Understanding the trouble with N_2 fixation in beans (*Phaseolus vulgaris*)

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Introduction

Phaseolus vulgaris (bean) is an important component of many diets in the world. In Sub-Saharan Africa (SSA) , it is the main source of dietary protein. However, average annual yields in SSA are only a fraction of potential due to nitrogen-deficient soils among other factors. Beans , like most legumes, are able to make combined nitrogen from the inert N in the air in association with bacteria called rhizobia. Whereas inoculation of beans with rhizobia has been done by farmers for decades to enhance yields, challenges with erratic responses to inoculation still persists. Some of the reasons suggested to cause this problem are inherent bean nitrogen-fixation limitations and strain competition where elite inoculant strains are out-competed by ineffective local strains for nodulation. This study will evaluate these factors in trying to achieve consistently high levels of nitrogen fixation in beans in Kenya.

Hypothesis One:

Kenyan soils harbor diverse indigenous and/or naturalized rhizobia strains effective in fixing N with beans.

Study approach

-Isolate strains from root nodules on beans from the main bean growing regions in Kenya and identify their phylogeny by multi-loci sequence analysis (MLSA).

-Screen isolates for N fixation on important Kenyan bean cultivars in an aseptic N-free vermiculite system in a glasshouse and compare with beans fed with different rates of inorganic N (KNO_3)

Preliminary results

-Sixteen isolated strains showed there is a genetically diverse rhizobia population in Kenyan soils (Figure 1). The strains are distributed in 3 broad groups among other rhizobia in GenBank based on 16 S rRNA sequences. Some strains GM146 (NAK 354) , GM2 (NAK 210), GM176 (NAK 384) and GM79 (NAK 287) clustered on unique clades and may represent novel undescribed organisms.

->100 strains are being screened for effectiveness alongside nitrogen demand experiments (Figure 2). Expected outcomes are shown in Figure 3 as scenario A and B. Scenario A confirms highly effective symbiosis while scenario B alludes to a suboptimal relationship which would require further investigation. Sections of nodules on beans inoculated with NAK 387 show normal determinate nodule structure with central infected cells interspersed with uninfected cells (Figure 4).

Figure 2. Beans growing in Nitrogen free systems. Left two rows inoculated with NAK 387. Right row uninoculated.

