International Journal of Scientific Research in Biological Sciences
Research Paper
Vol.7, Issue.3, pp.47-51, June (2020)
E-ISSN: 2347-7520
DOI: https://doi.org/10.26438/ijsrbs/v7i3.4751

Assessment of Nodulation Potential of *Bradyrhizobium japonicum* and *Bradyrhizobium elkanii* on *Glycine max* L. in Acidic Clay and Loam Soils

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Available online at: www.isroset.org

Received: 25/Apr/2020, Accepted: 04/June/ 2020, Online: 30/June/2020

Abstract - Poor soils and prohibitive cost of nitrogen fertilizers contribute to low soybean production in southern Africa. Soybean is a legume with high nitrogen demand during its development and is sensitive to acidic soils. This paper investigated the potential of two rhizobia species *Bradyrhizobium japonicum* strain 550 and *Bradyrhizobium elkanii* strain 510 in nodulating soybean variety SeedCo Status in acidic clay and loam soils. Strains of *Bradyrhizobiun japonicum* and *B. elkanii* rhizobia were each inoculated to surface sterilized soybean seeds prior sowing in acidic clay and loam soils. The inoculated seeds were sown in 1-litre pots with three replicates for each of six treatments. The soybean plants were evaluated for nodule colour, nodule number, shoot biomass and shoot N content. All means were subjected to ANOVA in STATA version 12.1. The observed nodule colour for plants inoculated with *B. japonicum* and *B. elkanii* strains was red brown. The results showed a significant difference in nodule number, shoot biomass and N content among the soybean plants inoculated with the rhizobia in both acidic clay and loam soils ($F=9.09$ $p=0.05$). Inoculating soybean seed with *B. elkanii* strain 510 provides more nodulation potential in acidic loam soil than *B. japonicum* strain 550 in the same soil type.

Key words- *Bradyrhizobium elkanii*, *Bradyrhizobium japonicum*, clay soil, loam soil, soybean.

I. INTRODUCTION

Glycine max L. (soybean) is a legume grown for its high essential amino acids, making the crop superior as feed for human, livestock [1] and fish [2]. The crop is best suited to soils with high clay content and is sensitive to soil acidity [3]. Soybean has a high nitrogen (N) requirement during its growth and development. The legume requires 80kg of N to produce 1000Kg of grain [4]. Therefore, the yield is usually limited by inadequate N supply. In sub-Saharan Africa including Zimbabwe, small holder soybean yields are reportedly constrained by poor soil fertility [5] and high cost of nitrogen (N) fertilizer [6, 7]. Apart from the prohibitive cost, the use of N-fertilizer poses threat to the environment through eutrophication of rivers. There are reports on the inhibitory effect of N-fertilizer on soybean performance resulting in delayed and/or inhibition of nodule development [8]. Several hypotheses have been suggested to explain the inhibition. They include inhibition of nitrogenase by nitrite on the synthesis of leghemoglobin [9, 10] resulting in the synthesis of a new molecule nitrosylleghemoglobin which affect leghemoglobin functioning [11]. This eventually limits diffusion of oxygen to nodules thereby reducing nitrogenase functioning [12]. Therefore, the application of N- fertilizer to soybean may pose undesirable results.

Soybean production may be enhanced by use of alternative sources N through application of symbiotic bacteria called rhizobia. Rhizobia convert nitrogen (N_2) in the air making it available to plants [13]. The highest rates of N demand in soybean are experienced at the onset of pod filling [14, 15]. World over, soybean has been inoculated with strains of *Bradyrhizobium* to supply the N demand of the legume. Since 1932 up to 1980, all bacteria that were able to produce nodules on soybean were termed *Bradyrhizobium japonicum*. It was after the 1980s that genetic, biochemical and physiological differences were noticed with the *B. japonicum* species [16, 17]. Extensive characterization of the soybean rhizobia led to a subdivision into two species *B. japonicum* and *Bradyrhizobium elkanii* [18]. The two rhizobia species are a source of commercial inoculant formulations in many countries [19, 20]. *Bradyrhizobium elkanii* bacteria are aerobic, motile Gram-negative rods with potential to be considered fertilizing agents [21]. Genetic studies on *B. elkanii* has shown its ability to assimilate ammonia and at the same time offering a possibility to grow in polluted soils [22]. Whereas *B. japonicum* has been reported to be sensitive to acidic soils, hence require the application of lime to the soil prior inoculation and sowing [23]. Objectives of the study were to identify the Bradyrhizobia strain that is best able to nodulate soybean in acidic loam and clay soil types and to assess the shoot total N content. The paper is organized into, section I which consist of the introduction covering the importance of biological fixation in soybean and the types of *Bradyrhizobium* species. Section II contains the material and methods used in assessing the Bradyrhizobia potential in nodulating soybean in acidic soils. Section III and IV describes the results and discussion of the study respectively. Finally, the conclusion and future scope of the study are in Section V.

