Arbuscular mycorrhizae play key role for mungbeans in low phosphorus soil

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Abstract
Mungbean has a high arbuscular mycorrhizal fungi (AMF) dependency so there is a risk to production if growers do not adequately address phosphorus (P) nutrition. Gaps exist in current understanding of mungbean P nutrient requirements to maximise productivity, particularly if soil AMF levels are low at planting. A glasshouse trial of mungbean (cv. Jade-AU) with factorial combinations of three AMF levels (*NIL*, *LOW* and *HIGH*) and eight P rates, was conducted. All mungbean plants increased in biomass and pod production as P level increased. However, lower AMF levels in the soil meant mungbean plants required higher rates of P to attain similar growth. If AMF levels are low following a long fallow of more than 12 months and/or soil P levels are deficient to low, the application of between 44 and 87 kg P/ha is required to maximise production.

Keywords
Fertiliser, symbiotic fungi, pulse, legume, phosphorus

Introduction
Ensuring adequate phosphorus (P) supply to plants is key for good mungbean production. Arbuscular mycorrhizal fungi (AMF), previously known as vesicular arbuscular mycorrhiza fungi (VAM), form a symbiotic relationship with plant roots to supply them with P and zinc (Zn). Mungbeans have a high mycorrhizal dependency (Thompson et al. 1997). Dry weather causing enforced long fallows, can reduce AMF levels in the soil. This is a possible risk to mungbean production if growers do not adequately address P nutrition. Recent experiments in the northern grain's region have provided only limited information of mungbean responses to deep-placed P. Gaps remain in our understanding of P nutrient requirements to maximize productivity of mungbeans, particularly if mycorrhizal levels are low. It is expected that mungbeans will respond differently to applied P at different levels of mycorrhiza colonisation; with higher soil levels of P required for growth when AMF are low or not present. The Grains Research and Develop Corporation, Department of Agriculture and Fisheries and New South Wales Department of Primary Industries funded Mungbean Agronomy project undertook research to investigate this topic.

Methods
A glasshouse trial under controlled temperature conditions was established using a randomised block design of a full factorial of three AMF levels (*NIL*, *LOW* and *HIGH*) and eight phosphorus rates (Table 1), with four replicates of each treatment.

Table 1. Rates of applied phosphorus into pots showing equivalent in kg P/ha.

Applied phosphorus (mg/kg)	0	5	10	20	40	80	160	320
Calculated equivalent rate of applied phosphorus (kg/ha)	0	22	44	87	174	348	696	1392

A vertosol soil from a cropping property south-east of Chinchilla Queensland, was used for this experiment due to its low P status (bicarbonate-extractable or Colwell- P of 16 mg/kg for surface 0 - 10 cm, 3 mg/kg for 10 – 30 cm). The top 0 – 10 cm of soil was removed and 10 – 30 cm layer of the profile was used for the trial. This site had recently grown sorghum, so moderate to high levels of AMF were expected in the soil. A PREDICTA®B test (South Australian Research and Development Institute, Urrbrae, South Australia) showed that 34 kDNA copies of AMFa/g soil and 1 kDNA copies of AMFb/g soil were present in the 10 - 30 cm layer at the time of soil collection.

Soil was sieved to <1 cm particle size. Untreated soil was reserved for use as the *HIGH AMF* treatment. The remaining soil was slightly moistened with water and then heated at 60 ºC for 24 hours to sterilise the soil. This soil was used for the *NIL AMF* treatments. The *LOW AMF* treatment was then created by combining

90% *NIL AMF* soil with 10% *HIGH AMF* soil. For each of the three AMF treatments 8 kg oven-dried equivalent of soil was mixed with each level of P in solution and then mixed thoroughly to give an even concentration of P throughout the soil. The soil was then placed into large pots (8 L capacity).

Four pre-germinated mungbean (cv. Jade-AU ϕ) seeds were planted on the 31 October 2019 and were thinned to two plants per pot ten days later. All seeds were inoculated at planting with a solution of *Bradyrhizobia* sp. (strain CB1015). The mungbeans were grown for 47 days, during which time they were supplemented with non-limiting levels of basal nutrients applied in solution to the soil surface. The solution contained magnesium sulfate ($MgSO_4$), potassium sulfate (K_2SO_4), calcium sulfate ($CaSO_4$) and trace elements (iron, zinc, copper, manganese, molybdenum and boron).

Glasshouse temperatures were maintained between 20ºC and 40ºC throughout the trial. No additional nitrogen or phosphorus was added during the trial. The plants were watered using deionised water as required. Above ground biomass was measured by cutting the plants just above the soil surface at harvest, each sample was dried at 60 ºC for 72 hrs. and then weighed. Roots were collected by wet sieving through a 500µm sieve to remove soil, and then roots dried at 60 ºC for 72 hrs before weighing to measure biomass. A subsample of root (0.25-0.3 g) was collected before drying and then stained using trypan blue in lactic acid (modified from Phillips and Hayman 1970). Roots were assessed by the grid intersect method (Giovanetti and Mosse 1980) to determine per cent root length colonised by AMF.