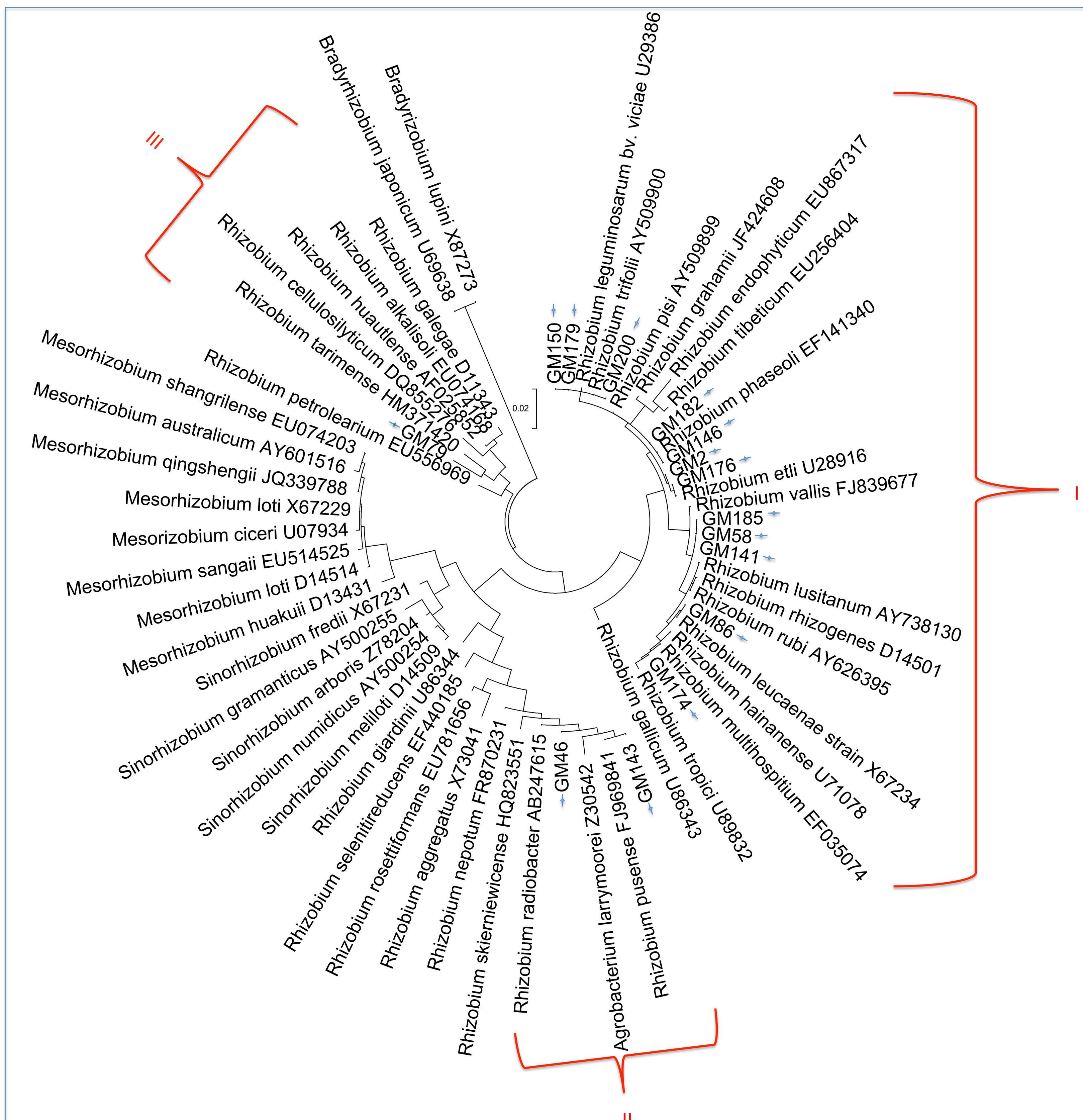

Figure1. Molecular phylogenetic analysis of a subset of study strains by Maximum Likelihood method in MEGA5 (Tamura et al., 2011)