II. MATERIALS AND METHODS

Soil description

Loam and clay soils used in this study were obtained from the Horticulture Institute, Marondera, Zimbabwe, with no previous history of soybean cropping or rhizobia inoculation. A full soil analysis was performed at the Chemistry and Soil Research Institute, Department of Research and Specialist services (DRSS), Ministry of Agriculture, Harare, Zimbabwe. Air-dried soil samples were passed through a 2 mm sieve and tested for various characteristics. The characteristics included soil colour, pH, amount of calcium (Ca), calcium chloride (CaCl), magnesium (Mg), Nitrogen (N), phosphorus (P) and potassium (K). The soils were used to prepare 1-liter pots for soybean inoculation studies by *B. japonicum* and *B. elkanii* strains.

Soybean seed sterilization

Seeds of determinate soybean SeedCo (SC) Status cultivar, of uniform size, shape and colour were selected. In addition, only seeds with intact seed coats were handpicked and used in the study. The cultivar was selected because its recommended area of production includes all agronomic regions of Zimbabwe. Sterilization of seeds was done with 0.1% (w/v) aqueous solution of mercuric chloride for 1 minute. The seeds were then washed thoroughly with sterilized water. Thereafter, the seeds were resuspended in sterile distilled water for 3 hours.

Preparation of microorganisms and seed inoculation

Rhizobia strains *B. japonicum* (550) and *B. elkanii* (510) were sourced from Soil Productivity Research laboratory Harare, Zimbabwe. Purity tests on the strains were carried out by inoculation on yeast mannitol bromthymol blue agar, nutrient agar and glucose peptone agar. To obtain colonies of distinct and consistent form, continuous sub culturing was done on yeast mannitol Congo red agar. The plates were incubated for 5-7 days at 28ºC. The number of Bradyrhizobia strains able to induce nodulation in soybean was determined by using the most-probable-number (MPN) technique [24]. An equivalent of 100g (dry mass) loam and clay soils were each resuspended in 900 ml deionised water. The suspension was rigorously shaken for 5 minutes. A 10^{-1} dilution was performed to the resultant suspension. Aliquotes of 2 ml of the dilution were added to surface sterilized seeds grown in prepared pots. Three replicates were prepared for each dilution. A negative control with uninoculated seed was also prepared. For all the treatments, the sterilized seeds were inoculated with an inoculant at a concentration of 5 × 109 cells g^{-1}, containing *B. japonicum* strain 550 and *B. elkanii* strain 510. Approximately, 1.2 × 106 viable cells of Bradyrhizobia were inoculated per seed. Adherence was improved by including 10% sucrose solution to the inoculant [14]. The study comprised of 6 treatments combinations. (i) Uninoculated soybean in loam soil (ii) uninoculated with soybean in clay soil (iii) inoculated with *B. japonicum* in loam soil (iv) inoculated with *B. elkanii* in loam soils (v) inoculated with *B. japonicum* in clay soil (vi) inoculated with *B. japonicum* in clay soil. All experiments were performed in a randomized block design with three replicates for each treatment.

Sampling, harvest and analyses

Three plants were collected from each pot six weeks after sowing for evaluation of nodule colour, number of nodules, above-ground (shoot) biomass and N content. Intact roots and nodules were collected by uprooting the plants carefully using a small lab spade. Adhering soil particles were removed from the entire root system by hand over a metal sieve. The nodules were detached from the roots, enumerated and their inside colour noted. Roots and shoots were separated, carefully washed with deionized water and dried 65 °C for 72 hours before the dry shoots were weighed and evaluated. Shoot N content was measured by Kjeldahl (wet oxidation) method [25].

Statistical Analyses

Tests for normality and variance homogeneity were performed on each data set and the data were analyzed by ANOVA in STATA version 12.1 [26].

III. RESULTS

Soil characteristics

The soil analysis report (Table 1), indicated that clay soils were light brown and the loam were pale brown. The calcium chloride pH values for clay soil was 5.1 while that of loam soil was 4.8. The mineral N ppm ammonia and nitrate N available in the soil before and after incubation was greater for clay the loam soils. The available P resin extract ppm of P205, was higher in clay than loam soils. This was the case for exchangeable cations K^{2+}, Ca^{2+} and Mg^{2+}.

Table 1 Soil analysis description of soil samples used in assessing nodulation potential of *B. japonicum* (550) and *B. elkanii* (510) in acidic clay and loam soils.

	Soil type	
	Clay	Loam
Texture	medium grained sand clay loam	medium grained loamy sand
Soil colour	LB	PB
CaCl pH	5.1	4.8
N(ppm)	54	15
P [ppm (P205)]	108	43
K (mg/100g)	0.62	0.15
Ca (mg/100g)	2.19	1.15
Mg (mg/100g)	1.15	0.76

Nodule colour

A visual inspection of insides of nodules collected from soybean plants whose seeds were inoculated with both Bradyrhizobia strains produced a red brown colour. The red brown colour was noticeable in all the plants sown in clay and loam soils.