Data were analysed using linear mixed models, and significant effects were further explored using the protect least significant difference (lsd) test. The level of significance was set at 5% for all tests.

Results

Mungbean growth, measured by the above ground biomass, showed a significant interaction between P rate and AMF (Fprob=0.002). The above ground biomass showed significant increases from 0 mg P/kg with the addition of 5 - 20 mg P/kg (22 – 87 kg P/ha) to the soil (Figure 1). With no added P, plants at all three levels of AMF were not significantly different in biomass. When soil P was very low i.e., at the 0 mg P/kg rate (just 3 mg available P/kg (bicarbonate extractable P)), the level of AMF made no difference to plant growth. At 5 and 10 mg P/kg, the plants with *NIL AMF* were significantly lower in biomass than those with *LOW* or *HIGH AMF* soil levels, hence the AMF allowed a response to the added P that was not seen at the rates of 20 mg P/kg and above. There was no significant difference in response to AMF above 20 mg P/kg rates until the P level reached the highest rate of 320 mg P/kg, where growth at the *LOW AMF* level was significantly higher than *NIL* and *HIGH AMF*. Higher AMF levels may start to drain carbohydrates from the plant at the higher rates of phosphorus, particularly in a pot. This is because the plant doesn't need AMF to assimilate P but if it is still supporting the AMF in relation to energy, then it may become a burden on the plant's resources and ultimately affect biomass production.

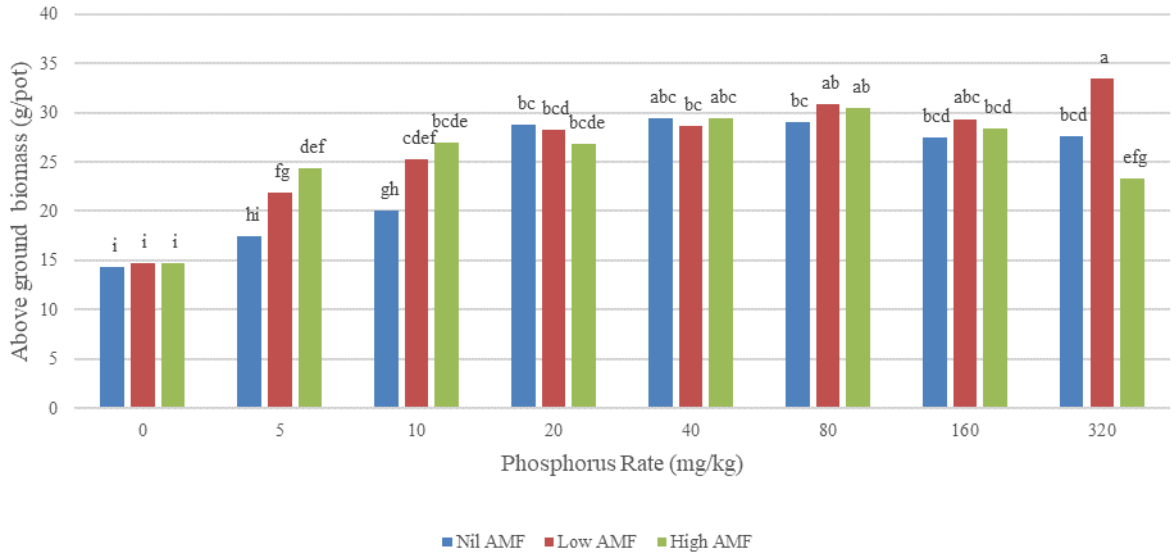

Figure 1. Above ground biomass including pods (biomass/pot of 2 plants) response of mungbean (cv. Jade-AU$^{\Phi}$) to applied phosphorus at different levels of AMF. Inoculated with rhizobia (CB1015). Means with the same letters are not significantly different at the P = 0.05 level.

Root weights recorded for each treatment showed a similar trend to the above-ground biomass results with a significant P rate x AMF statistical interaction (Fprob=0.05) i.e., no response to AMF level at 0 mg P/kg but a significant increase in root weight due to *HIGH AMF* when 5 or 10 mg P/kg was added. From 20 mg P/kg upwards, the plant roots were not significantly affected by levels of AMF until the reduction in root weight at 320 mg P/kg at high AMF levels (data not shown).

