By Dr Ahmed Idris Hassen

Using appropriate rhizobium strains for OILSEED LEGUMES

With about 2 000 species, legumes represent the most utilised plant family and are the most significant crop worldwide. Besides being an important dietary source constituting 33% of the dietary protein nitrogen (N) needs for humans and animals, they are also a source of income and livestock feed. Among the grain legumes, soya bean (*glycine max L.*) and peanut (*arachis hypogeae L.*) are the major sources of vegetable oil, producing more than 35% of the world's processed vegetable oil.

In order to meet their nitrogen requirements, fertiliser nitrogen has long been used as one of the major agricultural inputs. However, several reports of depressive effects of N-fertiliser that suppress nodulation and yield have been documented in many cases, including soya beans in the past. A combination of factors, including soil nitrogen (N-fertiliser), extremely low or high soil pH and moisture stress, affect grain yield negatively.

Biological nitrogen fixation (BNF) is an alternative process to the use of N-fertiliser where a group of bacteria called rhizobia, form a symbiotic association with the roots of legumes, form root nodules and fix atmospheric nitrogen (N2). The fixed nitrogen will then be available to plants in the form of ammonia and nitrate. Thus symbiotically fixed nitrogen either by indigenous or introduced strains of effective rhizobia is the most efficient source of nitrogen for legume grain production.

Like many other legumes, both soya beans and peanuts establish a symbiotic relationship with rhizobia. The major soya bean nodulating rhizobia that have so far been identified are *bradyrhizobium japonicum*, *bradyrhizobium elkani* and *sinorhizobium/Ensifer fredi*. The other significant oilseed peanut differs from soya bean as it is a highly promiscuous legume being nodulated by rhizobia that nodulate a diverse group of legumes.

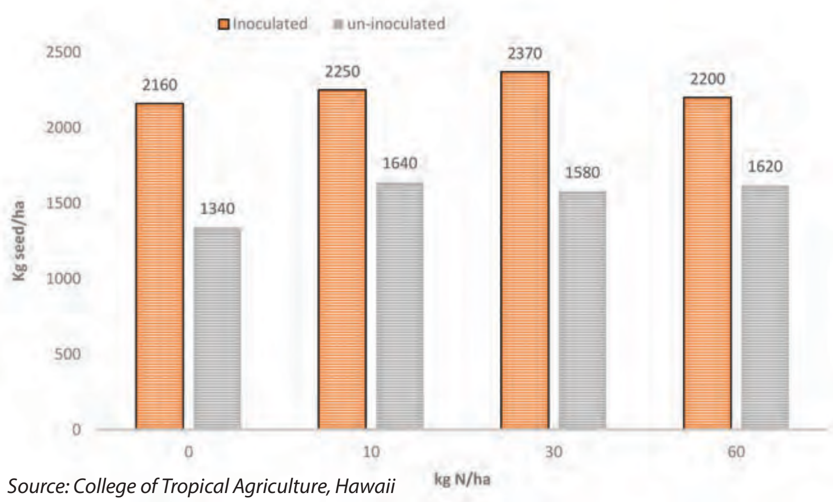
Benefits of inoculation

Ever since 1988, when Hellreigel and Wilfrath showed that root nodule bacteria were providing fixed nitrogen to the plants using N2 as a sole nitrogen source, much work has been done to improve methods of inoculation and inoculant strains. The major aim of inoculation is to provide a sufficient number of viable effective rhizobia and to induce rapid colonisation of the root. In doing so, it is possible to initiate nodulation soon after the completion of germination and to produce optimum yield.

Compared to nitrogen fertilisers, inoculation with rhizobium represents an economically sustainable, environmentally friendly resource that guarantees the nitrogen requirement of an ecosystem. As legumes are rich in protein with high nitrogen content, they have higher nitrogen requirements than cereal crops. Thus to meet all the nitrogen needs of the legume crops and to achieve high yield, farmers would have to apply more nitrogen fertiliser than the legume crop actually requires.

For instance, to obtain a soya bean yield of 2 000kg/ha farmers need to apply approximately 621 to 429kg nitrogen. The same yield could be obtained with inoculation (BNF) and no nitrogen fertiliser. Benefits of inoculation are not restricted to yield, but can also increase the protein content of seed, even if there is no yield increase. In addition, if crop residues are returned to the soil during harvest, more nitrogen will be available to the next crop, as legumes can obtain more nitrogen as a result of BNF.

Figure 1: Comparison of the effect of added fertiliser nitrogen on inoculated and non-inoculated soya beans. Inoculation with rhizobium results in increased yield. Added fertiliser nitrogen at high concentration affects nodulation and yield in inoculated rather than un-inoculated soya bean.



