Soya bean *Bradyrhizobium* persistence or an inoculation paradox?

The case of Mooigelegen Farm, east of Ermelo

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About 88% of the over 19 321 known species of the plant family *Fabaceae* (legumes) form a symbiotic association with a group of soil microbes known as rhizobia (Chaulagain and Frugoli, 2021). The association leads to the formation of specialised structures, namely nodules on the roots of legumes.

Inside the nodules, the rhizobia convert atmospheric nitrogen (N_2), usually unavailable to plants, into a usable form of nitrogen (N), namely ammonia (NH_3). The NH_3 is then converted to amino acids and amides within the nodules, after which it is transported in the xylem sap to other parts of the legume plant. This important interaction between the legumes and the rhizobia that results in converting N gas into a usable form of plant-available N is termed biological N fixation (BNF).

Using this process, it is estimated that 50 to 60 million tons of N are fixed annually by the symbiotic interaction between rhizobia, 250 million hectares of pulse and oilseed crops, and 220 million hectares of pasture legumes globally (Farquharson *et al.*, 2022).

Photo 1A) Soya bean roots uprooted from Mooigelegen Farm showing several prominent nodules formed mainly on the root crown regions (photo: Jantjie Randell). B) The same plants on the field on which the nodules in Photo 1A are shown (photo: Jantjie Randell). C) Dr Ahmed Hassen and Prudence Mtsweni of the ARC-BNF unit are pictured with Jantjie Randell at one of the fields planted to maize. D) Dr Abe Shegro Gerrano of ARC-VIMP (left) and Dr Ahmed Hassen collect soil samples from the maize rhizosphere.

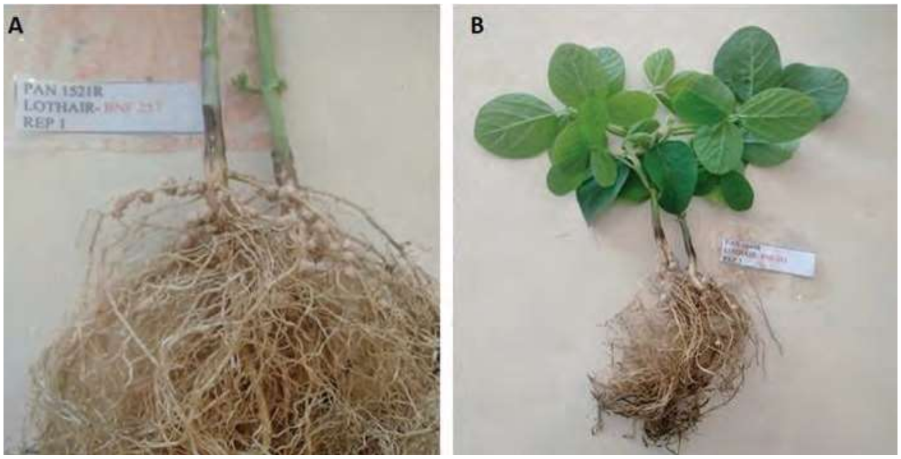
Exploring rhizobia inoculation

According to an early study by Van Rensburg *et al.* (1976) the indigenous soil rhizobia, which specifically nodulate and fix N in soya bean, are absent in many African soils, including South Africa. It is therefore essential to inoculate soya bean plants with the appropriate type of rhizobia, *Bradyrhizobium japonicum* (*B. japonicum*), in the form of liquid or powdered inoculants.

Inoculation with rhizobia is also required when a legume is grown in a particular soil for the first time or after a break of several years. This is because compatible, effective rhizobia will not be present in such circumstances and therefore inoculation with a high concentration of rhizobia is required (Farquharson *et al.*, 2022).

In December 2021, a team of research scientists from the Agricultural Research Council Plant Health and Protection (ARC-PHP), as well as the ARC-Vegetables, Industrial and Medicinal Plants (ARC-VIMP) visited Jantjie Randell, a soya bean producer at Mooigelegen Farm in Lothair, east of Ermelo, for a survey and soil sampling as part of an existing soya bean inoculants research project funded by the National Research Foundation (NRF).

Randell has been growing soya bean and other crops such as maize for several years. He used to treat his soya bean plants with *Bradyrhizobium* inoculants every second year, using mainly seed treatment and sometimes liquid application of different commercially available products of *B. japonicum* strains in furrows. However, he stopped inoculating his soya bean



Soya bean roots showing several big nodules on the crown region formed in a glasshouse soil trapping experiment after soya bean cultivar PAN 1521R (photo 2A) and PAN 1644R (photo 2B) were planted in soils collected from the rhizosphere soils on Mooigelegen Farm. The nodules were formed by the native soil rhizobia which were trapped by the roots (photo: Khumbudzo Ndhlovu).

seeds with *Bradyrhizobium* products as he claimed that his untreated seeds could do better in his soils.

The last time he inoculated his soya bean with rhizobium inoculants was in 2014. Nevertheless, the producer employs other management practices such as correcting soil with nitrogen, phosphorus and potassium (NPK) fertilisers in the ratio of 1:2:4 and later 1:2:6, and planting soya bean by rotating with maize every other season.

Randell plants mainly two types of cultivars at a time. In the past he used soya bean cultivars such as LS 6161R, PAN 1664R, PAN 1532R, PAN 1623R and P 64T39.

