



Improving mung bean profitability by better understanding the comparative effectiveness of wild and commercial rhizobium

Agri-Science Queensland Innovation Opportunity

July 2016

This publication has been compiled by <insert name/s> of <insert business group>, <insert department>.

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Summary

Mung beans are a profitable leguminous crop. Expansion of the cultivation of mung beans in many parts of Queensland could benefit growers, by increasing the value and profitability of primary production both through direct sales of seed from this high-value crop, and/or through other crops in the farming system benefiting from residual fixed nitrogen. Grower profits could be improved, by purchasing less, increasingly costly, nitrogen fertilizer. It could also benefit the environment. By maximising nitrogen fixation by legumes, the application of nitrogen for sugarcane, banana and other non-legume crops could be reduced, potentially leading to less run-off and better outcomes for Queensland's rivers and reef.

Simplifying the operations involved in growing mung beans may encourage more farmers to incorporate it into their farming system.

Anecdotal reports have suggested that wild rhizobia are as effective as commercial inoculum for mung bean in the Burdekin region of far north Queensland. To test this hypothesis, mung bean nodules were collected from mung bean plants from two sites in the Burdekin region. One site had been treated with commercial inoculum CB1015, the other that had never been treated with commercial inoculum. Analysis using Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) showed that up to 8 different wild strains of *Bradyrhizobium*, distinctly different to CB1015, were present in the nodules of these plants. A different wild strain dominated at each collection site. Mung bean plants grown in soils from Millmerran, on the Darling Downs, on the other hand, hosted only CB1015 when inoculated, and did not nodulate when not inoculated. Mung beans inoculated with the two dominant wild rhizobia strains in a controlled glasshouse experiment produced similar biomass compared with those inoculated with CB1015. The plant inoculated with the wild *Bradyrhizobium* strains however fixed significantly more nitrogen than those with CB1015. The two mung bean varieties and the black gram, responded similarly to the three *Bradyrhizobium* strains.

These results support the belief that wild rhizobia are as effective at promoting growth and may be superior in fixing nitrogen in mung beans as the commercial strain in some parts of Queensland. However, the application of commercial inoculum is necessary for a healthy crop in other parts of Queensland, and may still be considered a useful "insurance policy" to ensure healthy nodulation of mung bean crops in the Burdekin catchment area.

Table of contents

Background	1
Project Objectives	1
Methodology	1
Results	3
Conclusions/Significance/Recommendations	8
Key Messages	9
Where to next	9
Budget Summary	9

Table of figures

Figure 1 – Principal component analysis of MALDI-TOF MS spectra from Bradyrhizobium collected from mung beans from two sites in the Burdekin catchment area of north Queensland. Spectra from Cluster 5 are in grey, Cluster 9 in green and Cluster 7, including CB1015, in gold.	3
Figure 2. Top dry weight per plant (g) of mung bean and black gram plants uninoculated, uninoculated with added nitrogen or treated with CB1015, Brady 5 or Brady 9 inoculum, expressed as best linear unbiased estimates (BLUES).....	5
Figure 3. Nodule dry weight per plant (g) of mung bean and black gram plants uninoculated, uninoculated with added nitrogen or treated with CB1015, Brady 5 or Brady 9 inoculum, expressed as best linear unbiased estimates (BLUES).....	6
Figure 4. Nodule count per plant of mung bean and black gram plants uninoculated, uninoculated with added nitrogen or treated with CB1015, Brady 5 or Brady 9 inoculum, expressed as best linear unbiased estimates (BLUES).....	6
Figure 5. Root dry weight per plant (g) of mung bean and black gram plants uninoculated, uninoculated with added nitrogen or treated with CB1015, Brady 5 or Brady 9 inoculum, expressed as best linear unbiased estimates (BLUES). There was a significant interaction for root dry weight between treatment and variety.....	7
Figure 6. Nitrogen fixation of mung bean and black gram plants treated uninoculated, uninoculated with added nitrogen or treated with CB1015, Brady 5 or Brady 9 inoculum, expressed as best linear unbiased estimates (BLUES). There was a significant interaction for root dry weight between treatment and variety.....	8

Table of images

Image 1 – Glasshouse experiment comparing inoculation treatments for three mung bean varieties.....cover

Table of tables

Table 1. Summary analysis of variance Highlighted statistics are significant at the 5% level.....4

Table 2 – Budget summary.....9

Background

Mung beans are a profitable leguminous crop. Expansion of the cultivation of mung beans in many parts of Queensland could benefit growers, by increasing the value and profitability of primary production both through direct sales of seed from this high-value crop, and/or through other crops in the farming system benefiting from residual fixed nitrogen. Grower profits could be improved, by purchasing less, increasingly costly, nitrogen fertilizer. It could also benefit the environment. By maximising nitrogen fixation by legumes, the application of nitrogen for sugarcane, banana and other non-legume crops could be reduced, potentially leading to less run-off and better outcomes for Queensland's rivers and reef.