Source: College of Tropical Agriculture, Hawaii

Why, when and what to inoculate

Questions worth asking when it comes to inoculating legumes are why, when and what to inoculate? Many soils used for legume cultivation do not contain an adequate number of effective rhizobia. A typical example is the case of South African soils that lack the specific rhizobia that nodulate and fix nitrogen in soya bean. In such cases it is vital to inoculate the plants with highly effective rhizobial inoculants.

In general, inoculation is useful and necessary when there are no indigenous strains of the required rhizobia or when they are present at a very low concentration. However, inoculation may not be necessary and does not provide any benefit if there are already much rhizobia in the soil that can stimulate effective BNF.

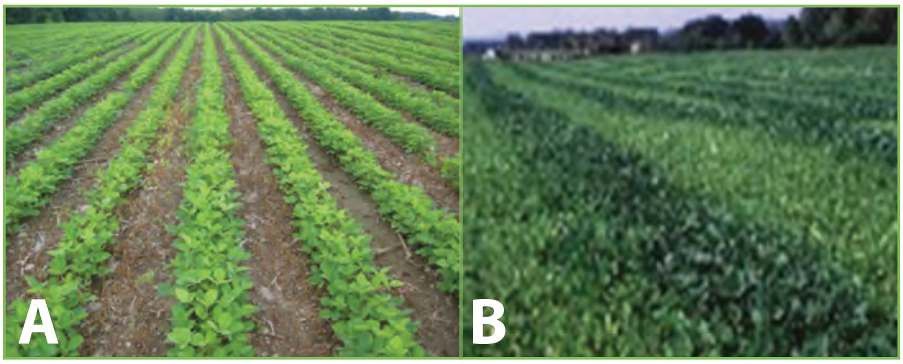
When performing inoculation with rhizobium, it is vital to ensure that the formulated inoculant product is registered for marketing. This will only be achieved if the efficacy of the inoculant strain has been confirmed both under glasshouse and field conditions, in which a trial is conducted for at least two seasons and two geographically different soils.

Registration

However, the fact that the selected inoculant strains pass the field nodulation screening trial, does not mean that the product can be automatically registered. This is because the survival and effectiveness of the inoculant strain may be affected due to low quality or sub-standard inoculant carriers such as peat, perlite or liquid formulations.

For instance, the survival of the rhizobia and its numbers decrease in the carrier as the age of a low standard inoculant product increases. The efficacy of the rhizobium in the product can also be minimised or lost if the pH of the inoculant is not optimum or if it contains contaminants at a level ≥ 100 000 cells/mℓ. Due to this, the quality of any inoculant product that contains effective rhizobium strains should be regularly controlled for at least six months.

Figure 2: Soya bean field not inoculated with rhizobia before showing symptoms of nitrogen fixation deficiency (A) and part of a soya bean plot inoculated with rhizobium shown as dark green strips (nodulation effective) and the un-inoculated plots shown as yellowish strips (B).



Source: Pioneer Hi-bred available at https://www.pioneer/home

The internationally accepted standard quality control protocol in South Africa suggests that:

- The number of rhizobia in powdered carrier products must be at least 5 x 108 cfu g-1 for peat carriers and at least 6,5 x 108 cfu g-1 for perlite and 2 x 109 cfu mℓ-1 for liquid formulations.
- The pH of the carrier must be between 6 and 7,5.
- The moisture content should be 35 to 45%.
- Contaminants should not exceed 100 000 cfu g-1, preferably much less than this.
- It should have good shelf life – rhizobia should be viable at acceptable number for at least six months.
- It must contain the desired strain – can be verified microscopically, serologically or molecular fingerprinting.

Conclusion

With special preference to the production of soya bean as the major oilseed legume in South Africa, an effective inoculant strain, *bradyrhizobium* strain WB1, was introduced in the 1970s to promote nodulation and yield. Then, another strain, *B. japonicum* WB74, a synonym of the Australian strain, *B. japonicum* CB1809, was selected and replaced the WB1 strain in the 1990s, following an extensive field trial and screening of different soya bean genotypes.

> Benefits of inoculation are not restricted to yield.

With over 35 soya bean genotypes in the country, relying only on one strain of *bradyrhizobium* may not render the desired result, nor does the introduction of various new inoculant products from abroad, without proper screening on South African soils, guarantee effective nodulation, nitrogen fixation or yield increase.

It is therefore recommended that emphasis is placed on future research projects to screen and select the most appropriate inoculant products and rhizobia strains across a wide range of soya bean cultivars. This does not only provide better results by improving nodulation and yield increase, but also maintains the ecological welfare and microbial diversity of South African soils.

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