Constraints to improving nitrogen fixation in chickpea in the Central Dry Zone of Myanmar

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Abstract
Myanmar is one of the largest pulse-producing nations, with chickpea being an important export crop. Despite this, there is very little knowledge of the extent to which soil rhizobial populations contribute to N_2 fixation by crop legumes. Chickpea rhizobial populations were estimated in 20 soils in the Central Dry Zone of Myanmar and seven field experiments were established to determine whether the combined inputs of phosphorus (P) fertiliser and rhizobial inoculation improved chickpea yields. Chickpea rhizobial populations in the sampled soils were generally small, indicating potential for increasing N_2 fixation through inoculation. Although the combined inputs of P fertiliser and inoculation increased nodulation and grain yield at some sites, the impacts were moderate and likely to have been constrained by effective agronomic management, multiple nutrient deficiencies and crop weeds and diseases.

Keywords
Legume, rhizobia, phosphorus, natural abundance, most probable number.

Introduction
The farming communities in Myanmar are poor, with many families spending 70% of their income on food. Approximately 70% of Myanmar's population is based in rural areas (Ministry of Immigration and Population 2015) and about half of those in the Central Dry Zone (CDZ) are engaged in crop and livestock farming activities as a key source of income (LIFT 2012). Improving agricultural systems in the CDZ is likely to benefit the livelihoods of rural households and contribute to poverty alleviation.

Myanmar is the world's second largest exporter of pulses, i.e. food legumes, after Canada. Myanmar farmers grow green gram (*Vigna radiata*), black gram (*Vigna mungo*), pigeon pea (*Cajanus cajan*), groundnut (*Arachis hypogaea*), chickpea (*Cicer arietinum*) and other legumes across an area of 4.4 million hectares, in rotation with rice, sesame, maize and other crops. Export markets are principally to India, with expanding markets in Vietnam and China. Pulses are widely grown in Myanmar's CDZ by small-holder farmers, typically with minimal inputs.

Chickpea is essentially grown as an export crop for the Indian market, and areas planted and production have increased rapidly since its introduction in the mid 1990's to the current 0.5 million tonnes annually (http://www.fao.org/faostat/en). Chickpea is grown on Vertosols in the Sagaing, Mandalay and Magwe regions of the CDZ, often in rotation with rice. Despite the importance of chickpea and other legumes to Myanmar, the use of rhizobial inoculants is extremely limited. The history of inoculation of chickpea by farmers in Myanmar is poorly understood and there is currently no established supply chain for rhizobial inoculants. Although chickpea plants are generally nodulated in the absence of inoculation, there is little information on the genetic diversity or effectiveness of chickpeas rhizobia in the soils of the chickpea-growing areas and almost no knowledge on how those rhizobia might have initially established. Finally, there has been an absence of research to determine if grain yields of chickpea could be increased through application of inoculants containing highly-effective rhizobia.

The research reported here aims to 1) quantify the abundance and symbiotic effectiveness of chickpea rhizobia in Myanmar soils, and 2) test the extent to which existing soil rhizobia contribute to N_2 fixation and 3) determine if inoculation with highly-effective rhizobia might improve N_2 fixation and grain yields.

Methods

Determination of rhizobial estimates in Myanmar soils

Field soils, 0-10 cm depth, in 20 locations in the Sagaing, Mandalay and Magway regions of the CDZ of Myanmar were collected and the number of rhizobia was determined using the most probable number of rhizobia (MPN), as described by Brockwell et al. (1963).

Field experiments

Field experiments were established at seven locations (Zaloke, Pankone, Myittha, Myingyan, Tatkone, Sebin and Kyaukse) in the CDZ. Soils at the sites were neutral to slightly alkaline Vertosols and the previous cropping history was chickpea and rice, except for Myingyan, which was sown to jatropha (*Jatropha curcas*). The experimental design was a 2 x 2 factorial comprising (+/-) inoculation using the Australian commercial chickpea strain (CC1192) applied as a slurry to seed just before sowing and (+/-) phosphorus (P) fertiliser (20 kg P/ha as triple super phosphate). Plots were arranged in complete randomised blocks with 5 replicates. Phosphorus was drilled into furrows next to the seed rows at approximately 10 cm depth. Chickpeas Yezin 6 (ICCV 92944 desi type) and Yezin 8 (ICCV97314 kabuli type) were hand sown in rows that varied from 30 to 37.5 cm. Plots were hand-weeded and fungicides were used as necessary to prevent disease.

