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Effectiveness of indigenous soyabean rhizobial isolates to fix nitrogen under field conditions of Zimbabwe

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Abstract

Ten promiscuous soyabean isolates that proved superior in nitrogen fixation effectiveness to commercial inoculant strains under greenhouse studies were evaluated for their symbiotic potential under field conditions. The promiscuous soyabean variety, Magoye and the specific variety Solitaire were used as test hosts. Five of the isolates were obtained from the nodules of Magoye and coded M1 to M5 while the other five were isolated from the promiscuous soyabean variety Hernon 147 and coded H1 to H5. Two commercial inoculant strains, MAR 1491 and 1495 were included for comparison. Parameters tested included nodulation effectiveness, yield, nitrogen content and nitrogen fixation effectiveness. Isolate M3 gave significantly higher nitrogen contents and N_2 fixation effectiveness in Magoye than the reference strains, while H1, H3 and H5 gave better nitrogen contents and N_2 fixation effectiveness with Solitaire. Nodule numbers and yields obtained were highly variable and not reliable indicators of effectiveness. There were strong harvest index effects of different rhizobial isolates, which closely followed the trend for grain yield. Isolates M3, H1, H3 and H5 were concluded to be potential commercial inoculants, while the other six need to be retested over many sites and seasons before they can be recommended.

Keywords: Nitrogen fixation effectiveness, promiscuous soyabean isolates, rhizobia

1. Introduction

Most smallholder farmers in Zimbabwe face the problem of limited access to rhizobial inoculants for commercial soyabean production. In an effort to solve this, promiscuous soyabean varieties were introduced to the smallholder sector of Zimbabwe (Javaheri, 1994). These are varieties that readily nodulate with indigenous rhizobia and hence do not require inoculation. Kasasa (1999) showed that promiscuous soyabean varieties performed well in the absence of rhizobial inoculation and gave comparable yields to those obtained from inoculated commercial varieties in soils of Hurungwe District, northern Zimbabwe. Promotion of promiscuous soyabean varieties was seen as a better alternative to using specific varieties that required inoculant, which was not readily available.

It was later noted that accessing seed of promiscuous varieties was a problem since local seed producers did not favour them. Use of specific varieties requiring inoculation was thus unavoidable.

Musiyiwa et al. (2005, 2006) assessed the symbiotic potential of indigenous rhizobia present in local soils. She found that indigenous rhizobia that readily nodulate promiscuous soyabean varieties were present in many Zimbabwean soils. Rhizobia isolated from nodules of the promiscuous soyabean varieties Magoye and Hernon 147, were characterized for cultural properties (Musiyiwa et al., 2006) and tested for nitrogen fixation potential under controlled conditions (Musiyiwa et al., 2005). Some of these isolates proved superior in N_2 fixation effectiveness to commercial inoculant strains under greenhouse conditions (Musiyiwa et al., 2005). It was essential to test for their performance under field conditions.

The aim of the present study was to screen promiscuous soyabean isolates, which proved superior to commercial

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Table 1. Soil characteristics for two smallholder fields in Goromonzi District, Zimbabwe, where the experiments were conducted.

Soil property	Chihwai	Masenyama
% Carbon	0.51	0.27
% Nitrogen	0.05	0.02
pH in Water	5.1	4.5
pH in $CaCl_2$	4.7	4.1
P (mg kg^{-1})	23	6
Texture	Sandy loam	Sandy loam
% Clay	11	12
MPN (cells g^{-1} soil)	37	23
MPN range at P [0.95]	8–176	5–112

inoculant strains under controlled conditions for their symbiotic performance under field conditions. Successful establishment of promiscuous soyabean isolates under field conditions would allow for the development of indigenous commercial inoculant strains and further exploit their wide host range and symbiotic capabilities. This would increase the inoculant genetic base and improve contributions of biological nitrogen fixation by soyabean to sustainability of cropping systems in Zimbabwe.

