Final Project Report to Protein Research Trust

DETERMINATION OF THE COMPETITIVE ABILITIES OF *BRADYRIIIZOBIUM JAPONICUM* INOCULANT STRAINS IN SOILS FROM LOCAL SOYBEAN PRODUCTION REGIONS

Executive Summary

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Introduction and objectives

In an effort to improve the protein content of soybean *[Glycine max* (L.) Merr.] cultivated in South Africa, strain CB 1809 (= WB74) was recently chosen to replace strain WB 1 in commercial inoculants. The criterion used for selection of CB 1809 was N_2-fixing effectiveness, either under N-free greenhouse conditions or in fields where rhizobial populations were small or absent. An additional characteristic required of inoculants is ability to compete for nodulation of the plant with other strains of *B. japonicum* that might be present in the soil. Strain competitiveness is influenced by the genetic diversity of both symbiotic partners as well as the soil environment in which nodulation occurs. Efforts to enhance competitiveness have consequently achieved limited success and selection remains an empirical process based on the ability of strains to nodulate roots in the presence of other competing rhizobia.

Although our soils do not contain indigenous rhizobia that effectively nodulate soybean, populations of *B. japonicum* have become established in soybean production regions as a result of previous inoculation. This is important as the success of an inoculant strain is often limited by the size of naturalized soil rhizobia. Inoculation is invariably futile in soils with populations greater than 1000 rhizobia g^{-1} soil, whereas a response to inoculation could be expected in soils with <1000 rhizobia $g^{-1.}$ Populations of in the aforementioned range are likely to occur in South African soils.

The present study compared CB 1809 and WB 1, as well as two other strains, for ability to compete for nodulation of soybean. Greenhouse experiments were carried out with plants grown in sterile sand as well as in five soils from different locations, each of which contained established populations of *B. japonicum.*

Method

Determination of strain competitiveness was previously seldom undertaken in inoculant selection programmes because identification of strains in nodules was

technically difficult. A new method of identification was recently developed based on insertion of the *gusA* marker gene, coding for the enzyme β-glucuronidase, into the desired strain. Nodules containing the marked strain are identified by colour formation after reaction with a suitable substrate. The marker was introduced into strains CB 1809, WB 1, USDA 110 and 965 and evaluated on soybean.

Results and discussion

A *gusA*-marked derivative of each strain was isolated that was equivalent to the wild type in N_2-fixing effectiveness and nodulation.

Relative competitiveness of the four strains was initially compared using plants grown in assemblies containing sterile sand. Overall results showed that strain CB 1809 was significantly superior to strain WB 1 in competitive ability while the other two strains were similar to CB 1809 in this property. Competitiveness of the strains was not significantly influenced by inoculation on three cultivars of soybean.

Subsequent experiments compared the ability of the *gusA*-marked strains to compete against rhizobia in five soils. The populations of *B. japonicum* were effective on soybean and varied from about 300 rhizobia g^{-1} (soil 1) to about 10^6 rhizobia g^{-1} (soils 25). Unexpectedly, serological methods detected strain WB 1 amongst only 8% of isolates from soil 3. Antibody to WB 61, an inoculant strain used before WB 1, reacted with 4-20% of isolates from soils 3-5. In contrast, 32-88% of the isolates in soils 1-5 reacted with antibody to WB 66, an inoculant that was discontinued in 1966. Soils 1-3 contained a substantial number of isolates (24-68%) that did not react with the antibodies tested, but 32% of the isolates from soil 1 reacted with antibody to USDA 110.

When *gusA*-marked strains were applied in peat inoculant at the rate of about 1000 rhizobia $seed^{-1}$, negligible nodulation by inoculant strains was detected in soils 2-5. Appreciable nodulation by inoculated strains was obtained only in soil **1,** which had an inoculant: soil rhizobia ratio of 1:600. Nodule occupancy data on each of three cultivars showed that strain WB 1 was the weakest competitor against the soil rhizobia. On average, strains ranked in the order 965>CB 1809>USDA 110>WB 1. The superior performance of strain 965 appeared related to significantly better competitiveness on one of the cultivars.

In a subsequent experiment, the *gusA*-marked strains were inoculated at a rate of about 5×10^6 rhizobia $seed^{-1}$. In soil 1, the ratio inoculant: soil rhizobia would have been 10:1. Most seeds failed to emerge in this compacted sandy soil and results could not be statistically analysed. Inoculant strains, however, occupied 95-100% of the nodules.

In soils 2-5 the inoculant: soil rhizobia ratio was 1:400. Strain x soil interactions were not significant. Strain WB 1 ranked lowest of all the strains in nodule occupancy in all four soils and its mean value was significantly less than that of the top ranking strain CB 1809. Nodule occupancy of the inoculated strains differed significantly between soils, being highest (81%) in soil 5, which had a low pH of 4.8. Intermediate occupancy (45%) was detected in soils 3 (pH 6.1) and 4 (pH 5.3) and lowest occupancy in soil 2 (22.3%) a sandy loam with pH 6.7 and high C content. The reason for the effect of soils on nodulation by the inoculated strains is not clear.

In summary, strain CB 1809 appeared superior to strain WB 1 in competitive ability. This finding supports the recent decision to replace WB 1 with CB 1809 in commercial inoculants.

The observation that four of the five soils from soybean production regions contained substantial populations (10^6 g^{-1}) of effective rhizobia is noteworthy. In such soils, establishment of inoculated strains is usually difficult and a response to inoculation would be unlikely. This was supported by the finding that an inoculant strain WB 66 discontinued as long ago as 1966 comprised a substantial proportion of each soil population, whereas subsequent strain WB 1 was scarcely detected although used over a period of 19 years. Both strains are less effective in N_2-fixing ability than CB 1809.

There is therefore a strong possibility that the recently introduced inoculant strain CB 1809 may fail to induce a significant response or become established in soils that already contain naturalized populations of *B. japonicum*. This aspect needs investigation together with critical assessment of the size, genetic composition and effectiveness of soil populations in soybean production regions. This information would enable the inoculation needs of individual soils to be predicted.

With the results of the present study and those of a co-project that is currently assessing other problems associated with soybean inoculation, we will soon be in an excellent position to carry out the evaluations suggested above. By that time the ability of CB 1809 to establish itself in the field should be evident as it will already have been applied for several seasons.

Aspects of this work will be presented at the Bio Y2K meeting, Grahamstown, January 2000 and a research paper is in preparation for submission to *Biology and Fertility of Soils.*

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