Number of nodules and above-ground biomass and shoot nitrogen content

Table 2 Number of nodules, above-ground (shoot) biomass and N content of soybean variety SC Status obtained in the assessment of nodulation potential of *B. japonicum* strain 550 and *B. elkanii* strain 510 in acidic clay and loam soils

	Clay soil			Loam soil		
Rhizobia strain	No. of nodules	Biomass (g)	Nitrogen content (mg)	No. of nodules	Biomass (g)	Nitrogen content (mg)
None	2	7	8.4	3	5.57	9
B. japonicum (550)	41[a]	7.50[a]	12.67[a]	55[a]	7.31[a]	16.33[a]
B. elkanii (510)	33	6.39	14.33	59	8.38	20.93

Values are mean of 3 replicates.
Means with the same superscript do not have significant difference at p=0.05.

The results (Table 2) show a significant difference in the number of nodules, shoot biomass and N content among soybean plants inoculated with *B. japonicum* with those inoculated with *B. elkanii* within each soil type and between clay and loam soil (F=9.09: p=0.05). In clay soil, plants inoculated with *B. japonicum* had a significantly higher number of nodules and biomass, however, the N content was significantly higher for soybean plants inoculated with *B. elkanii*. In loam soil, soybean plants inoculated with *B. elkanii* had a significantly higher number of nodules, shoot biomass and N content than plants inoculated with *B. japonicum*. A comparison of the soybean performance in loam and clay soil indicated that soybean inoculated with *B. elkanii* grown in loam soil had significantly higher number of nodules, shoot biomass and N content than their counterparts in clay soil. Soybean inoculated with *B. japonicum* showed no significant difference in performance in either clay or loam soil (F=0.4; p=0.05).

IV. DISCUSSION

The soils used in this study were acidic (Table 1) with clay soil being medium acidic and loam strongly acidic. Acidic soils within pH range between 5.2- 5.4 have been reported to limit rhizobial survival [13] therefore, we expected inefficient to absent nodulation of soybean after inoculation with both *B. japonicum* and *B. elkanii* strains. Clay soil had higher values of soil N, P, K, Ca and Mg an indication that the clay soil was generally more fertile. Phosphorus ppm (P205) was higher in the clay soil. High P is important for the growth of soybean since the mineral is responsible or the legume's N supply during biological nitrogen fixation (BNF) [27]. [28] demonstrated that rhizobium inoculation helps plants to acquire P from otherwise insoluble sources. Symbiotic N fixation process readily occurs in the presence of P [29]. Therefore, nodulation and N_2 fixation are constrained by low P. This potentially affects nodule number, plant biomass and nitrogenase activity [30, 31, 32].

A visual inspection of nodule colour in the soybean plants inoculated with both rhizobia strains and grown in either clay or loam soil were red-brown color. This coloration is an indication of nitrogen fixation in all the plants. In loam soil with low P ppm (P205), the inoculation with either *B. japonicum* or *B. elkanii* strains facilitated nitrogen fixation. The red brown colour is due to leghaemoglobin [33] which is an iron-containing protein. The primary role of leghaemoglobin is provision of sufficient oxygen for the metabolic functions of rhizobia.

Soybean plants inoculated with *B. japonicum* (550) and sown in clay soil produced significantly higher number of nodules and biomass but significantly lower N content (Table 2). This is probably due to the high P and N, that the plants were able to significantly fix the available N. In loam soil, the soybean plants previously inoculated with *B. elkanii* (510) had significantly higher number of nodules, shoot biomass and N content. The loam soil was characteristically acidic and with low soil fertility. However, the *B. elkanii* strain proved to be more effective in BNF than the *B. japonicum* strain. These notable differences in nodulating potential can be attributed to differences in the rhizobia competitiveness fitness and physiology. According to [16] *Bradyrhizobium* species have unique physiological characteristics that determine their BNF. Previous studies have reported failure of legumes to nodulate in acidic soil [34] but our results are not consistent with these findings. *Bradyrhizobium elkanii* (510) effectively utilized the low available P in loam soil to enhance BNF in a strongly acidic soil environment. Soybean inoculation with rhizobia enhances rhizospheric acidification [29] which in turn enabled P acquisition from low soluble P source. The performance of the soybean plants inoculated with *B. elkanii* (510) had significantly high N content within each soil type. This means that the *B. elkanii* strain is more effective in promoting the utilization of available soil resources to increase plant N content in either acidic clay or acidic loam soil when compared to the *B. japonicum* strain. However, *B. japonicum* is the most widely used rhizobia species for soybean inoculation in Zimbabwe [35].

V. CONCLUSION AND FUTURE SCOPE

We conclude that *B. elkanii* strain (510) is more effective at BNF when compared to *B. japonicum* (550) in low fertile acidic loam soils. We recommend that the study be extended to field trials with other locally available determinant and indeterminate soybean varieties. The results of this study may be used as a baseline for testing the resilience of the *B. elkanii* strain and other strains under different soil types, including the sandy soils.

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