Are mungbean-compatible wild bradyrhizobia more resilient to abiotic stress?

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Abstract

Bradyrhizobia required by mungbean for nitrogen fixation can be impacted by abiotic stresses, reducing nitrogen fixation and yield. Wild bradyrhizobia were collected from ten sites in the dry tropics of Queensland and compared with the commercial strain for performance of inoculated mungbeans, under neutral and acid soil conditions. We found thirteen of the fifteen strains tested promoted growth at least as well as the commercial strain under both acid and neutral conditions. Two significantly outperformed the commercial strain under neutral conditions. This study suggests that gains could be made in mungbean performance through use of better-adapted bradyrhizobia.

Keywords

Legumes, nitrogen fixation, acid soil, biodiversity, genetic resources

Introduction

Legumes, including mungbean, are becoming an increasingly important component of the coastal farming systems of Queensland. They are important in maintaining soil health and reduce the need for the addition of nitrogen fertiliser. Further, they provide additional cash flow, particularly for cane growers, to improve the profitability of farming systems (Garside et al. 2001). Currently, most growers inoculate crops with commercial rhizobia to promote nodulation and nitrogen fixation, but its effectiveness is impacted by abiotic factors, such as soil temperature, moisture, and pH. For mungbean, the recommended inoculum in Australia is the bradyrhizobia species strain CB1015, originally from India (Bullard et al. 2005). In some parts of Queensland, growers believe that wild rhizobia are as effective as commercial strains in promoting nitrogen fixation in mungbean. An earlier investigation found that some wild bradyrhizobia from the Burdekin region are indeed as effective as commercial strains for mungbean and black gram (Christopher et al. 2016).

Better adapted inoculum may improve nitrogen fixation in poorer seasons and allow legumes to be grown in environments currently considered unsuitable, for example in acid soils, improving grower outcomes. By maximising nitrogen fixation, the application of nitrogen for sugarcane and other non-legume crops could be reduced, potentially leading to less leaching of N into waterways, improving grower profits and improving the representation of legumes in rotation.

The aim of this study was to determine if wild bradyrhizobia were (i) as effective as CB1015 in promoting mungbean growth, and (ii) better adapted to acidic soils compared with commercial inoculum.

Methods

Bradyrhizobia collection

Soil was collected from ten locations in north Queensland: four locations on Mt Stuart, two on Castle Hill, and one each at Kalamia, Alva Beach, Rowes Bay and Pallarenda. One of the soil samples from Mt Stuart was associated with a native mungbean, *Vigna radiata* var. sublobata. Soil pH for the various sites ranged from 4.48 to 5.66. Mungbean-compatible rhizobia were lured from the soil using the variety Jade-AU and cultures were established and maintained on yeast mannitol broth and agar, using standard protocols (Vincent, 1970). Three bradyrhizobia cultures originally collected from commercial mungbean crops at Airdmillan and near the Australian Agricultural College in the Burdekin region in 2016 (strains 13, 14 and 15) were also included in the study. These samples were re-cultured from storage.

MALDI-TOF MS analysis and diversity of bradyrhizobia collected

Seventy-eight distinct healthy cultures collected from north Queensland soils or plants were sent to MALDI-ID Pty Ltd for matrix assisted desorption ionization time of flight mass spectrometry (MALDI-TOF MS) analysis. They identified species, where possible, by comparison with their database. The MSP dendrogram function of the MALDI-Biotyper 3.0 was used to perform cluster analysis (Zeigler et al. 2015). Seventeen clades were identified, using the 380-distance level of the MPS cluster analysis to discriminate difference. These groups were used to select thirteen diverse bradyrhizobia strains for testing in neutral and acid soil conditions in the glasshouse. Within groups, selections were informed by diversity of collection site.

Comparison of bradyrhizobia strains under neutral and acid soil conditions

A factorial combination of eighteen treatments by two soil acidities (neutral pH, acid pH) was used in the glasshouse experiment. The experiment had three replicates of each inoculated treatment, four replicates of each of the uninoculated treatments (four with no nitrogen and four with added nitrogen). Treatments were randomised into four incomplete blocks, and latinised with respect to rows and columns.

The eighteen treatments consisted of sixteen strains of rhizobium, one no nitrogen treatment and one added nitrogen treatment. The sixteen rhizobium strains consisted of thirteen of the bradyrhizobia strains from soils collected from north Queensland, two wild rhizobia strains collected in the Burdekin region in 2016, and the commercial bradyrhizobia strain, CB1015. The soil acidity treatments were achieved by applying neutral water (pH 7.40) or acidified water (0.2g/l iron sulphate pH 5.15) for the acid pH treatment.

Two plants were grown in 13 cm diameter pots filled with autoclaved 2:1 sand: vermiculite mix. After planting and applying inoculum, an extra 2 cm layer of autoclaved sand was applied to prevent dust contamination on the soil surface. Plants were watered via a capped PVC watering tube. Extra nutrients, excluding nitrogen, were applied in luxury amounts weekly during plant growth. Nitrogen was added as 10% KNO_3 solution to uninoculated controls. The experiment was conducted in a glasshouse between 2 July and 17 August 2020, under natural light and humidity (25% to 100%) conditions, at temperatures ranging between 6.5°C and 32.5°C.

