MODULE 3: DEMONSTRATION 1

THE CROSS-INOCULATION CONCEPT:
LEGUMES REQUIRE SPECIFIC RHIZOBLIA

PURPOSE

- Demonstrate that some legume species require different rhizobial species for effective nodulation.
- For effective nodulation, the rhizobia and legume must be properly matched following the "cross-inoculation groups" concept.
- When inoculation is successful and effective, the legume is well nodulated, leaves are green, and nodules, when cut open, are red inside.

CONCEPTS OF DEMONSTRATION

This exercise illustrates that not every rhizobia nodulates all legumes. There is no one type of rhizobia that can be used for all legumes. For example, when the inoculant for soybean seeds are out of supply, do not buy or use inoculants meant for inoculating other legume species. Correct matching of the legume to its recommended inoculant will result in effective nitrogen fixing nodules. When the legume is green and healthy, effective nodules will have developed on the roots. Green plants indicate self-sufficiency in nitrogen. Effective nodules will appear red to pink when cut open.

RECOMMENDATION TO FARMER FROM RESULTS OF THIS DEMONSTRATION

- Always use the correct inoculant for your legume crop.
- To understand what successful inoculation is, the farmer should excavate carefully a few healthy, green plants, look at the nodules, cut open a few and see the red or pink interior.
- Nodules formed by the rhizobia in the inoculant are nourishing his legume crop.
- Nodulation is not a root disease harming his crop.
- Nodules formed by rhizobia have red to pink interiors compared to nodules formed by nematodes.
CONDUCTING THE DEMONSTRATION

It is not necessary to conduct the demonstration to cover the entire range of rhizobial species and their appropriate cross-inoculation groups of legumes as shown in Table 1 of Module 3. Only legumes which are of local or regional economic and agricultural importance need to be selected for the demonstration. The demonstration can be set up in a greenhouse/glasshouse with the legumes grown in potted sand or soil. Greenhouse experiments should be evaluated at 30-35 days.

MATERIALS

Seeds: Soybean (Glycine max), bean (Phaseolus vulgaris), peanut (Arachis hypogaea) and lima bean (Phaseolus lunatus) are used in this exercise. Seeds should have at least a 90% viability rate and be free of insect or mechanical damage. To facilitate inoculation and planting of the seeds at later stages, the seeds need to be mixed in batches and each batch surface sterilized separately. Prepare four batches of seeds, each batch consisting of 20 lima bean, 20 soybean, 20 bean and 20 peanut seeds.

Rhizobia: Obtain peat-based inoculants for soybean (Bradyrhizobium japonicum), bean rhizobia (Rhizobium leguminosarum biovar phaseoli) and cowpea rhizobia (Bradyrhizobium sp.).

Potting (growth) medium: Washed and dried river-sand is suitable because it can be steam sterilized (autoclaved) to kill off contaminating rhizobia. Subsoil free of native rhizobia is a better alternative. Soils which are known to have low numbers of ineffective rhizobia can also be used. Sand or soil should be contained in 5 l capacity pots. Ceramic or clay pots can be autoclaved and are preferred for use when sterilizing sand. Soil can also be contained in plastic pots. A total of 24 pots are needed for the exercise.

Sterilization materials: Seed used in this experiment should be surface sterilized to ensure that rhizobia on the surface of the seeds are destroyed. A 2.5% bleach solution (commercial sodium hypochlorite) is needed. Sterile or boiled water must be available. One liter capacity glass containers are required for seed sterilization and inoculation.

PROCEDURE

1) Potting (growth) medium preparations: Determine the water-holding capacity of the soil, adjust the soil pH, and add nutrients as described in Demonstration 2 of Module 7.

If sand is used it can be autoclaved or heated in the pots to at least 50°C for 5 hours. Cover the top of the pots with aluminum foil during autoclaving. Keep the sterilized pots in a cool and clean spot in the greenhouse/glasshouse where contamination from insects and airborne rhizobia can be controlled. Pots should be prepared two days before planting.

2) Seed sterilization and inoculation: Place each batch of legume seeds in a container. Add sufficient bleach solution to immerse the seeds. Swirl the flask gently and set aside for
2 minutes. Drain off the bleach completely and rinse the seeds with at least eight changes of sterile water. Label the flasks with the strain treatments and an uninoculated control.

3) **Inoculation**: To remove the inoculant(s) from the bag (TAL 182, for example), cut open one corner of the bag with a pair or scissors. (Scissors are sterilized by dipping into a beaker of alcohol and burning off the alcohol with a spirit flame.) Remove a small spoon of inoculant and transfer it to the container of seeds. Swirl the flask until the seeds are coated with the inoculant. Inoculate the rest of the seeds in the flask with the appropriate inoculant, as indicated on the labels. (Remember to sterilize the scissors and spoons before opening the next bag containing a different inoculant.)

4) **Planting the seeds**: For planting the seeds, follow the scheme shown in Figure 1. Plant the uninoculated treatments first. Plant three to five seeds of each species per pot. Ensure that seeds are planted at least an inch deep in the soil (or sand). To prevent cross-contamination when planting the inoculated treatments, it is important to disinfect your hands by spraying them with 75% ethanol or washing them with soap and water. Hands and plant accessories must be disinfected between inoculated treatments. Complete planting all the treatments.

5) **Maintaining the potted plants**: To maintain the water-holding capacity of the soil, water the pots with tap water whenever necessary. For pots with soil follow instructions in Demonstration 2, Module 7. Water plants grown in potted sand alternately with tap water and half-strength nutrient solution to prevent salt build-up. A nutrient solution formulation is provided in *Methods of Legume-Rhizobium Technology*.

6) **Harvest and recording of data**: Harvest the experiment after 30-35 days of plant growth. Record plant color, nodulation, and plant weight. Cut open nodules to note the color of the interior. Use Table 1 to record your observations. From the results, analyze the ineffective and effective legume species-inoculant combination.