Simplifying the operations involved in growing mung beans may encourage more farmers to incorporate it into their farming system.

Anecdotal reports have suggested that wild rhizobia are as effective as commercial inoculum in promoting growth and nitrogen fixation for mung bean in the Burdekin region of far north Queensland.

The aim of this research was to deliver to Queensland mung bean growers, and potential new growers, information about the benefit or otherwise of commercial inoculation in their growing region. By understand any advantages offered by artificial inoculation of mung bean crops growers will be able to make informed decisions about whether to invest the time and funds in inoculation. Where natural rhizobia provide optimum inoculation, growers may improve efficiency and profitability by sowing without commercial inoculum. Conversely, the research may show that some growers who currently do not use commercial inoculum could significantly improve profitability if they did. Thus, the value and profitability of primary production will increase through increased profitability of mung beans resulting from informing growers about their most efficient and effective choices for crop inoculation, thus "the value and profitability of primary production will increase" (DAF strategic plan). This work addresses the NPIRDEF priority of improving productivity, contributing to a profitable, competitive and sustainable grains industry.

Project Objectives

The objective of this project was to deliver to growers a better understanding of the consequences of artificial inoculation versus natural inoculation of their mung bean crops. This may improve outcomes for growers regarding nitrogen fixation, and profitability of their crops. By maximising nitrogen fixation by legumes the application of nitrogen for sugarcane and other non-legume crops could be reduced, potentially leading to less run-off and better outcomes for the rivers and reef, and grower profits could be improved, by purchasing less, increasingly costly, nitrogen fertilizer. This could benefit exiting mung bean growers, and encourage other growers to incorporate legumes into their farming systems.

The aim of the project was to test the hypothesis that wild rhizobia are as effective as commercial inoculum for mung bean in the Burdekin region of far north Queensland. Based on the findings information can be provided to growers on the effectiveness of commercial inoculation compared with not inoculating and relying on wild rhizobia for nodulation and nitrogen fixation.

Methodology

Whole mung bean plants were collected from two sites in the Burdekin catchment area of north Queensland in November 2015. Uninoculated mung bean plants were collected from a site at 19°46'44.17"S and 147°11'39.84"E, with a black cracking clay soil type, and a cropping history that did not include legumes. The variety at this site was Jade. Inoculated plants were collected from a site at 19°31'54.53"S and 147°26'36.52"E, with a sandy loam soil overlaying delta sand. Mung bean

had been grown at this site 6 years prior, followed by sugarcane. They were inoculated with the recommended type I (CB1015) inoculum. The variety was Crystal. Plants were at the mid- to late-flowering growth stage.

Ten nodules were collected from each of 4 plants from each Burdekin site for testing protein profiles by MALDI-TOF MS (Mabritec). Reference culture plates of CB1015, CB756 (the previously recommended commercial inoculum) and CB1809, used to inoculate soybean. An additional ten nodules from each of 4 plants were used to establish cultures on Yeast Mannitol Agar (Hungria et al 2016)

Nodules from mung bean plants grown in soil from Millmerran on the Darling Downs were collected from plants grown in pots in a glasshouse. The sandy loam soil had been collected from a site at approximately 27°46'29"S and 151°14'49"E which had never grown legumes and had never been inoculated. Five nodules were collected from each of six Jade mung bean plants grown in soil from the inoculated site. Three from pots which had been inoculated with CB1015 and three from pots left uninoculated. Plants were at the early- to mid-flowering stage.