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Plant sampling and harvest

Chickpea crops were sampled at early pod fill, approximately 80 days after sowing (DAS), except at the Kyaukse site, where plants suffered from a soil borne disease. Plant biomass was measured from 1 m^2 quadrats/plot. Plant N_2 fixation was assessed at the Myittha site using the ^{15}N natural abundance method, as described in Denton et al. (2013). Plants were excavated and nodules assessed using the methods of Corbin et al. (1977). Grain yield was determined at approximately 100 DAS by hand harvesting plants and threshing to extract grain.

Results

Rhizobial populations in the 20 chickpea-growing fields in the CDZ were generally low with estimated numbers ranging from $\log_{10}$ 0.7 to 4.5/g soil (equivalent to 5-32,000/g soil) (Figure 1). At 17 of the 20 sites, the chickpea rhizobial populations were sufficiently small that increased grain yields would be expected as a result of inoculation, according to Thies et al. (1991).

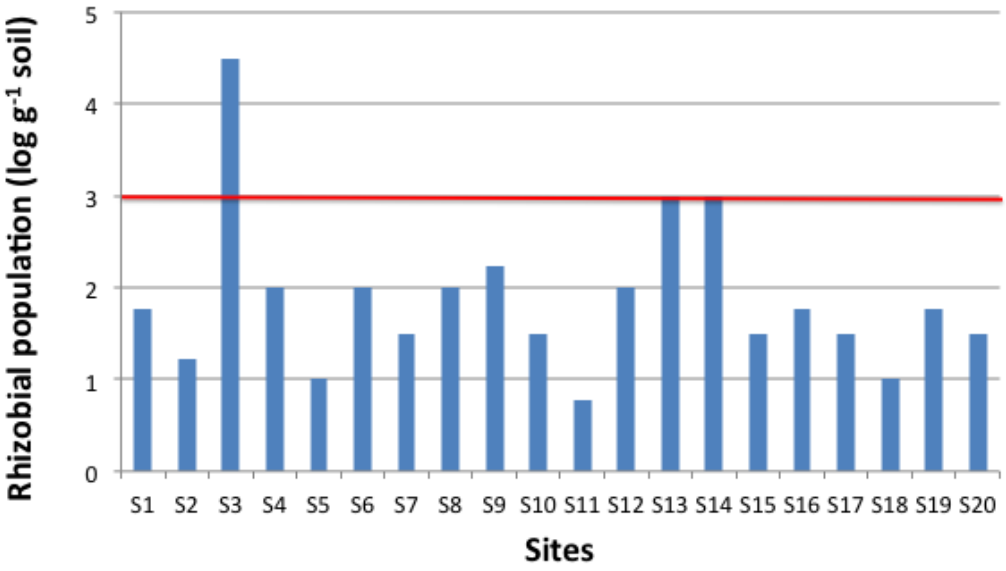

Figure 1. Chickpea rhizobial numbers in the soil (top 10 cm) at 20 sites in chickpea-growing areas in the Central Dry Zone of Myanmar. Populations were estimated using the most probable number (MPN) procedure as outlined in Brockwell 1963. The threshold soil rhizobial population above which an inoculation response of improved yield would not be likely (Thies et al. 1991) is indicated by the red line.