2. Materials and Methods

Site selection

Testing for the symbiotic performance of ten promiscuous soyabean isolates under field conditions was done from November 2001 to April 2002 at the Chihwai and Masenyama sites in Goromonzi District, about 50 km NE of Harare. The sites had no known history of soyabean production but had been under continuous maize cropping for 9 years. The total rainfall received at the study sites in the 2001/02 season was 460 mm (far below the normal annual range in this region of 750–1000 mm). Characterization of soil properties and rhizobial population sizes prior to planting showed both soils to be very sandy (less than 15% clay) with small quantities of nitrogen (<0.05%), carbon (<0.5%), pH (<5.5, pH water) and small populations of indigenous rhizobia of <50 cells per gram of soil (Table 1). Phosphorus availability (measured by extraction with acidified NH_4F and colorimetric analysis) at both sites was below 30 mg kg^{-1}, the critical limit for most plants (Table 1).

Selection of promiscuous soyabean isolates

A total of ten promiscuous soyabean isolates five from the variety Magoye (coded M1–M5) and five from variety Hernon 147 (H1–H5) were tested for their symbiotic performance in comparison with two reference commercial inoculant strains, MAR 1491 and MAR 1495, which are currently marketed as inoculants in Zimbabwe. The ten promiscuous soyabean isolates originated from four different districts in the country. M1, M3 and M4 originated from Guruve district while M2 and M5 were from Chikomba district. The Hernon isolates H2, H3, H4 and H5 were from Mhondoro district while H1 was from Chiweshe district. The isolates were stored on agar slants at 4°C.

Population counts of soyabean isolates

Isolates and reference strains on agar slants were initially grown in tubes containing yeast extract mannitol broth (YEM) on a rotary shaker until turbidity was observed. They were then sub-cultured on to YEM agar containing Congo red dye until uniform colonies of each isolate were obtained. The ten promiscuous soyabean isolates were quantified and standardized to equal cell populations before testing for their symbiotic N_2 fixation effectiveness in the field. Cell counts of soyabean isolates were determined using the Petroff-Hausser counting chamber under a light microscope. Each broth culture had approximately 10^9 cells per ml of broth.

Testing for symbiotic performance of isolates in the field

Two soyabean varieties (Magoye, a promiscuous variety; and Solitaire, a specific soyabean variety) were inoculated with each of the ten isolates and assessed for symbiotic performance at two smallholder sites. Two commercial inoculant strains MAR 1491 and MAR 1495 and an uninoculated control were included for comparison. Field sites were first ploughed with an ox-drawn mould board plough then levelled with a spike harrow before marking out 4 × 4 m field plots. Compound L basal fertilizer at a rate of 150 kg ha^{-1} was applied on all plots prior to planting by bending into furrows (to give N, P_2O_5, K_2O, S and B contents of 7.5, 25.5, 15, 12 and 0.78 kg ha^{-1}, respectively).

Inoculation was done by thoroughly mixing seed with 5 ml of inoculum in a plastic bag, before hand planting at a rate of 100 kg of seed ha^{-1} at each site. The 5 ml of inoculum contained 10^9 cells per ml of inoculum for each of the ten isolates and reference strains. Treatments were arranged on a completely randomized block design. Since the experiment took up a large area, only 3 replicates were used per site. Blocks were made across the slope.

Assessment of the symbiotic potential of ten promiscuous soyabean isolates

Nodulation of the two soyabean varieties was examined at eight weeks after planting (WAP) by carefully digging out plants with their entire root system from within 2 m of the row next to the guard row in each plot. Ten of these

plants were randomly picked and their roots separated from the plant tops, placed in plastic bags and transported to the laboratory. Nodule numbers per plant were counted.

Nodulation effectiveness was assessed by randomly sampling nodules from 5 plants per plot and noting their internal colour. Nodules with a red internal colour were classified as effective, pink weakly effective, while white or green colours were ineffective. At physiological maturity, a 2 × 2 m net plot consisting of 4 planting rows was harvested and assessed for grain yield and total dry matter yield by weighing, while total nitrogen was determined using the Kjeldahl method. Stover and total dry matter yields excluded the leaves since most of them had fallen off at the time of harvest.

All the data collected was analysed for variance of treatment means by carrying out an ANOVA test using the Genstat for Windows Statistical Package (Lawes Agricultural Trust, 1997).