Root nodule formation

By the time this survey was done, the soya beans had just been planted and there was not yet an opportunity to collect and observe the nature and abundance of the root nodule formation. Randell was asked if he had any record or information on the amount of N fixed per hectare by his soya bean plants without having to treat the soya beans with any *Bradyrhizobium* inoculant products.

Except for the lack of information available on the amount of biologically fixed N, he indicated that on the best soya bean fields it was possible to obtain a yield of as much as three tons per hectare per year.

Photo 1A to *1D* shows soya bean root nodules and the plants in the field on Randell's farm in 2021. As can be seen in *Photo 1A*, several large nodules were formed on the crown region of the root, which Randell confirmed were active nodules owing to the pink colour inside and, of course, the yield that was achieved.

This observation made by Randell appeared paradoxical on the surface regarding the necessity of inoculation with rhizobia, especially under South African conditions in which the soils are devoid of the specific rhizobia that nodulate soya bean and fix atmospheric nitrogen.

Testing rhizosphere soil samples

To validate this and as part of our research objective, we collected rhizosphere soil samples from three different sites on the Mooigelegen Farm which were planted to soya bean and maize in the 2021/22 growing season. The soil samples were taken to the laboratories and were subjected to a glasshouse trapping experiment to verify the presence of native rhizobia isolates in the collected samples, or possibly persistent rhizobia isolates from past inoculations that might potentially colonise the roots and form root nodules by planting different soya bean cultivars.

The results obtained were very interesting in that most of the soya bean cultivars, including PAN 1532R used by producers in the past, were able to form intact green shoots with seemingly effective nodules, i.e. large pink nodules mainly on the crown root region when grown in the native soils collected from the rhizosphere (*Photo 2A* and *2B*).

The next task was to investigate if the trapped nodules harbour rhizobia (particularly *B. japonicum* and other *Bradyrhizobia* species) commonly involved in the nodulation and N fixation of soya bean. Thus, we conducted a molecular identification method for each bacterial isolate retrieved from the root nodules of each soya bean cultivar.

This identification shows that *B. japonicum*, *B. elkani* and other *Bradyrhizobium* spp. survive in the native soils and were trapped by more than one soya bean cultivar used in the glasshouse trapping experiment (*Figure 1*).

Some other bacteria also retrieved from the nodules such as *Pseudomonas, Paenibacillus* and *Bacillus* are not root nodulating and N fixing symbionts, but could be living in the nodule tissues as endophytic bacteria. Generally, in our observation, the relative abundance of non-*Bradyrhizobium* spp. such as *Mesorhizobium*, *Rhizobium* and other non-*Rhizobium* endophytes was very low.

Concluding remarks

Soya bean (*Glycine max* L.) is not a crop native to South Africa and was first introduced and cultivated as an economic crop in 1903. When an exotic legume such

Figure 1: Identification of species and genus level of rhizobia strains isolated from the root nodules of soya bean after being trapped from the rhizosphere soil in a glasshouse experiment. (Source: Khumbudzo Ndhlovu, Biological N_2 Fixation unit, ARC-PHP)

as this is introduced into new soil, its growth and yield will only be improved by inoculating it with rhizobia, hence the necessity of inoculation.

Inoculation is also essential when the soil contains a higher population of rhizobia that are only competing for nodulation, but are not effective in N fixation. Therefore, it is always recommended to conduct a 'need to inoculate experimental trials' in order to decide whether or not inoculation is required in a given soil before growing legumes, as reported by Howieson and Dilworth (2016).

Despite the fact that soils at Mooigelegen Farm have not been planted to soya beans inoculated with rhizobia for the past eight years, quite a large population of rhizobia could be detected from the native rhizosphere soil. Most of the rhizobia detected in the native soil belong to *B. japonicum* and *B. elkani*, as compared to other fast-growing rhizobia.

This could be an indication that the inoculant strains (seed treated or in furrows application) applied many years ago could have persisted and survived in the soil for up to eight years since the last time Randell treated his seeds with rhizobia inoculants.

Such persistence of inoculant strains of rhizobia has been reported before (Lindstrom *et al*., 1991). The report indicated that two inoculant strains of *Rhizobium galegae* that nodulate and fix N in the perennial forage legume, *Galega officinalis* (goat's rue), remained persistent in the soil for five years.

Similarly, another inoculant strain, *Lotononis bainsii*, remained in the soil for 12 years. It is therefore imperative to note that, although in some instances non-inoculated soya bean plants perform better than the *Rhizobium* treated ones, this is most probably only because of the occurrence of a large population of naturalised *B. japonicum* and *B. elkani* strains, which persisted and survived in the soil from the previous inoculation events.

It is worth noting that if native rhizobial populations are absent or not effective, it is always important to inoculate exotic legumes such as soya beans with exotic rhizobial strains of known potential. That is why the exotic strain *B. japonicum* WB74 (=CB1809) was introduced in South Africa in 1998 as a soya bean inoculant after confirming its efficacy under field conditions.

Finally, while modern agriculture plays a significant role in the attainment of food security, it is our belief that producers' indigenous knowledge has a huge contribution to agricultural research to improve the global food sustainable system.

Acknowledgements

We would like to acknowledge the NRF for funding this research under Grant Nr 135456 to Dr Ahmed Hassen, and for the PhD postgraduate bursary to Khumbudzo Ndlhovu. Due acknowledgement also goes to Jantjie Randell, soya bean producer at Mooigelegen Farm in Mpumalanga, for allowing us to do a survey and sample collection on his farm.

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