The AMF colonisation levels taken at 47 days (represented by mycorrhiza root length) did show significant differences between colonisation levels for *NIL*, *LOW* and *HIGH AMF* treatments, however showed no clear trends across P rates (Figure 2). It is hypothesised that the differences in biomass accumulation (Figure 1) observed under low P conditions could be attributed to the heat treatment reducing (but not eliminating) the AMF propagules to a point that still impacted on the level of P uptake in the early stages of plant development. Colonisation levels then caught up over the course of the experiment such that *NIL*, *LOW* and *HIGH AMF* treatments were found to be equal at the time of removing the plants, except at the highest rate of applied P.

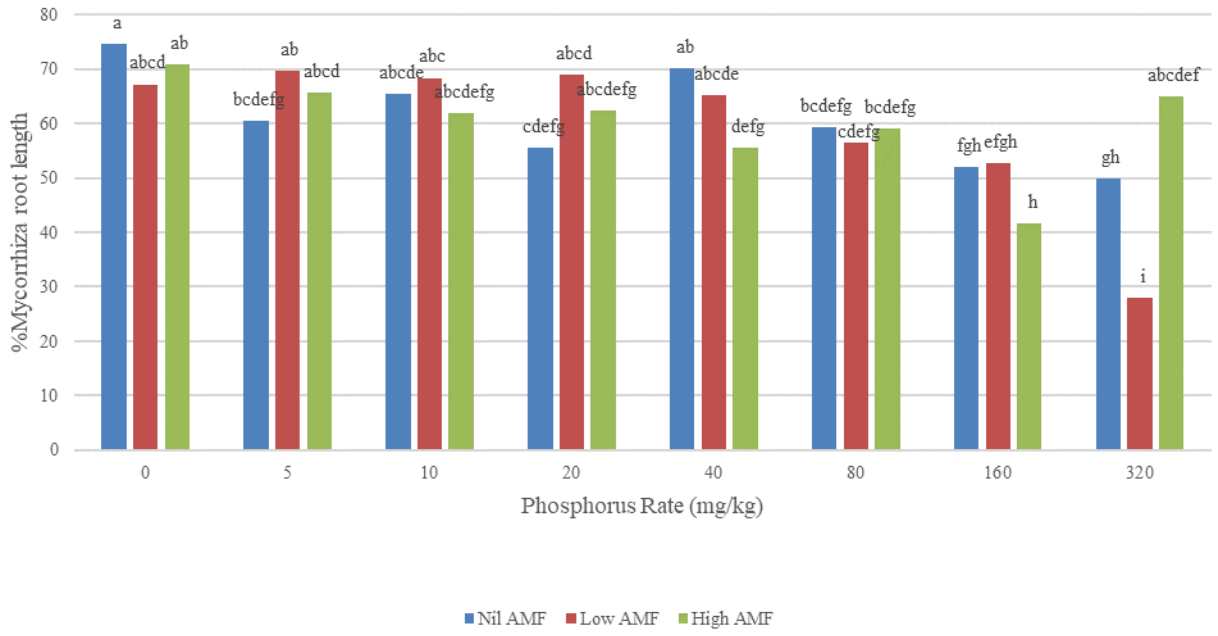

Figure 2. Percentage of Mycorrhiza in roots of mungbeans (cv. Jade-AU$^{\Phi}$) response to applied phosphorus at different levels of AMF. Inoculated with rhizobia (CB1015). Means with the same letters are not significantly different at the P = 0.05 level.

Alternative methods such as steam sterilisation of the soil are recommended to completely kill AMF propagules but were not available to use at the time of setting up this trial. The slightly lower (not significant) colonisation levels combined with the lower root weights at 5 and 10 mg P/kg is a possible reason for the lower biomass measured for the *NIL AMF* plants and the relative increase in biomass for *LOW* and *HIGH AMF* treatments at these two P levels. The results show that as the P rates increased above 40 mg P/kg, the percentage of AMF in the roots tended to decrease, however not significantly different until the 160 mg P/kg rate. This indicates that as P rates increased, the mungbeans relied less on AMF to extract P.

The weight of pods (Figure 3) only showed a significant main effect of P rate (Fprob<0.001), which followed a similar trend to above ground biomass (Figure 1). As P was added at 5 and 10 mg P/kg pod weight increased significantly above the 0 mg P/kg with slightly higher increases up to 80 mg P/kg. Pod number showed a significant interaction between AMF levels and P rate (Fprob=0.006) with *HIGH AMF* giving significantly more pods at the 40 and 160 mg P/kg levels (data not shown). Although this trial was not taken through to maturity, pod number and weight were used to indicate yield. It could be hypothesised that to maximise yield, mungbeans need an available P level of around 40 mg P/kg, The addition of *HIGH AMF* at

low levels of P assisted the mungbeans P uptake however above 20 mg P/kg the mungbeans were able to access P directly from the soil with no impact to growth. Therefore, at low levels of soil P, AMF levels need to be taken into consideration when planning to plant mungbeans as P access will be restricted if there are limited AMF populations.

