**Plant-rhizobia experiment**

 Name\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Date\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Question 1: How do rhizobia affect plant growth?**

**Question 2: How does nitrogen fertilization affect plant-rhizobia interaction?**

1. Write down your hypotheses for Question 1 and 2:
2. Plot prediction graphs for Question 1 and 2.
3. **Experimental set-up:**

**Materials:**

|  |
| --- |
| *To set up the experience:*  Soybean seeds |
| Potting soil |
| 10-12 oz plastic cups or pots |
| Small plastic plates as saucers |
| Slow-release fertilizer |
| Rhizobia inoculum |
| Plastic disposable pipette |
| Masking tape |
| Permanent markers  dice |
| F*or harvesting:*  Tray or bucket to dispose soil |
| Ruler |
| Balance |
| Weigh boats or paper towel |
| Micro-centrifuge tubes |
| Forceps |
| Stereo microscope (optional to look at the roots) |

**Methods:**

1. Label each pot as shown below.
2. Add soil in each of the pots to 4/5 full.
3. Add 6g of fertilizer pellets on the pots labeled #5- #8
4. Place each pot on individual saucer and water all the pots until soil is completely saturated and water drips out drain holes.
5. Make a 1cm indentation with your finger
6. Add 3 seeds and cover loosely with moist soil. NOTE: We plant 3 seeds to ensure germination.
7. Add 5ml of rhizobium inoculum using pipette to pots labeled #3, #4, #7, and #8
8. Add sterilized media (5ml) using pipette to pots labeled #1, #2, #5, and #6, as control.
9. Randomize the location of the pot using a dice.
10. Place all plants in a warm, light location

#1

N (-)

Rhiz (-)

# 3

N (-) Rhiz (+)

# 5

N (+)

Rhiz (-)

# 7

N (+)

Rhiz (+)

#2

N (-)

Rhiz (-)

# 4

N (-)

Rhiz (+)

# 6

N (+)

Rhiz (-)

# 8

N (+)

Rhiz(+)

**Maintenance:**

\*This experiment will be carried out for one month. Make sure to apply the same amount of water to each pot every other day (approx. 100 ml/pot). Avoid water splash to minimize contamination.

\*One week later, make sure that there is only one seedling per pot. If there are extra seedlings, remove them gently.

**4. Results:**

One month after setting up the experiment, measure all the traits below and plot the results:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Treatment | Height (mm) | Number of Leaves | Number of Nodules | Aboveground Biomas  (g) | Belowground Biomass  (g) |
| 1 | Control |  |  |  |  |  |
| 2 | Control |  |  |  |  |  |
| 3 | Rhizobia (+) |  |  |  |  |  |
| 4 | Rhizobia (+) |  |  |  |  |  |
| 5 | Fertilizer (+) |  |  |  |  |  |
| 6 | Fertilizer (+) |  |  |  |  |  |
| 7 | Rhizobia (+), fertilizer (+) |  |  |  |  |  |
| 8 | Rhizobia (+), fertilizer (+) |  |  |  |  |  |

1. **Conclusions: Based on the data you collected answer these questions:**

Question 1: How do rhizobia affect plant growth?

Question 2: How does nitrogen fertilization affect plant-rhizobia interaction?