For the glasshouse experiment, five treatments were applied to three varieties, which were replicated in random blocks four times in 60cm pots of sterile sand and vermiculite soil. The varieties were Crystal and Jade mung beans and Regur black gram. Sorghum was included as a ¹⁵N control. Treatments were: uninoculated, uninoculated with added nitrogen (KNO₃ applied to the +N pots was at concentration of 10g/L added 5 ml x 4 and 10 ml x 3 over the experimental period), CB1015 inoculum added, Brady 9 and Brady 5, where the wild inoculum was added to the pots. Inoculum concentration was 8.25 × 10⁹ colony forming units per ml (CFUml⁻¹) for CB1015 and 1.70 × 10¹⁰ CFUml⁻¹ for Brady 9, calculated using the method of Vincent (1970). Concentration for Brady 5 was not able to be determined. Seed was surface sterilised, pre-germinated and planted four per pot. 30ml per pot nutrient solution, as described by Howieson et al (1995) was applied 8 times and water was applied as needed during the 6 weeks of the experiment. All water, nutrients and treatments were applied through a capped tube embedded in the soil and surrounded by inert beads to minimise contamination of pots from bacteria on dust particles.

At six weeks plants were assessed for top weight, top dry weight, root dry weight, number of nodules and nodule dry weight. Nodules were collected for assessment by MALDI-TOF MS, and plant tops sent to the University of New England, NSW, for ¹⁵N analysis.

Nitrogen fixation was calculated using the following formula:

$$\%Ndfa = \frac{\delta^{15}N \text{ of reference plant} - \delta^{15}N \text{ of } N_2 \text{ fixing legume}}{\delta^{15}N \text{ of reference plant} - B} \times \frac{100}{1}$$

B values were -2.5 for mung bean, -1.75 for black gram, based on Unkovich et al (2008) and 7.37 for the reference plant, sorghum, as determined by ¹⁵N analysis.

Each measurement was analysed separately using a linear mixed model. Inoculum and variety were treated as fixed along with their interaction, with a random term fitted for the replicate blocks. To allow for spatial trends within the glasshouse, the residual errors were modelled with an autoregressive variance structure of order 1 in the row or column direction where appropriate.

As a key focus of this experiment was to investigate whether there were differences between the inoculated treatments, the analysis was extended to allow the treatments to be partitioned into uninoculated (uninoculated and uninoculated +N) and inoculated (CB1015, Brady5, and Brady9) treatments. The fixed effects for each measurement in the model described previously were altered to allow the interaction between variety and inoculum within inoculated and uninoculated treatments to

be investigated. An overall inoculum term to allow for the differences between inoculated and uninoculated treatments was also fitted.

Best linear unbiased estimates (BLUEs) were generated from these models for each measurement.

Results

***Bradyrhizobium* strains other than CB1015 were common at the two sites tested in the Burdekin**

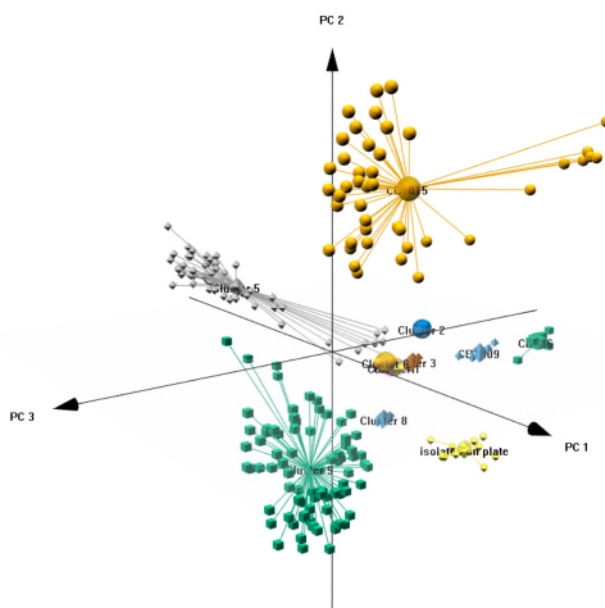
A total of 80 nodules were assessed using MALDI-TOF MS from 10 plants collected from the Burdekin, 40 from the inoculated and 40 from the uninoculated site. The cluster analysis showed 9 separate clusters (Figure 1). Clusters 1, 2, 3, 4, 6 and 8 were represented by one nodule each. Cluster 5 was represented by 29 nodules, Cluster 9 by 42 nodules and Cluster 7, which was identified as including CB1015 was represented by only 3 nodules.

From the uninoculated site most nodules carried *Bradyrhizobium* assigned to Cluster 5 (28 Cluster 5 and 8 Cluster 9). At the inoculated site most nodules carried *Bradyrhizobium* assigned to Cluster 9 (34 Cluster 9, 1 Cluster 5 and 3 Cluster 7 ie CB1015).