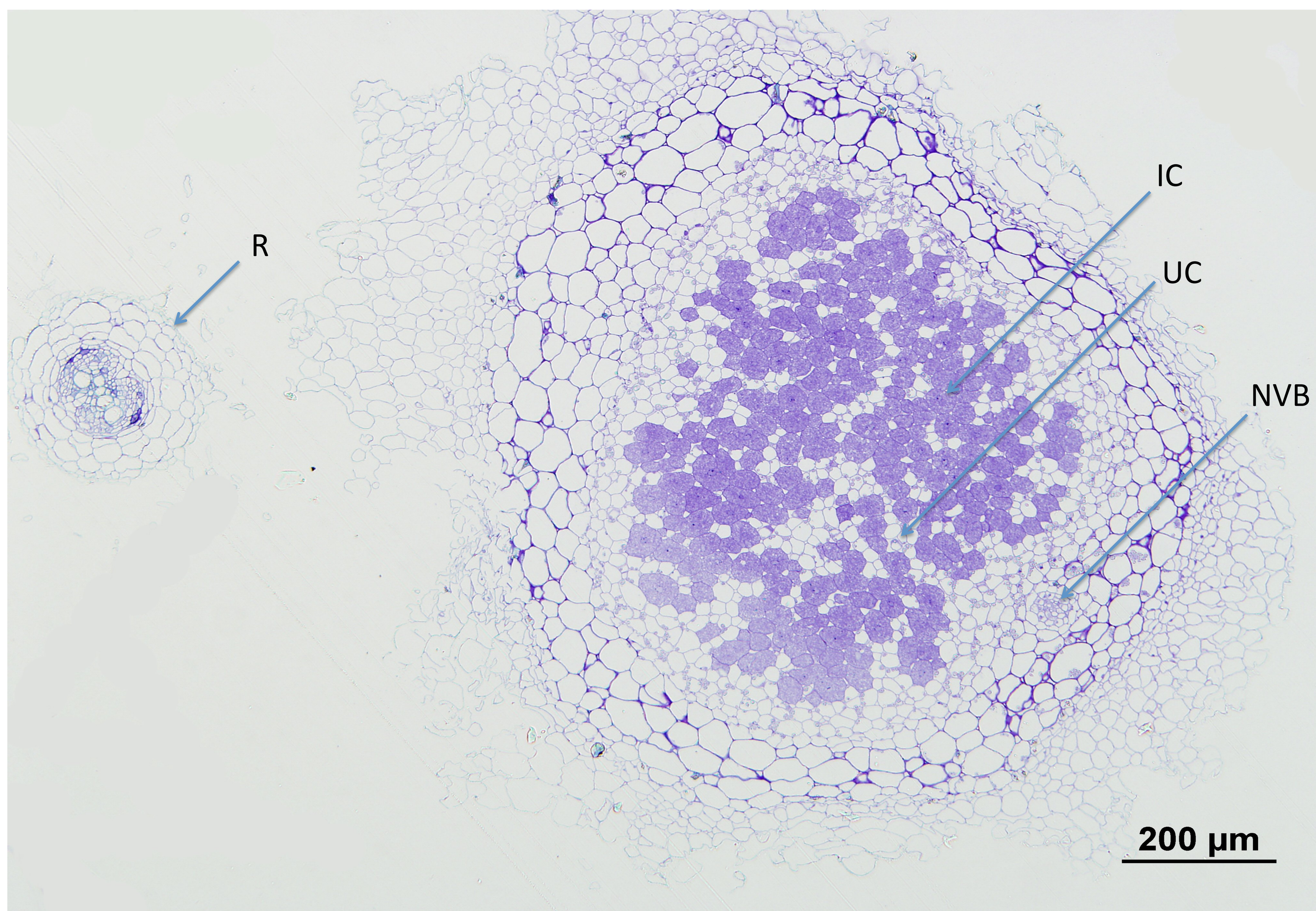

Figure 4. Nodule section of a bean root nodule well occupied by GM79 bacteroids Abbreviations: R , root; IC, infected cell; UC, uninfected cell; NVB, nodule vascular bundle