Plants were assessed for shoot biomass, number of nodules, nodule biomass, nodule weight and root biomass. Linear mixed models were used to analyse the data and pair-wise comparisons of significant effects were further investigated using the protected least significant difference (lsd) test. All tests used 5% level of significance.

Results

Comparison between selected bradyrhizobia for shoot dry weight under neutral and acid conditions

The bradyrhizobia strains differed in their ability to promote the growth of mungbean shoot biomass, measured as shoot dry weight of mungbeans (Fig 1). There was a significant interaction between the strains and acidity ($P = 0.019$) reflecting the varying differences between shoot weight from normal and acidic soils for each treatment.

Under neutral conditions, significant differences were found between strains ($P<0.001$) Thirteen of the fifteen collected strains promoted mungbean growth at least as well as the commercial strain (CB1015). Strains 7 (*B. yuanmingense* collected from Kalamia pH 5.04), 8 (*B. sp.* collected from Alma Beach pH 5.58) and 9 (another *B. sp.* collected from Alma Beach pH 5.58) significantly outperformed the commercial strain CB1015. Strains 4 (*B. sp.* collected from Mt Stuart pH 4.68) and 6 (*B. yuanmingense* collected from a different location on Mt Stuart pH 5.03) were significantly poorer than CB1015. The other eleven strains were not significantly different to CB1015. The applied nitrogen treatment had a significantly greater mean shoot dry weight than the CB1015 treatment under neutral conditions. This was likely to be partly due to the low initial temperatures experienced in the experiment, delaying development of all bradyrhizobia and thus nitrogen fixation. Further, fixation of N is an energetic process requiring more carbon and other input than direct uptake of N.

Under acid conditions, no significant differences were found between performances of strains ($P = 0.41$). All fifteen collected strains promoted mungbean growth, at least as well as the commercial strain (CB1015).

When comparing the neutral and acid treatments, bradyrhizobia strains 7, 8, 9 and 10 (*B. sp*. collected from Castle Hill pH 4.59) and the nitrogen treatment showed significant differences in shoot dry weight. So largely, it was the better performing bradyrhizobia under neutral conditions that had the greatest reduction due tothe acid soil conditions but these strains were still some of better performers overall in the acid soil.

No significant difference was found in shoot dry weight between neutral and acid soil for CB1015. It was the nitrogen treatment that was most affected. We could speculate that was because the added nitrogen was less available under the acid conditions (Mkhonza et al. 2002), or that the presence of bradyrhizobia was buffering the effect of the acid (Ferguson et al. 2013).