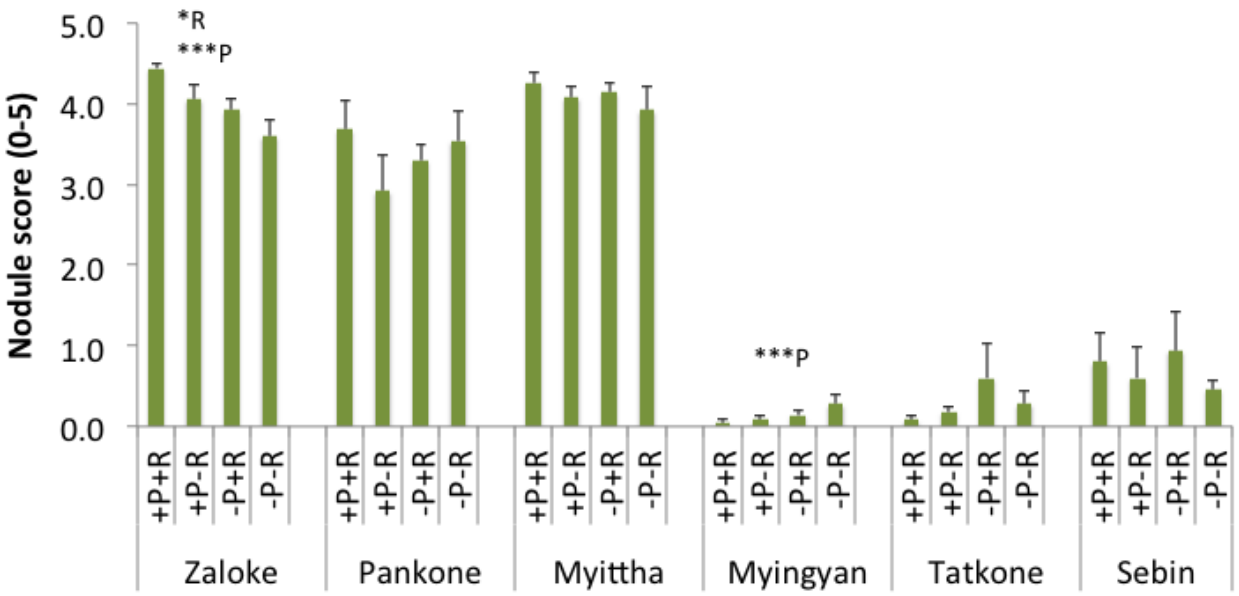

Figure 2. Nodulation scores of chickpea grown at six locations in the Central Dry Zone of Myanmar and treated with factorial combinations of fertiliser P and rhizobial inoculation with strain CC1192. Nodulation scores are based on Corbin et al. (1977). Significant differences are indicated for either inoculation with rhizobia (R) or fertiliser phosphorus (P), where * *P*<0.05, ** *P*<0.01, * *P*< .001.**

Chickpea nodule scores were influenced by site, with more nodules/plant at Zaloke, Pankone and Myittha, and reduced nodule numbers at Myingyan, Tatkone and Sebin (Figure 2). Addition of P increased nodule scores significantly at Zaloke and reduced nodule scores at Myingyan, while inoculation increased nodule scores at Zaloke. There were no interactions between inoculation and P addition at any site.