Of the 4 plants from the uninoculated site, 2 had nodules colonised mostly by *Bradyrhizobium* from Cluster 5, 1 had nodules colonised exclusively by *Bradyrhizobium* from Cluster 5 and 1 had nodules colonised mostly by *Bradyrhizobium* from Cluster 9. No nodules were identified as carrying CB1015.

Of the 4 plants from the inoculated site, 4 had nodules colonised mostly by *Bradyrhizobium* from Cluster 9, 1 had nodules colonised exclusively by *Bradyrhizobium* from Cluster 9. Of these, 1 plant had 1 of the 10 nodules collected identified as carrying Cluster 7 *Bradyrhizobium* (CB1015), and 1 plant had 2 of the 10 nodules collected identified as carrying Cluster 7 (CB1015).

Figure 1. Principal component analysis of MALDI-TOF MS spectra from *Bradyrhizobium* collected from mung beans from two sites in the Burdekin catchment area of north Queensland. Spectra from Cluster 5 are in grey, Cluster 9 in green and Cluster 7, including CB1015, in gold.



Bacterial cultures were established from nodules from plants from which only Cluster 5 or Cluster 9 *Bradyrhizobium* had been identified. Unexpectedly, when subjected to MALDI-TOF MS analysis, these cultures did not all cluster in their predicted culture group, that is in the cluster where all of the other nodules tested from that plant was assigned (Figure 1).

CB1015 was the only *Bradyrhizobium* found in nodules from mung beans grown in soil from Millmerran.

A total of 30 nodules were assessed using MALDI-TOF MS from 6 plants grown, in a glasshouse, in soil collected from a site that had never grown commercial legumes or been inoculated, at Millmerran on the Darling Downs, Queensland. Three plants had been inoculated with CB1015. No nodules were seen on the 3 plants grown in uninoculated soil. All nodules collected from the inoculated pots were identified as belonging to Cluster 7, that is, as *Bradyrhizobium* sp. CB1015.

The two wild non-CB1015 strains of *Bradyrhizobium* were as effective in promoting mung bean plant growth for most measures as the commercial CB1015 strain.

Five treatments were applied to two varieties of mung bean, Crystal and Jade, and a black gram, Regur, in a four times replicated glasshouse experiment. The treatments were uninoculated, uninoculated with added nitrogen, inoculation with CB1015, inoculation with Cluster 5 *Bradyrhizobium* (Brady 5) and inoculation with Cluster 9 *Bradyrhizobium* (Brady 9).

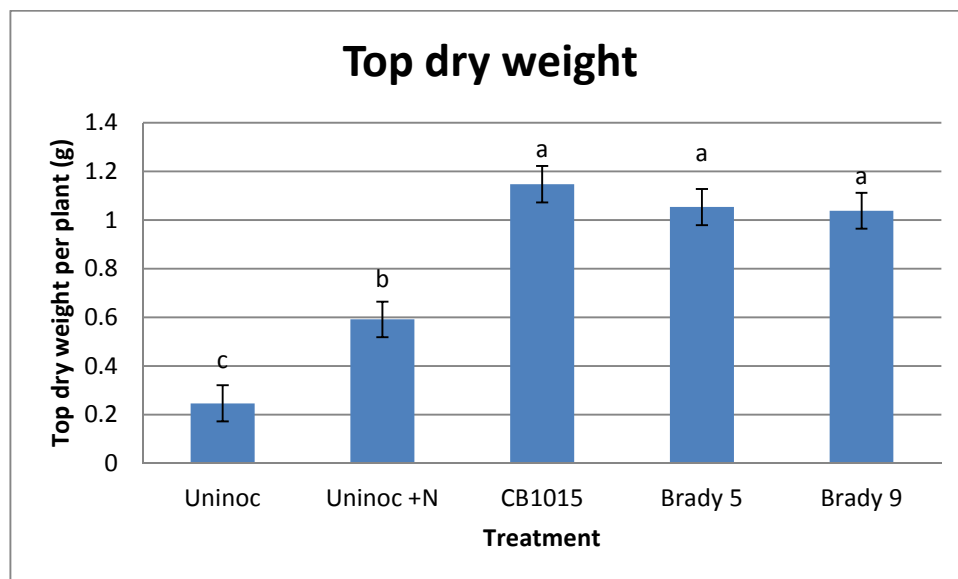
Table 1. Summary analysis of variance. Highlighted statistics are significant at the 5% level.