3. Results

Nodulation of Magoye and Solitaire soyabean varieties inoculated with different isolates

Throughout the experiment differences were observed in the measured parameters based on the variety, isolate used and site. The effect of the isolate for example showed inoculation with strain MAR 1491 to result in the highest nodulation for Magoye at both sites than with all the ten soyabean isolates which varied considerably with site (Table 2). Five isolates i.e. H1, M2, M4, H4 and M5 resulted in significantly higher nodulation and three isolates M1, H2 and H5 gave equal nodule numbers to the standard strain MAR 1495 at Chihwai. The isolates H1, M2 and H4 resulted in significantly higher numbers while M1, H2, M4, M5 and H5 did not significantly differ in nodule numbers from MAR 1495 at Masenyama. Nodule numbers obtained were however generally low (<10 per plant) in all treatments.

Isolate M3 (together with M1, H3, M4, M5, H5 and the strain MAR 1491) proved to be very effective on Magoye since it gave nodules with a red internal colour at Chihwai and good soyabean biomass yield. Isolates M2, H2 and strain MAR 1495 were weakly effective (pink internal colour) and H1 and H4 ineffective at Chihwai. At the Masenyama site, M2, H2, M3, H4 and both reference strains formed effective nodules while M1, M4 and M5 were weakly effective and H1, H3 and H5 ineffective.

In the case of variety Solitaire, only two isolates, i.e. H4 and H5 resulted in significantly higher nodule numbers than obtained after inoculation with the reference strains MAR1491 and MAR1495 (Table 2). Isolates H1 and M2 failed to nodulate the variety Solitaire at either site. Unlike Magoye, the uninoculated Solitaire crop also failed to nodulate at either site. There were no significant differences in nodule numbers of the two varieties at the 2 sites. Four isolates H1, M3, H3 and H5 formed the most effective nodules with Solitaire, three, H4, M4, M5 and the reference strains were weakly effective while three M1, M2 and H2 were ineffective at both sites (Table 2).

Grain yield

Isolate M5 gave the highest grain yield for Magoye at Chihwai, while the remaining isolates gave significantly lower grain yield than obtained after inoculation with reference strain MAR1491 (Table 3). At Masenyama, M1, M4 and H1 gave higher yields than the reference strains. Grain yield attained in both the inoculated and uninoculated crop was quite poor for both soyabean varieties, i.e., less than 0.5 t ha^{-1} (Table 3).

In the case of the variety Solitaire, inoculation with isolate H1 significantly enhanced grain yield compared with the reference strains at Chihwai (Table 3). Isolate H5 also did not differ with the reference strains at both sites. Solitaire generally produced more grain than Magoye, ($P<0.001$). There were no significant site differences (Table 3).

Total dry matter yield

Isolate H2 gave a higher total dry matter yield for Magoye than the reference strains MAR1491 and MAR1495 at either site (Table 4). Dry matter yield response of Magoye to inoculation with only two isolates, i.e., H4 and M5, resulted in lower yield while the remaining isolates did not significantly differ in performance from the reference strains.

Inoculating Solitaire with isolate M1 resulted in lower yield than reference strains MAR 1491 and MAR 1495 at Chihwai, while H2, H3 and H5 gave higher yields than the strain MAR1491 at Masenyama (Table 4). Dry matter yield for Magoye was generally higher than that of Solitaire for most isolates except H4 ($P<0.001$).

Grain harvest indices (GHI)

Grain harvest indices (ratio of grain to total dry matter yield) were generally low (<0.2) and closely followed the trend for grain yield (Table 5). Isolate H2, which resulted in the highest total dry matter yield for Magoye but attained very low grain yield at either site also had a correspondingly low HI. Isolates M5 at Chihwai and M1 at Masenyama that produced high grain yield with Magoye also gave High GHI, while H1 at Chihwai, then M1 at Masenyama gave better GHI with Solitaire than the Reference strains. The variety Solitaire also had higher GHI than Magoye for most isolates except M5 and H4 at Chihwai.

Table 2. Nodulation response (number of nodules/plant) to inoculating 2 soyabean varieties with promiscuous soyabean isolates at 2 field sites in Goromonzi District. Zimbabwe.