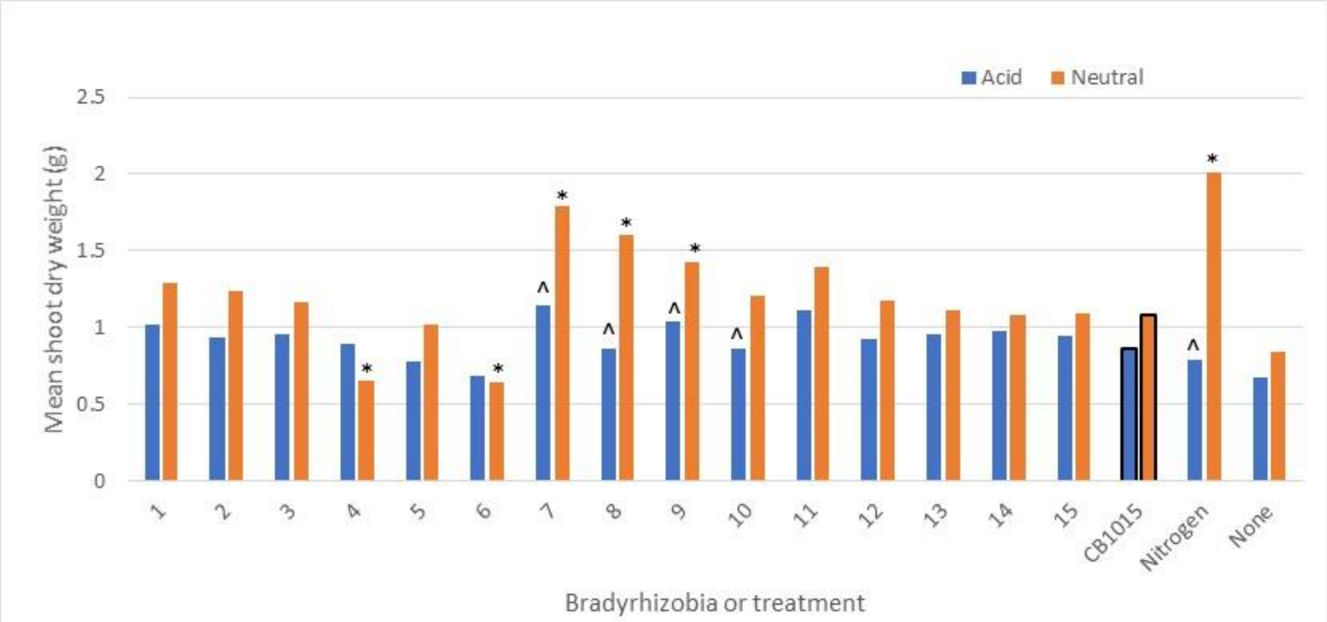

Figure 1. Predictions from top dry weights of mungbeans treated with inoculum from fifteen wild bradyrhizobia and commercial inoculum CB1015, or uninoculated with added or no nitrogen, under acid and neutral soil conditions (blue bars and orange bars, respectively). '*' indicates significant differences to CB1015 under neutral soil conditions. '^' indicates a significant difference between acid and neutral within the same bradyrhizobia treatments. There was a significant interaction with average least significant difference of 0.31g.

Comparison between selected bradyrhizobia for root weight under neutral and acid conditions

For neither fresh or dry root weight respectively were there significant differences between bradyrhizobia strains (P = 0.26, P = 0.06), acidity treatments (P = 0.12, P = 0.11) or their interactions (P=0.99, P=0.61). This suggests that the top weight differences were likely driven by differences in nitrogen fixation, but root weights are not similarly impacted.

Comparison between selected bradyrhizobia for nodulation under neutral and acid conditions

For number of nodules per pot, there was a significant interaction between the sixteen rhizobia strains and acidity (P = 0.0384) (Fig 2a). In the neutral soil, strains 4, 5 (*B. yuanmingense* from Mt Stuart pH 4.48), 7, 13, 14 and 15 were not significantly different to CB1015, with the remainder having fewer nodules per pot. In the acid soil, all but bradyrhizobia strain 6 had significantly fewer nodules per pot compared with CB1015.

For nodule fresh weight, there was also a significant interaction between soil acidity and bradyrhizobia strain (P = 0.028) (Fig 2b). In neutral conditions, nodules from plants treated with bradyrhizobia strains 3 (*B. pachyrhizi* from Mt Stuart pH 4.68) and 10 were significantly heavier than those treated with CB1015. Under acid conditions nodules from plants treated with bradyrhizobia strain 10 were significantly heavier than those treated with CB1015.

Total fresh weight of nodules (data not shown) showed a significant main effect for bradyrhizobia strain/nitrogen and acidity (P<0.0001, P<0.0001 respectively). However, only plants treated with bradyrhizobia strain 3 had a significantly greater total nodule weight compared with CB1015.

A complex relationship is indicated between nodule size, nodule number and shoot dry weight, plus the tolerance of different bradyrhizobia strains to soil acidity.

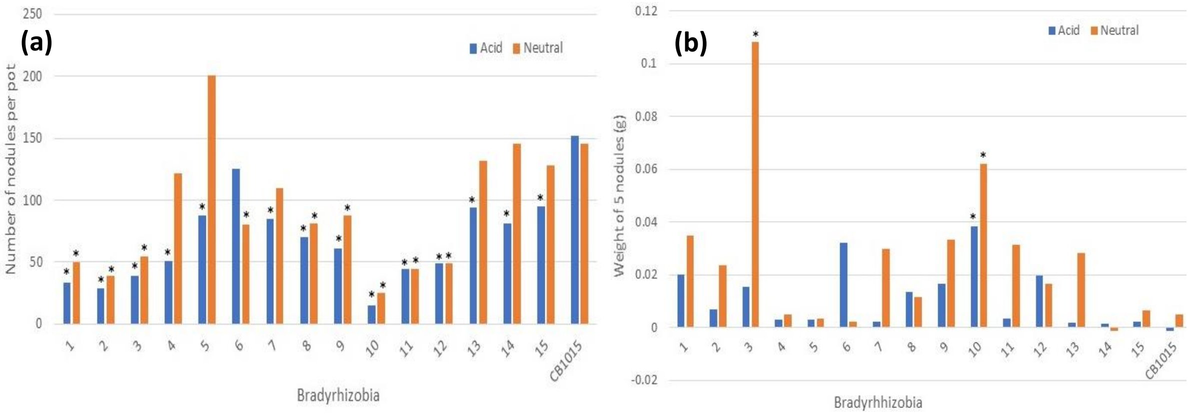

Figure 2. Predictions of (a) number of nodules per pot and (b) fresh weight of five nodules. Both measures produced significant interactions. The bradyrhizobia indicated by '*' are significantly different to CB1015 under the same soil conditions. Lsd values are 50.0 nodules per pot and 0.036 g respectively.

Conclusion

This study suggests that gains could be made in mungbean performance through use of better-adapted bradyrhizobia. A diverse range of mungbean-compatible bradyrhizobia strains were found in a small sample of soils from north Queensland. Some of those were found to be superior to the commercial inoculum CB1015 in promoting mungbean growth under neutral soil conditions. Under acid conditions, bradyrhizobia strains differed in their ability to promote mungbean growth, but no wild bradyrhizobia strain tested here was significantly superior to CB1015. Results from this small survey suggest that further untapped resources of wild bradyrhizobia may be found that could be exploited to improve performance of legume crops in northern Australia.

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