Treatment	Nodule Count	Top Dry Weight	Root Dry Weight	Nodule Dry Weight	%Ndfa
Inoc	<0.001	<0.001	<0.001	<0.001	<0.001
Variety	0.74	<0.001	0.002	<0.001	<0.001
Inoc:Variety	0.192	0.218	0.007	0.137	<0.001

There were significant differences between the five treatments for all measures (Table 1). Varieties were significantly different for all traits except nodule count. There was a significant interaction between variety and inoculum for root dry weight and for nitrogen fixation, measured as the fractional contribution of N₂ fixation to N uptake (%Ndfa).

As expected, the nitrogen added treatment was significantly different for top dry weight, nodule dry weight and nodule count, compared with the no added nitrogen (Figures 2, 3 and 4). Variety was significant for every measure, except nodule count (Table 1), reflecting the different plant architectures. There was a significant treatment by variety effect for root dry weight, with Jade-AU showing a greater response to the added nitrogen than Crystal, and Regur performing particularly poorly without added nitrogen (Figure 5). There was a significant treatment by variety effect for nitrogen fixation, with Regur sowing virtually no activity in the uninoculated treatment. Many individual plants from the uninoculated treatments were nodulated due to rhizobia contaminants (Figure 4), though they showed generally poorer growth (Figure 2, 3, 4 and 5). This reflects the fact that some individuals were not contaminated and the later development of nodules in those that were, compared with those plants to which inoculum was applied. These contaminant nodules were found to be colonised either by CB1015, Brady 5 or Brady 9 *Bradyrhizobium*.

Figure 2. Top dry weight per plant (g) of mung bean and black gram plants uninoculated, uninoculated with added nitrogen or treated with CB1015, Brady 5 or Brady 9 inoculum, expressed as best linear unbiased estimates (BLUES).



There were no significant differences between CB1015 and either of the wild rhizobia treatments for top dry weight, root dry weight, nodule dry weight or nodule count (Figures 2 – 5). For top dry weight and nodule dry weight there were no significant differences between treatments. For nodule count the Brady 5 treatment had significantly more nodules than Brady 9, but neither was significantly different to CB1015 (Figure 4).

A significant interaction occurred for root dry weight between inoculation treatment and variety (Figure 5). Jade-AU performed similarly within the inoculated treatments, but Crystal and Regur both performed poorer when treated with Brady5.

So, for most measures of plant growth, the wild inoculum was as good as CB1015, though Brady5 was poorer for root dry weight for some Crystal and Regur.

Figure 3. Nodule dry weight per plant (g) of mung bean and black gram plants uninoculated, uninoculated with added nitrogen or treated with CB1015, Brady 5 or Brady 9 inoculum, expressed as best linear unbiased estimates (BLUES).

