether an inoculant contains live or dead rhizobia because the two look alike. The following are other key considerations:

1. Rhizobium bacteria are sensitive to heat and sunlight. Do not leave the packages lying in the sun or under a **metallic roofing sheet!!!** Unused inoculant should be stored in a cool location; a refrigerator is best.



2. Use legume inoculants before their expiry date and avoid or discard all expired stock. Many inoculant producers offer retailers opportunity to replace expired stock. Inoculants can be stored for up to six months in a well-sealed ceramic jar and buried in a shady spot underground. The jar should be covered with a thick wooden lid to serve as protection as well as insulation from heat.
3. Once inoculants are opened, reseal inoculant package to avoid contamination and moisture loss.
4. Never store inoculated seeds for next planting because it is best to inoculate seeds just before planting.

Under What Conditions Will an Inoculant Fail?

- Application of an inappropriate strain may lead to inoculation failure.
- Use of poor quality inoculant can also lead to inoculation failure.
- In soils where large populations of infective rhizobia are present, inoculation failure is a common problem due to the competition presented by the native rhizobia.
- Anything that suppresses the growth of the host legume also affects the rhizobia. The success or otherwise of rhizobia inoculation is dependent upon healthy plant demand for symbiotic nitrogen.
- Pest and diseases, fertility status of the soil, and competition from native rhizobia are all constraints to legume host productivity, and can reduce nodulation and biological nitrogen fixation.

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