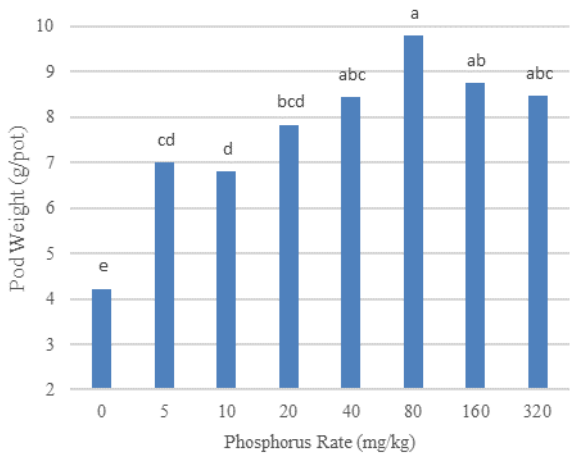

Figure 3. Weight of pods greater than 5 cm in each pot (2 plants) of mungbeans (cv. Jade-AU Φ). Inoculated with rhizobia (CB1015). Means with the same letters are not significantly different at the P = 0.05 level.

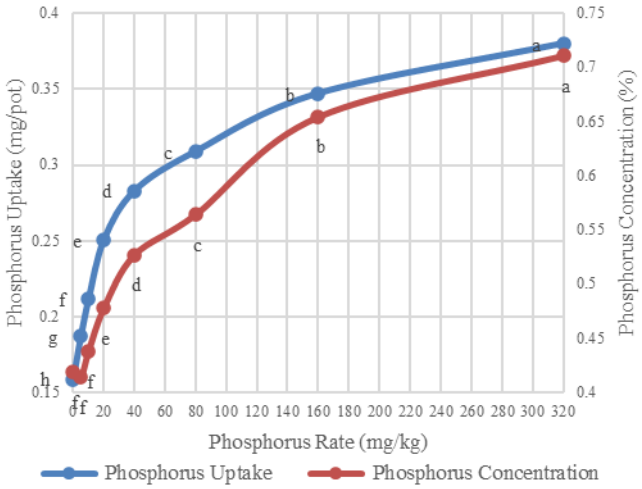

Figure 4. Phosphorus concentration (%) and uptake (mg per pot) of mungbeans (cv. Jade-AU Φ) in response to increasing levels of applied phosphorus averaged over at different levels of AMF. Inoculated with rhizobia (CB1015). Means with the same letters are not significantly different at the P = 0.05 level.

Plants continued to significantly increase in P concentration and P uptake as applied P increased up to 320 mg P/kg (Figure 4), with both showing no significant interaction but significant main effects of AMF level (Fprob=0.019 and 0.043 respectively) and P rate (Fprob<0.001 for both).

Plants in all pots were well nodulated, with no difference in nodule scores between treatments, indicating that nitrogen was not limiting biomass or yield

Conclusion

These results show applying P to mungbeans does increase biomass production and pod weight, particularly when AMF levels are low. Phosphorus concentration and uptake increased significantly for each level of phosphorus up to 320 mg/kg. Whilst this trial couldn't show differences in levels of AMF colonisation by the end of the experiment, the soil heating may have reduced AMF levels so that colonisation was slower at the start of the experiment in the *NIL AMF* treatments. This in turn did show a significant contrast to the plants growing in the *LOW* or *HIGH AMF* treatments in the low P level soil (5 and 10 mg P/kg).

This trend is akin to mungbeans being grown after a long fallow where AMF levels may be low and so colonisation is reduced in the early crop stages. If arbuscular mycorrhizal fungi levels are low at planting and/or soil phosphorus is low, consider applying a higher rate of phosphorus fertiliser (approximately 10 mg P/kg or 44 kg P/ha or above) to improve growth and production. The immobility of P in the soil means fertiliser placement and AMF levels are important for root access to P. Growers need to understand their soil P levels and the cropping history of the paddock to accurately develop fertiliser programs for their mungbean crops.

References

Giovanetti M, Mosse B (1980). An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots pp. 489-500. New Phytologist

Phillips JM, Hayman DS (1970). Improved procedures for clearing roots and staining parasitic and versicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Vol. 55 pp. 158-161, Department of Soil Microbiology,

Thompson JP, Bowman R, Seymour NP, Peck DM and Clewett TG (1997) VAM boosts crop yields. The natural way to healthy crops. Farming Systems Institute Crop Link Research Focus 8pp

Government of South Australia, Department of Primary Industries and Regions, PredictaB https://pir.sa.gov.au/research/services/molecular_diagnostics/predicta_b