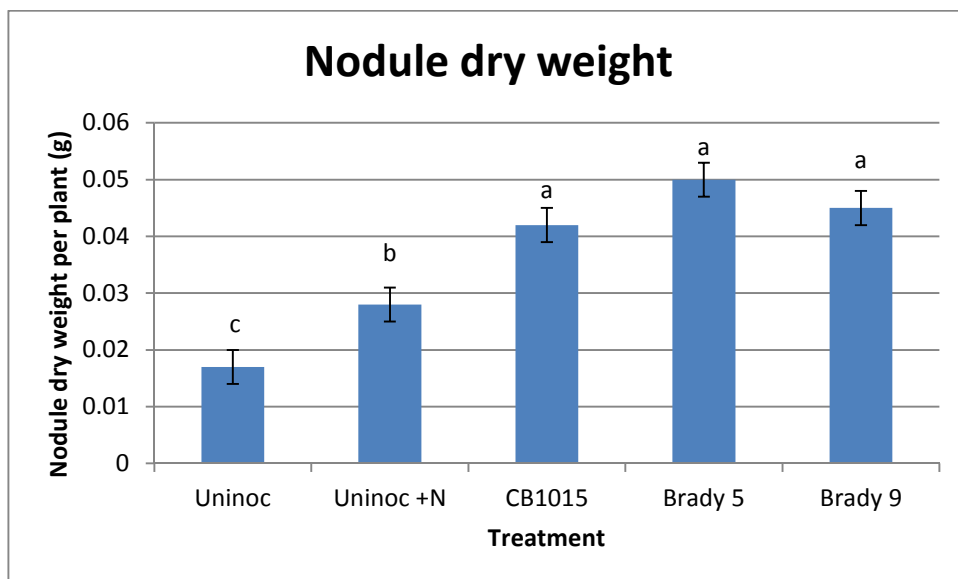


Figure 4. Nodule count per plant of mung bean and black gram plants uninoculated, uninoculated with added nitrogen or treated with CB1015, Brady 5 or Brady 9 inoculum, expressed as best linear unbiased estimates (BLUES).

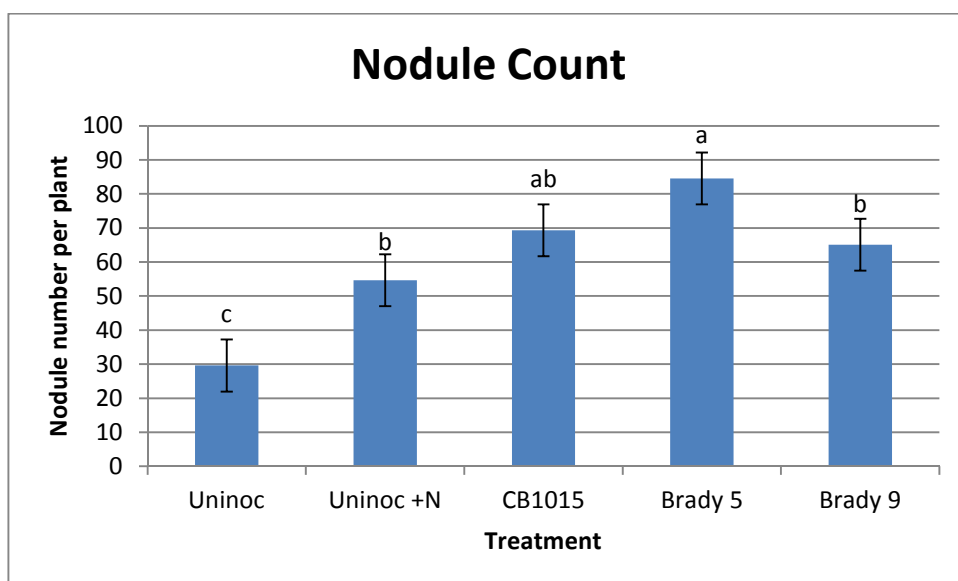
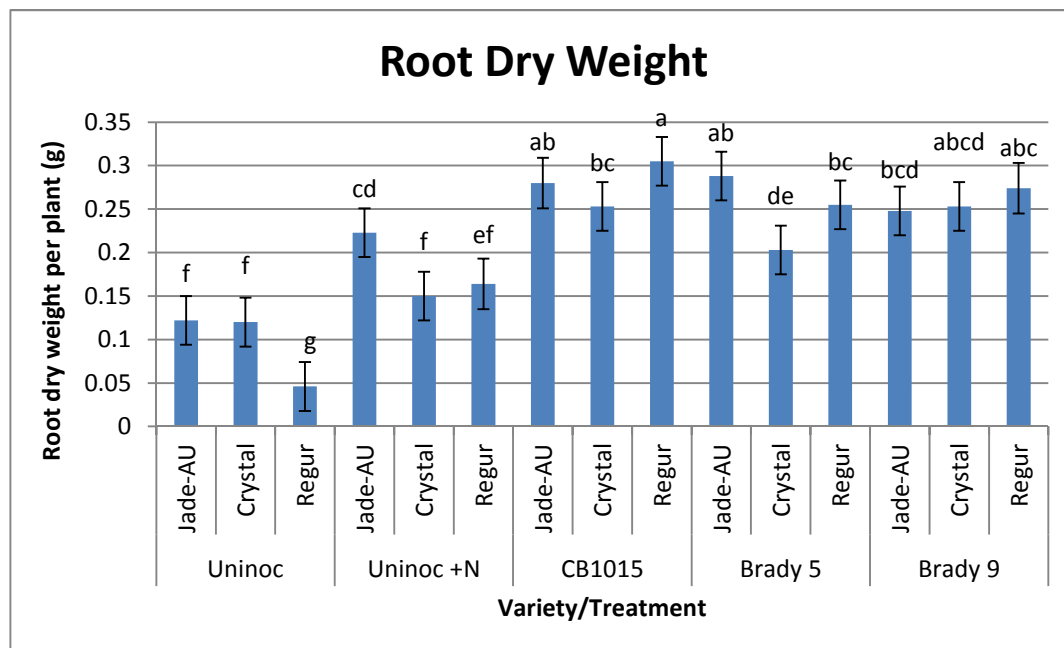