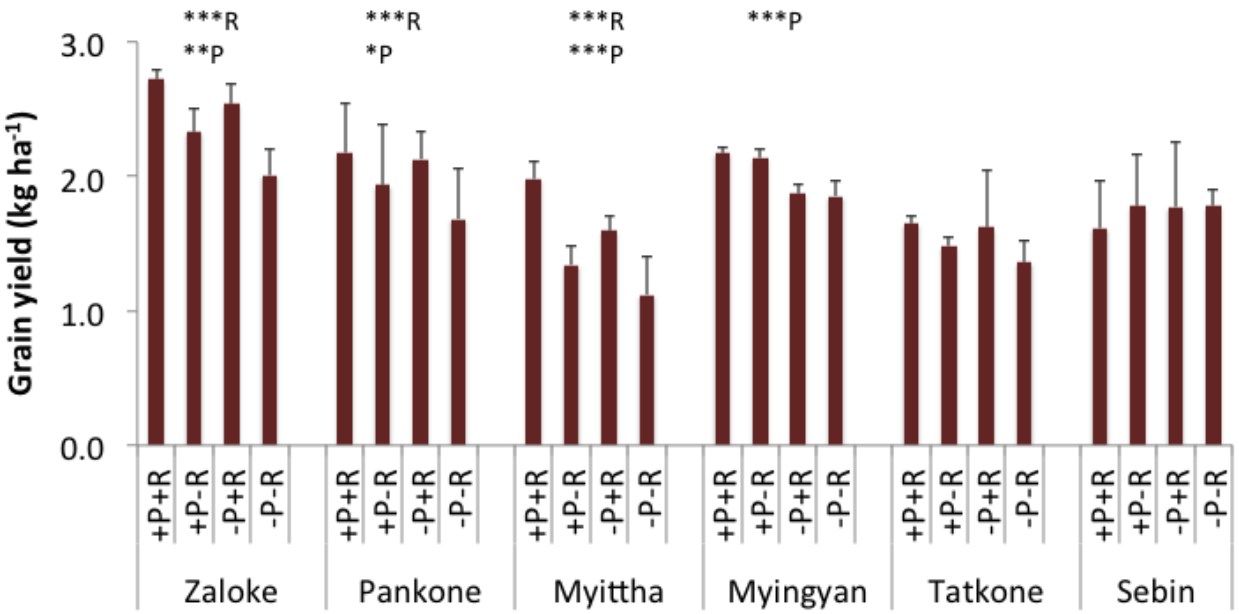

Figure 3. Grain yields of chickpea grown at six locations in the Central Dry Zone of Myanmar and treated with factorial combinations of fertiliser P and rhizobial inoculation with strain CC1192. Significant differences are indicated for either inoculation with rhizobia (R) or phosphorus addition (P), where * *P*<0.05, ** *P*<0.01, * *P*< .001.**

Grain yield was increased by both fertiliser P and inoculation, but not their interaction (Figure 3). At Zaloke, Pankone and Myittha, P and inoculation increased yield, while at Myingyan there was only a response to fertiliser P. There was no influence of either inoculation or P on grain yield at the Tatkone and Sebin sites.

The proportion of nitrogen fixed (%Ndfa) at Myittha indicated a strong differentiation between $\delta^{15}N$ from chickpea relative to that of reference plants (Figure 4). The calculated %Ndfa values were between 82 and 83%. There were, however, no treatment effects from either fertiliser P or inoculation. Our values are greater than those estimated by Herridge et al. (2008) of 63% and within the range of 41- 90% Ndfa reported by Unkovich et al. (2010). Our values indicate that chickpea at the site were highly dependent on N_2 fixation and it was likely that they did not have access to significant mineral N, which can reduce these values (Turpin et al. 2002).

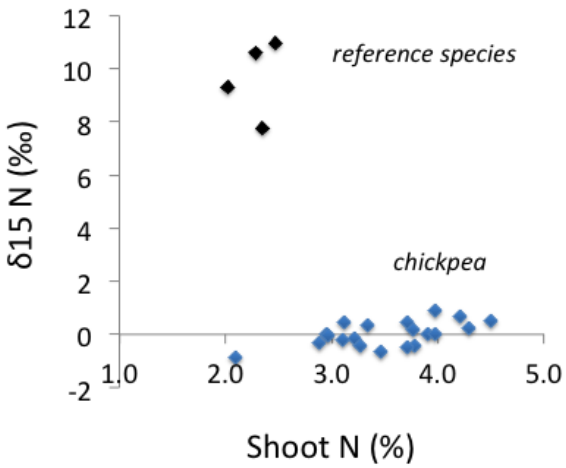

Figure 4. Shoot %N and $\delta^{15}N$ from chickpea and reference plants, used to calculate the proportion of chickpea N at the Myittha site derived from N_2 fixation. Chickpea values represent the fertiliser P x inoculation treatments while reference plants were broadleaf weeds growing at the site.

Conclusion

Chickpea rhizobia were recovered from all 20 sampled sites in Myanmar's CDZ, albeit at generally very low numbers. Nodulation of chickpea was likely to have been influenced by soil type and previous cropping history as there was little impact of inoculation, despite the low populations of chickpea rhizobia observed in the soils of the region. Grain yields were increased by P addition and inoculation at some sites. The %Ndfa estimates at the one site, Myittha, were not affected by the inoculation and fertiliser P treatments. Additional work suggests that multiple nutrient deficiencies and limited crop protection may be impacting chickpea production in the CDZ, and on-going work will address this issue.

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