Site	Variety	Control	Magoye isolate					Hernon isolate					Ref. strains	
		Uninoculated	M1	M2	M3	M4	M5	H1	H2	H3	H4	H5	R1	R2
Chihwai	Magoye	2	3	5	2	4	4	7	3	1	5	3	8	4
	Solitaire	0	1	0	1	1	2	0	2	3	5	7	1	4
Masenyama	Magoye	3	3	6	2	4	4	7	4	1	5	3	8	3
	Solitaire	0	0	0	1	0	3	0	2	4	6	9	2	5
SED							2.4							

Table 3. Grain yield (t ha^{-1}) response to inoculating 2 soyabean varieties with promiscuous soyabean isolates at 2 field sites in Goromonzi District. Zimbabwe.

Site	Variety	Control	Magoye isolate					Hernon isolate					Ref. strains	
		Uninoculated	M1	M2	M3	M4	M5	H1	H2	H3	H4	H5	R1	R2
Chihwai	Magoye	0.22	0.28	0.23	0.26	0.27	0.35	0.29	0.2	0.28	0.29	0.25	0.33	0.31
	Solitaire	0.30	0.35	0.32	0.23	0.32	0.29	0.46	0.28	0.28	0.22	0.37	0.34	0.36
Masenyama	Magoye	0.18	0.31	0.23	0.17	0.30	0.21	0.31	0.20	0.20	0.17	0.20	0.23	0.20
	Solitaire	0.25	0.32	0.28	0.34	0.3	0.28	0.31	0.28	0.24	0.32	0.34	0.33	0.32
SED							0.04							

Table 4. Dry matter yield (t ha^{-1}) response to inoculating 2 soyabean varieties with promiscuous soyabean isolates at 2 field sites in Goromonzi District. Zimbabwe.

Site	Variety	Control	Magoye isolate					Hernon isolate					Ref. strains	
		Uninoculated	M1	M2	M3	M4	M5	H1	H2	H3	H4	H5	R1	R2
Chihwai	Magoye	2.76	2.76	2.96	2.96	2.96	2.6	2.96	3.5	3.17	2.40	2.77	3.13	3.30
	Solitaire	2.16	2.03	2.43	2.43	2.43	2.43	2.56	2.6	2.6	2.43	2.60	2.43	2.60
Masenyama	Magoye	3.09	2.95	3.39	3.39	3.22	2.73	3.02	4.10	3.36	2.63	2.95	3.44	3.30
	Solitaire	2.33	2.13	2.66	2.66	2.66	2.66	2.69	2.9	2.9	2.66	2.90	2.66	2.73
SED							0.49							

Table 5. Comparison of the harvest indices (HI) of the 2 soyabean varieties at 2 field sites in Goromonzi District, Zimbabwe in response to inoculation with different soyabean isolates.

Site	Variety	Control	Magoye isolate					Hernon isolate					Ref. strains	
		Uninoculated	M1	M2	M3	M4	M5	H1	H2	H3	H4	H5	R1	R2
Chihwai	Magoye	0.08	0.10	0.08	0.09	0.09	0.13	0.10	0.06	0.09	0.12	0.09	0.11	0.09
	Solitaire	0.14	0.17	0.13	0.09	0.13	0.12	0.18	0.11	0.11	0.09	0.14	0.14	0.14
Masenyama	Magoye	0.06	0.11	0.07	0.05	0.09	0.08	0.10	0.05	0.06	0.06	0.07	0.07	0.06
	Solitaire	0.11	0.15	0.11	0.13	0.11	0.11	0.12	0.10	0.08	0.12	0.12	0.12	0.12

Table 6. Total N (kg ha^{-1}) accumulation of two soyabean varieties in response to inoculation with promiscuous soyabean isolates at 2 field sites in Goromonzi District of Zimbabwe.

Site	Variety	Control	Magoye isolate					Hernon isolate					Ref. strains	
		Uninoculated	M1	M2	M3	M4	M5	H1	H2	H3	H4	H5	R1	R2
Chihwai	Magoye	63	67	60	93	61	58	63	74	66	65	64	70	75
	Solitaire	51	38	47	67	62	62	68	55	72	59	68	60	61
Masenyama	Magoye	52	53	54	74	53	49	55	59	55	59	54	57	59
	Solitaire	45	32	46	57	59	51	62	47	64	50	60	55	50
SED							14							

Total N

With reference to Magoye inoculation with M3 resulted in the highest plant total nitrogen at both sites. At Masenyama isolates H2 did not significantly differ from the reference strains. Inoculation with only three isolates M2, M4 and M5 at Chihwai gave significantly lower total N contents than that of the uninoculated crop (Table 6).

