

## Development of a DNA test to quantify the number of *Rhizobium leguminosarum* bv. *viciae* in soil

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DNA tests to quantify rhizobia in soil are being developed. These tests will help growers determine if rhizobia inoculation is required. They will also provide researchers with an alternative test to the time consuming Most Probable Number plant nodulation bioassay, to help better understand how agronomic practices affect rhizobia populations. They may have application in the quality control of rhizobial inoculants, particularly of granular products where more efficient tests are needed to support independent quality control.

The most advanced test targets the rhizobia that nodulate pea, bean, lentil and vetch; *Rhizobium leguminosarum* bv. *viciae* (*Rlv*). A quantitative real time PCR assay for the specific detection of *Rlv* was designed and a calibration standard prepared to estimate *Rlv* DNA copy number/g soil.

The specificity of the test was verified using pure cultures of rhizobia. The test detected various strains of *Rlv*, but did not detect strains of the closely related clover nodulating rhizobia, *Rhizobium leguminosarum* bv. *trifolii*.

Sensitivity of the DNA test was determined using moist peat cultures of *Rlv*, similar to commercial inoculants. Number of rhizobia was determined in eight 10-fold dilutions of peat cultures in sterile water, using the DNA test and by counting colony forming units (CFU) on agar. The DNA and CFU results were highly correlated ( $R^2 = 0.97$ ,  $n=48$ ) and showed high precision above 1000 CFU/g.

The DNA assay detected *Rlv* in 19 of 42 soils collected from grower paddocks. In comparison, *Rlv* was detected in 28 of the soils using a plant nodulation bioassay. However, there was no instance where *Rlv* was detected by the DNA test, but not by the plant nodulation test. The results indicate that the DNA assay is specific and unlikely to detect organisms other than *Rlv*, but is less sensitive than the plant nodulation test, which is able to detect 10 rhizobia/g soil.

When used to assess a field trial examining the colonisation of an acidic soil by strains of *Rlv*, the DNA test clearly showed that colonisation by the rhizobia was limited and that the extent of colonisation varied according to the rhizobia strain. In general, the DNA results were consistent with nodule numbers measured in parallel plant bioassays, but improved the levels of significance and provided results within days of sampling.

Further validation and calibration of the test to Most Probable Number counts of rhizobia will be completed using field soils representative of major soil types, to ensure its reliability in a field setting. Confidence is needed to ensure that where the DNA test result indicates a large rhizobia population, there is no possibility of nodulation failure occurring if growers decide not to inoculate.

A test for chickpea rhizobia is currently under development. If successful, similar tests could be developed for other crop and pasture legumes.