Figure 5. Root dry weight per plant (g) of mung bean and black gram plants uninoculated, uninoculated with added nitrogen or treated with CB1015, Brady 5 or Brady 9 inoculum, expressed as best linear unbiased estimates (BLUES). There was a significant interaction for root dry weight between treatment and variety.

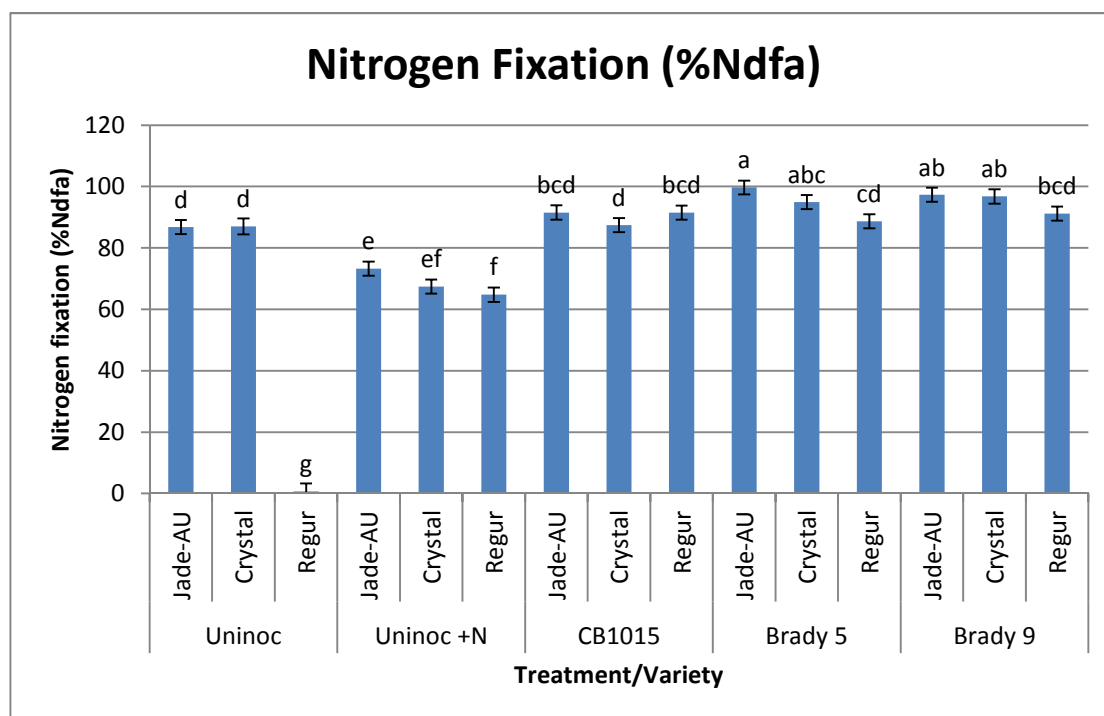


The two wild strains of *Bradyrhizobium* were equally or more effective in promoting nitrogen fixation by mung beans as the commercial CB1015 strain.

There was a significant interaction for nitrogen fixation between treatment and variety. Crystal showed significantly greater nitrogen fixation for each wild rhizobium compared with CB1015. Jade-AU fixed more nitrogen when treated with Brady5, but not Brady9, compared with CB1015. Regur did not fix significantly more nitrogen with any of the three inoculated treatments. Two pots were excluded from this analysis as outliers.

When the inoculated and uninoculated treatments were analysed separately, %Ndfa was the only parameter showing a significant difference between inoculated treatments and there was no treatment by variety interaction for any of the traits (Appendix 1).

Figure 6. Nitrogen fixation of mung bean and black gram plants uninoculated, uninoculated with added nitrogen or treated with CB1015, Brady 5 or Brady 9 inoculum, expressed as best linear unbiased estimates (BLUES). There was a significant interaction for root dry weight between treatment and variety.