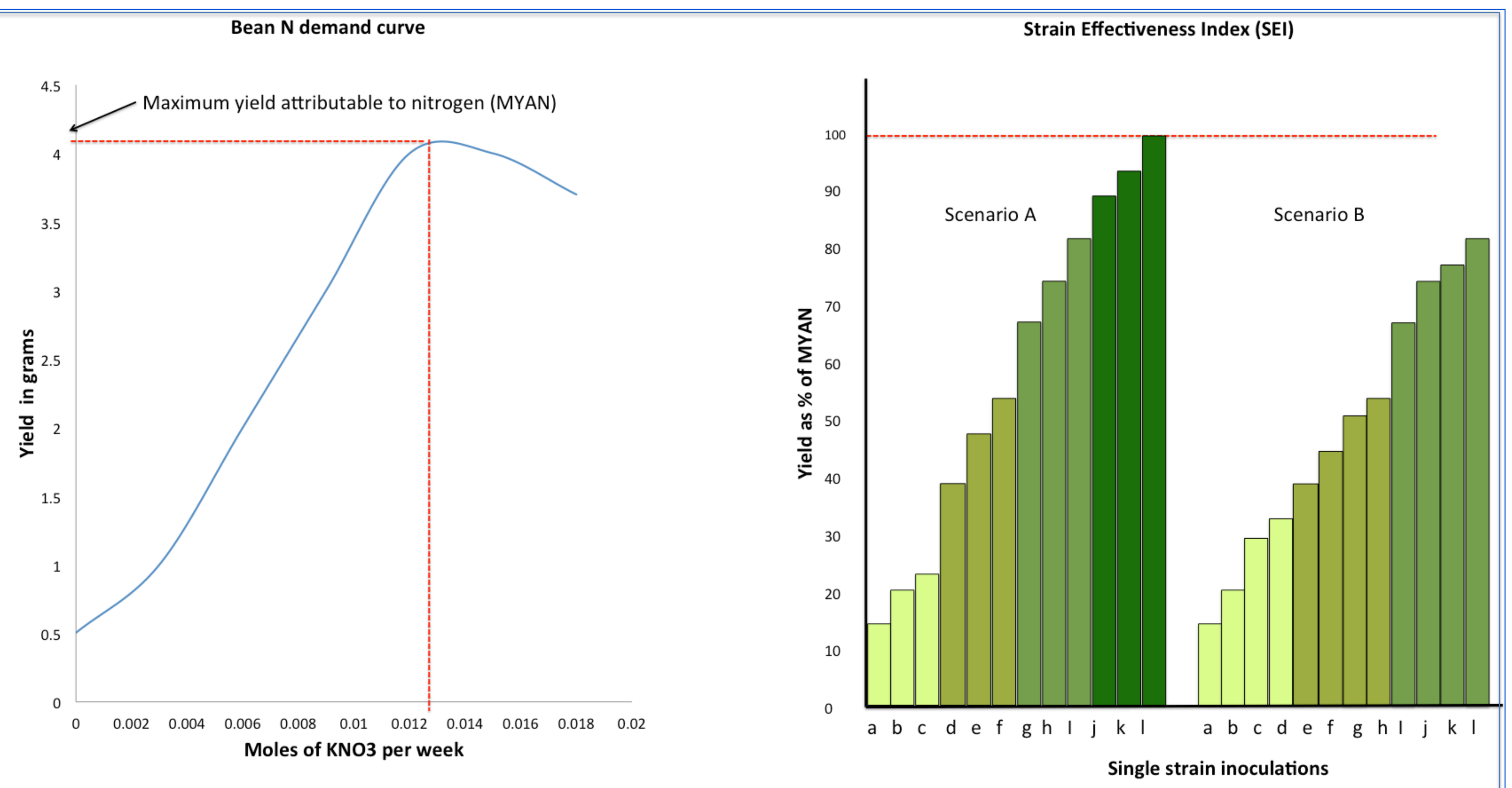

Figure 3. Hypothetical charts showing beans' demand for N and ability of rhizobia strains to meet this demand

Hypothesis Two:

Kenyan strains are more competitive at nodulating Kenyan bean cultivars than introduced commercial strains

Approach:

- Mark the chromosome of CIAT899 with a P_{tac}-driven *gusA* reporter.
- Clone the P_{tac}-driven *celB* reporter into the stable expression vector pJP2.
- Mobilise the pJP2-*celB* construct into Kenyan isolates.
- Co-inoculate marked CIAT899 and marked Kenyan isolates onto beans
- Use a combinational staining approach (X-Glc and Magenta-Gal) to determine whether endemic bean RNB are able to competitively nodulate *Phaseolus.*
- Competitive Kenyan isolates will then be tested in field trials in Kenya.

Expected results:

-Development of a marker system for evaluation of competitiveness in beans, which can easily be adapted for use in other N_2-fixing legume-rhizobium interactions.

-Determination of competitiveness of Kenyan isolates on Kenyan bean cultivars in comparison to a widely used competitive commercial strain

Acknowledgements

Study funded through a scholarship by N2Africa and Murdoch University

References

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011). "MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods". *Mol Biol Evol* **28** (10): 2731–9.

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