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EVALUATION OF INDIGENOUS RHIZOBIAL ISOLATES IN SEARCH FOR CANDIDATE STRAIN FOR COMMERCIAL PRODUCTION

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ABSTRACT
High cost of Nitrogen (N) fertilizer coupled with its potential deleterious effect on soil properties necessitates the need for alternative sources. Grain legumes in association with rhizobia contribute immensely in nitrogen accrual to agricultural system by fixing N from the atmosphere. An essential component for increasing the use of grain legumes is research leading to the selection and development of effective strains of rhizobia for commercial use. Fifty (50) indigenous rhizobial strains were collected from the gene bank of Soil Microbiology Laboratory at Institute for Agricultural research (IAR), Ahmadu Bello Zaria and screened for their symbiotic effectiveness. The test crop was Soybean variety TGx 1448-2E grown in 3 kg pot in screenhouse at the same Institute. Treatments included the 50 different strains, 3 commercial strains and 2 controls (-N and +N controls). Nodulation and dry biomass production were used to assess the performances of the inoculants vis-à-vis the commercial ones and the controls. The result showed that 70% of the strains outperformed the controls and commercial strains in terms of nodule number, 74% nodule dry weight while 18% recorded higher dry matter yield over control. However only 14%, 26% and 1% recorded significantly higher nodule number, nodule dry weight and dry matter yield respectively over the controls and commercial strains. Outstanding strains include SAMFIX036, SAMFIX074, SAMFIX113, SAMFIX306, SAMFIX369, SAMFIX381 and SAMFIX221. Hence, further characterization and field evaluation across multiple locations need to be carried out on these strains, as they may be suitable candidates for commercial production.
Keywords: Candidate strain, Dry-biomass, Nodulation, Rhizobia isolates, SAMFIX.

INTRODUCTION

Sustainable agriculture depends on soil fertility and health. But low yield are often experienced due to continuous cropping without replenishment. One major way of replenishing soil fertility is through application of mineral fertilizers. However, because of the cost involved, many farmers cannot afford it thereby applying below recommendation. Consequently, research efforts have been directed towards integrated nutrient management (INM), in which leguminous crops play a key role (Mafongoya *et al.*, 2007) due to their nitrogen fixing ability. Biological Nitrogen Fixation (BNF) is a key source of N for farmers who use little or no fertilizer, especially for legumes such as soybean (Smaling *et al.*, 2008). Based on the several other studies, it has been widely shown that up to 80% of the aboveground N accumulation in soybean is due to BNF (Hungria *et al.*, 2006). Depending on the legume variety, net soil N accrual from the incorporation of grain legume residue can be as much as 140 kg N ha^{-1} if only seeds are harvested (Giller, 2001). This is by far more than 50 kg nutrient ha^{-1} recommendation of the summit meeting of African head of states held in Abuja, Nigeria in order to improve agricultural productivity and hence food security. Biological Nitrogen fixation has therefore been used in farming systems to cut down on fertilizer expenses (Shamseldin, 2007). Grain legumes fix atmospheric nitrogen by working symbiotically with special bacteria, rhizobia, which live in their root nodules. The fixed nitrogen is then used by the grain legumes or carried-over in the soil for the next year's crop. Therefore, the successful use of grain legumes in cropping system depends upon appropriate formation of effective symbioses with these bacteria. An essential component for increasing the use of grain legumes is the appropriate research leading to the selection of elite strains of rhizobia.

Evidence from various strain screening programs indicates that yields of several grain legumes can be improved substantially by inoculation with compatible rhizobia. However, research to select the best strains is often piecemeal, lacking rigorous screening of large numbers of isolates. The aim of this research therefore, is to screen large number of isolates in searching for candidate strain(s) for commercial production.

MATERIAL AND METHODS

Bio-prospection

The screening exercise starts with bio-prospection in which nodules of soybean were sampled from three agro-ecological (Southern Guinea Savanna (SGS) Northern Guinea Savanna (NGS) and Sudan Savanna (SS)) zones of Nigeria. Sampling sites were spread in various geographical locations (Table 1). The sampled nodules were stored in a plastic vials containing cotton wools underneath which containing silica gel. The vials were kept in the lab at room temperature until isolation. The isolates were kept in the gene bank at – 80˚C.

Table 1: Inoculant strains and description of the sampling sites (2016).