Nodules were collected from plants grown in the glasshouse and subjected to MALDI-TOF MS. All nodules were identified as carrying *Bradyrhizobium* from the group with which it was inoculated. That is, they again clustered with their original nodule derived group.

Conclusions/Significance/Recommendations

The hypothesis that potentially wild *Bradyrhizobium* strains are as effective as the commercial CB1015 strain in promoting plant growth and nitrogen fixation in mung beans was largely confirmed. The two particular wild strains investigated did not seem to be significantly superior to the commercial inoculum in promoting plant growth but were superior to CB1015 in fixing nitrogen. The fact that CB1015 was rare, even at the inoculated site, suggests that the wild strains may be out-competing the commercial strain in some environments. Other wild strains may be superior to those we tested. The fact that all nodulated mung beans grown in the Millmerran soil had nodules infected with CB1015 only, suggests that mung bean compatible wild strains of *Bradyrhizobium* are not present in all regions of Queensland.

It would be useful to further investigate these *Bradyrhizobium* strains, particularly with respect to their competitiveness with CB1015 and with other wild strains. It would also be worthwhile sampling sites more widely to understand the distribution of these wild *Bradyrhizobium* and to collect further wild strains to understand the diversity of wild *Bradyrhizobium* in mung bean growing regions of Queensland. The potential for commercial development of one or more of these strains could be investigated if one or more are found to have characteristics significantly superior to CB1015. This would require understanding of how the competitiveness and effectiveness of these strains may be affected by environmental factors such as soil type, temperature and rainfall.

Based on these results recommendations made to growers of mung beans with respect to inoculation in some areas of north Queensland may be reconsidered in the future. However, further investigation of the effectiveness and pervasiveness of these wild rhizobia is required before any major practice change could be recommended.

Key Messages

These results support the belief that wild rhizobia are as effective at promoting growth and nitrogen fixation in mung beans as the commercial strain in some parts of Queensland. However, the application of commercial inoculum is necessary for a healthy crop in other parts of Queensland, and may still be considered a useful “insurance policy” to ensure healthy nodulation of mung bean crops in the Burdekin catchment area

Where to next

This was a pilot study only, and to understand the full implications of inoculation strategies a significantly larger project would be required. Results from the pilot study may be used in an application to suitable funding bodies for a more comprehensive study.

Questions arising from this study include:

- In which regions of Queensland are mung bean compatible wild rhizobium present?
- What factors influence if wild rhizobia are present and which are present?
- Do some of these wild rhizobia out-compete the commercial strain, when it comes to colonising mung bean roots?
- Are some wild rhizobia better at promoting growth and/or nitrogen fixation of mung beans than the current commercial strain?
- What are the native or weed hosts of the wild rhizobium?

Budget Summary

Table 2 – Budget summary

Budget item	Cost (\$A)	Running total
• Laboratory consumables initial purchase	1,531	1,531
• Postage (Australia Post)	93	1,624
• MALDI-TOF MS 1 (Mabritec)	4,471	6,095
• POSTAGE	61	6,156
• MALDI-TOF MS 2	736	6,892
• Postage	103	6,995
• MALDI-TOF MS 3	1,636	8,631
• Postage	20	8,651
• IR-MS (UNE)	900	9,551
• Laboratory consumables replace stores	534	10,085

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Acknowledgements:

This report was prepared by Mandy Christopher, Nikki Seymour, Bethany MacDonald (DAFQ) and Dominik Ziegler (Mabritec)

Appendix 1 – Summary analysis of variance with inoculated and uninoculated treatments analysed separately. Highlighted statistics are significant at the 5% level.

Treatment	Nodule Count	Top Dry Weight	Root Dry Weight	Nodule Dry Weight	%Ndfa
Y	<0.001	<0.001	<0.001	<0.001	<0.001
at(Y, Inoc):Inoc	0.061	0.335	0.059	0.154	0.021
at(Y, Inoc):Variety	0.384	<0.001	0.004	<0.001	0.004
at(Y, Inoc):Inoc:Variety	0.885	0.838	0.193	0.182	0.081
at(Y, Uninoc):Inoc	0.005	<0.001	<0.001	0.014	<0.001
at(Y, Uninoc):Variety	0.052	0.006	0.001	<0.001	<0.001
at(Y, Uninoc):Inoc:Variety	0.225	0.143	0.048	0.09	<0.001