Induction of root symbioses in common bean

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Abstract

A hallmark of legume plants is their ability to establish mutualistic symbioses with *Rhizobium* bacteria and arbuscular mycorrhizal fungi (*Rhizophagus irregularis*). These symbionts help in providing nutritional benefits to the host plants. Herein, we provide a protocol to inoculate the common bean for the induction of symbiont colonization. This protocol has been routinely used in our laboratory with high reproducibility.

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Protocol

Induction of mycorrhizal symbiosis in common bean by Rhizophagus irregularis fungi

Step 1.

About one gram of *Rhizophagus irregularis* spores (Symplanta®, Germany) were dissolved in 10 ml sterile water.

Induction of mycorrhizal symbiosis in common bean by Rhizophagus irregularis fungi

Step 2.

Using hemocytometer adjust the spore count to 1000 spores/ml by dilution with sterile water.

Induction of mycorrhizal symbiosis in common bean by Rhizophagus irregularis fungi

Step 3.

To induce mycorrhizal colonization, inoculate 1 ml of culture (prepared from step 1 & 2) at the root zone of 5 day old common bean seedlings maintained in pots containing sterile vermiculite.

Induction of mycorrhizal symbiosis in common bean by Rhizophagus irregularis fungi

Step 4.

Maintain the inoculated plants in growth chambers with a 16-h photoperiod and 65% relative humidity at 28 °C.

Induction of mycorrhizal symbiosis in common bean by Rhizophagus irregularis fungi

Step 5.

Irrigate the plants regularly with B&D solution to promote mycorrhizal colonization.

Induction of mycorrhizal symbiosis in common bean by Rhizophagus irregularis fungi

Step 6.

In wild type plants mature arbuscules will be seen form 14 days post inoculation.

Induction of root nodule symbiosis in common bean by Rhizobium bacteria

Step 7.

For induction of root nodules, inoculate *Rhizobium tropici* strain CIAT899 (this species is compatible to common bean) 100 ml of PY medium (0.5 g peptone, 0.3 g yeast extract) supplemented with 7 mM ml^{-1} $CaCl_2$ and 20 mg ml^{-1} nalidixic acid and incubate at 30 °C for 24 h with shaking at 200 rpm. NOTE: Any specific antibiotics could be used in case of transgenic *Rhizobium*.

Induction of root nodule symbiosis in common bean by Rhizobium bacteria

Step 8.

Pellet the cells in a centrifuge for 3 min at 5000 rpm at room temperature and discard the supernatant.

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Step 9.

Resuspend the pellet in 10 mM $MgSO_4$ or sterile water.

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Step 10.

Adjust the OD of rhizobia cells to 0.05 at OD_{600} by dilution with 10 mM $MgSO_4$ or sterile water.

Induction of root nodule symbiosis in common bean by Rhizobium bacteria

Step 11.

To induce nodulation, inoculate 1 ml of culture (prepared from step 3 & 4) at the root zone of 5 day old common bean seedlings maintained in pots containing sterile vermiculite.

Induction of root nodule symbiosis in common bean by Rhizobium bacteria

Step 12.

Maintain the inoculated plants in growth chambers with a 16-h photoperiod and 65% relative humidity at 28 °C.

Induction of root nodule symbiosis in common bean by Rhizobium bacteria

Step 13.

Irrigate the plants regularly with B&D solution to promote nodulation.

Induction of root nodule symbiosis in common bean by Rhizobium bacteria

Step 14.

In wild type plants the mature nodules will be seen form 14 days post inoculation (dpi) to 21 dpi.