ILLUSTRATED CONCEPTS IN AGRICULTURAL BIOTECHNOLOGY

A joint publication of the NifTAL Project*/MIRCEN**

Inoculating Tree Legume Seeds and Seedlings With Rhizobia

Root nodules contain rhizobia which fix nitrogen from the air (nodulated root of A. mangium).

Introduction

Many tree legumes have the ability to form a symbiotic relationship with root-nodule bacteria (rhizobia) which are able to convert nitrogen in the air into a form usable by the plant. This conversion is called biological nitrogen fixation (BNF) and is carried out by the rhizobia inside root nodules. BNF eliminates the need to apply expensive nitrogen fertilizer for tree growth.

Under conditions of low nitrogen, most legumes respond well to inoculation (plant on left).

Inoculants

In many soils the nodule bacteria are absent or are not adequate in either number or quality to meet the nitrogen requirements of legumes. Under these conditions, it is necessary to inoculate the seed or seedling with highly effective rhizobia cultures. Nodule bacteria are cultured in the lab and mixed with a suitable carrier material, such as peat, to make an inoculant. The process of adding this inoculant to seeds or seedlings is called inoculation.

Different legumes require different rhizobia in order to produce an effective symbiosis[1]. Some trees (such as, *Leucaena leucocephala*, *Gliricidia sepium* and *Calliandra calothyrsus*) require fast-growing *Rhizobium*, while others (such as, *Acacia mangium*, *Acacia auriculiformis* and *Albizia saman*) require slow-growing *Bradyrhizobium*. Further specificity exists between species in these two broad groups. Therefore, the user should be sure that the correct inoculant is supplied to a given tree.

A well nodulated seedling of Acacia koa *ready for outplanting.*

Inoculating Seeds

To insure good adherence of the inoculant, it is recommended that the seed be coated with a sticker before the peat is added. Any of the following stickers can be used:

Gum arabic. Dissolve 40 grams of gum arabic in 100 ml of hot water (complete dissolving requires stirring for several minutes) and allow to cool before use. Add 2 ml of the solution per 100 grams of

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seed. **Sugar.** Prepare a sugar-water solution (1 part sugar to 9 parts water). Add 2 ml of the solution per 100 grams of seed. **Vegetable oil.** Add 2 ml per 100 grams of seed. **Water.** Add 2 ml per 100 grams of seed.

Even distribution of the sticker on all seeds is obtained by mixing in a plastic bag or bucket. The peat-based inoculant is then added at the rate of 50 grams per kilogram of seed. The inoculated seeds are ready for planting after a short period of air drying (to eliminate any stickiness). Do not store inoculated seed. Inoculation should be done just prior to planting.

Inoculating Seedlings

Tree legume seedlings can be readily inoculated in the nursery. A 50 gram bag of peat inoculant has the capacity to inoculate 10, 000 seedlings (regardless of the species). This can be easily achieved by mixing the peat in cool water and using the suspension to irrigate the rooting medium of the seedlings. Use a sufficient volume of water to ensure the suspended inoculant is washed into the root zone of the seedling. Keep the suspension agitated.

Inoculant Storage

The peat inoculant contains living rhizobial cells which need to be protected from heat and direct sunlight. Prior to its use, store the packet at room temperature or cooler, preferably in a refrigerator. Also, the inoculant should be used by the expiration date marked on the label.

Relationship of Seed Number per Kilogram to the Number of Rhizobia per Inoculated Seed

Tree species	No. seeds per kg.	No. Rhizobia Per seed*
Acacia angustissima	100,000	1.5×10^6
Acacia auriculiformis	45,000	3.3×10^6
Acacia crassicarpa	44,000	3.4×10^6
Acacia mearnsii	71,000	2.1×10^6
Acacia mangium	100,000	1.5×10^6
Albizia lebbek	8,000	18.7×10^6
Albizia saman	5,100	29.4×10^6
Calliandra calothyrsus	20,000	7.5×10^6
Chamaecytisus palmensis	39,000	3.8×10^6
Desmanthus virgatus	263,000	5.7×10^5
Enterolobium cyclocarpum	900	16.7×10^7
Erythrina poeppigiana	5,000	30×10^6
Faidherbia albida	20,000	7.5×10^6
Flemingia macrophylla	50,000	3.0×10^6
Gliricidia sepium	8,100	18.5×10^6
Leucaena diversifolia	49,000	3.1×10^6
Leucaena leucocephala	14,000	10.7×10^6
Mimosa scabrella	83,000	1.8×10^6
Paraserianthes falcataria	48,000	3.1×10^6
Paraserianthes lophanta	10,000	15×10^6
Pithecellobium dulce	17,000	8.8×10^6
Sesbania grandiflora	20,000	7.5×10^6
Sesbania sesban	94,000	1.6×10^6

*Based on use of 50 grams of inoculant per kilogram of seed and a rhizobial density of 3×10^9 cells per gram of peat.

[1]For additional information, request a copy of NifTAL's Illustrated Concept in Agricultural Biotechnology Number 4, "Biological Nitrogen Fixation: Commonly asked Questions and Answers."