Inoculating Solitaire with four isolates H1, M3, H3 and H5 gave higher while the two isolates M1 and M2 gave lower N values than the reference strains at Chihwai. At Masenyama however, three isolates H1, H3 and H5 gave significantly higher total N values than both reference strains with M3 and M4 also performing better than strain MAR1495. Magoye attained higher total N than Solitaire after inoculation with most isolates except three Hernon isolates H1, H3 and H5 at both sites ($P<0.05$).

4. Discussion

Nodulation

The below average nodule numbers obtained in this study could be due to the very low moisture and poor soil conditions at the 2 sites. Marino et al. (2007) showed drought to induce a decline in nodule water potential that result in a cell redox imbalance in legume plants. The total rainfall received during this study was 460 mm, far below a normal average of 750–1000 mm. This could have caused water stress on the plants giving very few nodules. The soils also had very low nutrients amounts, i.e., low N and P (Table 2), that could also have contributed to the poor nodulation.

Eight out of ten isolates did not significantly differ from the reference strain MAR 1491 in their ability to nodulate the promiscuous variety Magoye. Five isolates namely H1, M2, M4, H4 and M5 were actually superior in nodulation to the reference strain MAR 1495 at Chihwai while H1, M2 and H4 were superior to MAR 1495 at Masenyama. This shows that the promiscuous variety Magoye had a wide host range. Magoye also nodulated even in the uninoculated crop.

The specific variety Solitaire on the other hand nodulated better after inoculation with 2 Hernon isolates H4 and H5 than after inoculation with the reference strains MAR 1491 and MAR 1495. It did not nodulate without inoculation. This failure by Solitaire, a specific soyabean variety to nodulate without inoculation indicates that the indigenous rhizobia present in the soils were inadequate or could not nodulate this variety. Ruiz Sainz et al. (2005) noted that some soyabean varieties while highly effective with a particular set of rhizobial strains, are also poorly nodulated by the indigenous rhizobial populations of a particular soil due to their very specific nature. Studies in Zimbabwe also revealed host-strain specificity can lead to lack of detection of indigenous rhizobia present in the soil where a specific soyabean variety is used was use (Mpepereki and Makonese, 1995).

The Magoye isolate M3 formed the most effective nodules on both varieties despite its poor nodulation showing that nodule number is not a good measure for assessing the symbiotic effectiveness of an isolate (Ruiz Sainz et al., 2005). This study was carried out in the 2001/02, a season characterized by erratic rainfall and mid-season dry spells which could have resulted in poor nodulation of the soyabean crop. Giller (2001) noted that the absence of nodules on legume roots is not clear evidence of poor N_2 fixation potential of a rhizobial isolate, since environmental constraints such as inadequate moisture may lead to poor nodulation. Solitaire however, formed the most effective nodules after inoculation with three Hernon isolates H1, H3 and H5 and also the Magoye isolate M3 showed a better symbiotic performance with the Hernon than Magoye isolates.

Previous studies have shown that both Hernon and Magoye isolates form effective nodules with Magoye under controlled conditions (Musiyiwa et al., 2005). In the present study some isolates formed ineffective nodules (e.g. M1) or failed to nodulate Magoye (H3), indicating variability in performance when an isolate is placed in real field conditions where environmental constraints such as inadequate moisture, nutrient and low soil pH are limiting as opposed to the ideal conditions in the greenhouse. Soils from the two smallholder sites were characterized by low levels of nutrients and acidic soil pHs. O'Hara (2001) highlighted that rhizobia require C substrates and nutrients such as P and bases to enable their growth and survival in the soil. The poor nutrient status at the smallholder sites might have been unfavourable for good establishment and symbiotic performance of the rhizobial isolates.

