Workshop Activity 3

Legumes & Nitrogen: Genotype To Phenotype

BACKGROUND

A unique characteristic of legumes is their ability to “fix” nitrogen because of a special relationship with soil bacteria called rhizobia. Plants, animals and humans need nitrogen to make proteins, nucleic acids such as DNA and other compounds necessary to build an organism. Although 78% of the air is nitrogen, plants and animals cannot convert gaseous nitrogen into proteins or DNA. The harmless soil bacteria live in nodules on the plant roots. They contain an enzyme called nitrogenase that converts atmospheric nitrogen into ammonium, which the legume can take up and use. This symbiotic relationship means that legumes such as clover, lupin and alfalfa make good natural fertiliser for pastures. Legume seeds are also rich in protein making them an important food source.

***MUTANT PLANTS**: The mutant plants used in this experiment each have a single point mutation (or a change in one nucleotide) in one gene. The soybean genome is 1115 megabases in size – that’s 1 115 000 000 nucleotides big.*

Legumes have tight control over rhizobia and only let them invade their roots in certain conditions. This is because the plant cannot make unlimited amounts of sugar to feed the bacteria. Legumes have a number of genes that instruct the plant on how many nodules to make in various conditions. Scientists are trying to uncover these genes to ultimately improve legumes as crops.

In this activity you will analyse the effect of gene mutation on nodulation and plant growth in the legume, soybean (Glycine max). The visible characteristics of the plant or phenotype are determined partly by its genes or genotype. Environmental factors such as heat, salt and nitrate, also play a role but we do not have enough time to look at these today.

RESOURCES

- Wild-type soybean plant (normal)
- Supernodulating mutant soybean plant (loss of function of a gene called GmNARK [Glycine max nodule autoregulation receptor] that controls nodule number)
- Non-nodulating mutant soybean plant (loss of function of a gene called NFR [Nod factor receptor] that controls nodule formation)
- Ruler
- Scalpel

All plants have been inoculated with Bradyrhizobium japonicum CB1809 (CB stands for CSIRO Brisbane)

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Workshop Activity 3 (cont.)

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PROCEDURE

1. **Select three plants each of a wild-type, supernodulating and non-nodulating soybean**. Remove them carefully from their pots and gently wash off the vermiculite. The plants are clearly labelled. Can you see any differences between them?

2. **Count the number of nodules on the root of each plant**. Record your results in the table below.

3. **Measure the shoot height of each plant using the ruler**. Record your results in the table below (in approximate centimetres).

4. **Measure the length of the longest root of each plant using the ruler**. Record your results in the table below (in approximate centimetres).

5. **Carefully using a scalpel cut open a nodule**. The nodule interior should appear to be reddish-brown. This is the infected zone. The red substance is a protein called leghaemoglobin, which is structurally similar to haemoglobin found in blood. Leghaemoglobin is produced by the plant and transports free oxygen in the cytoplasm of infected plant cells to ensure the proper function of root nodules by supplying oxygen at a slow and steady rate.

$$N_2 + 16\,ATP + 8H^+ + 8e^- \longrightarrow 2NH_3 + H_2 + 16ADP + 16PO^-_4$$

Note: the equation shows the high amount of ATP needed, H2 gas is produced. ATP is made by respiration needing oxygen.

EXPECTED RESULTS:

The supernodulating plant will have the most nodules because it has lost the ability to regulate nodule formation through a mutation in a gene that controls nodule numbers. The roots are supernodulated by the rhizobia.

The normal or wild-type soybean plant controls the number of nodules to the minimum necessary to sustain the nitrogen it needs.

The non-nodulating plant has lost the ability to form nodules through a mutation in a gene that tells the plant to make nodules in response to invasion by rhizobia.

How much variability was there between plants? Was your sample size big enough? What conclusions do you draw? What explanations exist?

Plant no	WT			Supernod			Non-nod		
	Nodules	Shoots	Roots	Nodules	Shoots	Roots	Nodules	Shoots	Roots
1									
2									
3									
average									

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