Total dry matter and grain yield

Differences in relative amount of grain to stover production (harvest index) were observed with some isolates resulting in more grain than stover while the reverse was true for others. A good example was the Hernon isolate H2, which resulted in the highest total dry matter yield for Magoye but gave very poor grain yield at both sites. This means H2 was superior in facilitating more stover production with Magoye than grain. High stover production is desirable in providing livestock fodder and residual soil fertility benefits to the following crop in a rotation (Kasasa, 1999). The Magoye isolate M5 on the other hand, which gave the least total dry matter, resulted in the highest grain yield especially at the Chihwai site. This is beneficial to a farmer whose aim is to maximize yields but might offset the N balance in the soil since most of the N is removed when the grain is harvested.

The Hernon isolates H2, H3 and H5 proved superior through higher dry matter yield for Solitaire than strain MAR1491 while H1 and H5 resulted in high grain production for this variety. H4, which had previously appeared to be superior through better nodulation, biomass yield and comparable total dry matter yield, attained the least grain yield for Solitaire at the Chihwai site. This indicates that H4 induces more nodulation and stover production than grain production. The greater total dry matter of promiscuous Magoye than the specific Solitaire after inoculation with most isolates shows a wider host range by Magoye than Solitaire.

A very poor grain yield of less than 0.5 t ha^{-1} compared with high total dry matter yield of above 2 t ha^{-1} was recorded at both sites for both varieties. This could be attributed to inadequate soil moisture at the flowering and pod-filling stages. Patterson and Hudak (1996) noted that moisture stress during seed filling caused a reduction in duration of seed fill and the early senescence of leaves. They attributed this to lack of water in the plant for translocating photosynthates and nitrogen to the developing seed. Water deficit during pod filling also reduces photosynthetic activity of the plant as well as its nitrogen fixation potential leading to reduced seed yield (Cure et al., 1985).

The GHI was generally low and closely followed the trend for grain yield. This was also due to the very low rainfall (460 mm) experienced in this season. Sadras and Calviño (2001) observed significant association between yield and water deficit for the early vegetative period in wheat and for the early vegetative and grain-filling periods in soybean and maize. There is a need for further evaluation of the isolates over several seasons since the season in which this experiment was carried out was relatively dry, receiving a total rainfall of only 460 mm. A better performance by the isolates might be realized in a more favourable season.

Total plant N

In addition to giving high yields, Isolate M3 proved to be the most efficient N accumulater with Magoye since it resulted in the greatest total N. It even outperformed the reference strains MAR 1491 and MAR 1495 at Chihwai but was not significantly different from MAR1491 at Masenyama. The lower nodulation despite high yields and nitrogen content could be because nodulation is more sensitive to water stress than are general root and shoot metabolism (Zahran, 1999). The response of nodulation and N_2 fixation to water stress depends on the growth stage of the plants, with water stress imposed during vegetative growth being more detrimental to nodulation and nitrogen fixation than that imposed during the reproduction stage (Zahran, 1999). High N accumulation by M3 might also mean that this isolate induces more N uptake by the root system showing a great potential for use as a commercial inoculant strain. Daramola et al. (1994) noted that inoculation of soyabean with the most effective test strain resulted in greater N accumulation because of the substantial input from N_2 fixation, which consequently enhanced dry matter yields. Isolate H2 was also efficient at N_2 fixation effectiveness since it did not significantly increase the total N accumulated by Magoye with the reference strains at either site.

Inoculating Solitaire with isolates H1, H3 and H5 at both sites and M3 at Chihwai resulted in significantly higher total N than with the reference strains showing high potential for use as commercial inoculants. Isolates M1, H2 and M2 and also H4 and strain MAR1495 at Masenyama however were less efficient at fixing N with Solitaire. Magoye generally portrayed a wider host range since it attained increased total N than Solitaire after inoculation with most isolates except the Hernon isolates H1, H3 and H5 ($P<0.05$).

5. Conclusion

Isolate M3 was superior in N_2 fixation effectiveness with variety Magoye than the reference strains MAR 1491 and 1495 while isolates H1, H3 and H5 were superior with Solitaire. These four isolates have a high potential to be used as commercial inoculants. Even though the remaining isolates sometimes gave high yields and nodule numbers, the increases were too inconsistent so they cannot be recommended for commercial inoculant production since they ultimately resulted in lower total N values. Large differences were found between the isolates in harvest index with the soyabean genotypes, indicating an interesting potential to manipulate partitioning of dry matter and N in grain legumes through selection of rhizobial strains.

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