S/N	Inoculant	Ecological zone	Location	Longitude	Latitude
1	SAMFIX097	SS	Warawa	E008.690167	N11.9729
2	SAMFIX224	SS	Garko	E008.812417	N11.67942
3	SAMFIX205	SGS	Kachia	E007.973617	N9.843383
4	SAMFIX339	SS	Dawakin kudu	E008.719967	N11.83513
5	SAMFIX245	SS	Garko	E008.93825	N11.59038
6	SAMFIX302	NGS	Tudun Wada	E008.3566	N11.24617
7	SAMFIX256	SS	Garko	E008.92625	N11.58275
8	SAMFIX287	SS	Minjibir	E008.667133	N12.14323
9	SAMFIX275	SGS	Kachia	E007.969133	N9.8112
10	SAMFIX347	SS	Albasu	E008.983	N11.66993
11	SAMFIX326	SS	Dawakin kudu	E008.58725	N11.85205
12	SAMFIX315	SS	Wudil	E008.9215	N11.6556
13	SAMFIX163	SS	Albasu	E009.19255	N11.676
14	SAMFIX154	SS	Albasu	E009.132783	N11.67317
15	SAMFIX306	NGS	Giwa	E007.388283	N11.34945
16	SAMFIX129	SS	Bichi	E008245217	N12.22797
17	SAMFIX135	SS	Bichi	E008.29685	N12.20575
18	SAMFIX174	SS	Dawakin kudu	E008.58725	N11.85205
19	SAMFIX036	NGS	Soba	E007.919883	N11.02645
20	SAMFIX043	NGS	Soba	E007.92	N11.02662
21	SAMFIX003	NGS	Giwa	E007.3817	N11.27788
22	SAMFIX080	NGS	Igabi	E007.643	N10.94175
23	SAMFIX113	SS	Wudil	E008.9215	N11.6556
24	SAMFIX074	NGS	Igabi	E007.687117	N10.7816
25	SAMFIX221	SS	Garko	E008.812417	N11.67942
26	SAMFIX359	NGS	Kubau	N10. 37708	E 008.40692
27	SAMFIX353	SS	Warawa	N11.88573	E 008.74815
28	SAMFIX397	SS	Warawa	N11.88573	E 008.74815
29	SAMFIX355	SS	Warawa	N11.86805	E 008.74295
30	SAMFIX399	SS	Warawa	N11.86805	E 008.74295
31	SAMFIX365	SS	Bunkure	N11.69337	E 008.56103
32	SAMFIX406	SS	Bunkure	N11.69337	E 008.56103
33	SAMFIX366	NGS	Giwa	N11.24892	E 007.25378
34	SAMFIX407	NGS	Giwa	N11.24892	E 007.25378
35	SAMFIX368	NGS	Giwa	N11.25678	E 007.25215
36	SAMFIX409	NGS	Giwa	N11.25678	E 007.25215
37	SAMFIX369	NGS	Giwa	N11.26011	E 007.27394
38	SAMFIX371	SS	Shanono	N12.03446	E 008.00910
39	SAMFIX373	SS	Shanono	N12.04047	E 008.02526
40	SAMFIX375	SS	Shanono	N12.04438	E 008.02065
41	SAMFIX414	SS	Shanono	N12.04438	E 008.02065
42	SAMFIX376	SS	Shanono	N12.03934	E 008.02147
43	SAMFIX379	SS	Shanono	N12.04260	E 008.02147
44	SAMFIX381	NGS	Tudun Wada	N11.28076	E008.42105
45	SAMFIX382	NGS	Tudun Wada	N11.33968	E 008.40641
46	SAMFIX383	NGS	Tudun Wada	N11.28018	E008.42166
47	SAMFIX418	NGS	Tudun Wada	N11.33968	E 008.40641
48	SAMFIX422	NGS	Tudun Wada	N11.31669	E008.43719
49	SAMFIX388	NGS	Tudun Wada	N11.33701	E 00.40556
50	SAMFIX392	NGS	Igabi	N10.80128	E 007.67453

SGS= Southern Guinea Savanna; NGS = Northern Guinea Savanna; SGS= Southern Guinea Savanna; and SS= Sudan Savanna

Isolation, growing and multiplication of rhizobial strains

Firstly, a yeast mannitol ager (YMA) was prepared as outlined by Woomer *et al*. (2010). A nodule from each vial was selected and surface sterilized to remove all the surface microorganisms (Woomer *et al*., 2010). Each of the nodules was then transferred into fresh and sterilized petri dish and crushed open using flame sterilized spatula thereby making the rhizobia to break loose into solution in the petri dish. A flame sterilized wire loop was used to scoop a loopful of rhizobia solution and then streaked on the already prepared YMA plate. Finally the plates were incubated at room temperature inside an incubator. Plates were observed every day to see if there were growths. Plate for each isolate was observed and colonies were confirmed. The grown colonies were then stored in a vial of YMA at 4˚C for further use. This makes the bank of the isolates, which contains as much as 600 strains isolated from different grain legumes including soybean, groundnut, cowpea and Bambara groundnut. This screening study focused only on soybean isolates as the aim was only to produce soybean inoculums. Hence, 50 strains isolated from soybean nodules were randomly selected from the bank (Table 1) and used for the screening exercise.

The rhizobial strains were grown and multiplied in a yeast mannitol broth (YMB) as outlined by Woomer *et al*. (2010). Each of the selected strain was then inoculated into respective 250 ml Erlenmeyer flask containing YMB. The inoculated YMB for each isolate was incubated in a rotatory shaker for at least a week for the isolates to mature.

Screenhouse experiment

The experiment was conducted in a screenhouse at the Department of Soil Science, Institute for Agricultural Research (IAR), Ahmadu Bello University Zaria (latitude $11^0$12''N and longitude $07^0$37''E on the elevation of 638m) Nigeria. The soil was taken from IAR's field at 0-15cm depths, bulked, air-dried, sieved through 4mm mesh and weighed into plastic pots (3 liters). Three kilograms of soil were put in the plastic pots. Optimum amount of nutrients solutions were applied into the soil and left to equilibrate for 24 hours. This was then followed by planting a soybean seeds at rate of 3 seeds/pot and thinned down to 1 plant / pot a week after germination. The treatments included 50 isolates from the gene bank (Table 1), 2 controls (-N (control) and +N (Nitrogen)) and 3 reference strains (GraphEx, Legumefix and Nodulaid) replicated 3 times and laid in a completely randomized design (CRD). The experiment was managed by irrigating the plants every day as required. At two weeks after planting (WAP), the seedlings were inoculated at the root zone (5ml of each inoculant was injected with a syringe at around rhizosphere).

Harvesting

Plants were harvested at eight weeks after planting. The plants were carefully removed from the pots, the shoots were cut at the soil surface level using sharp knife. The shoots were taken to the laboratory and oven dried at 60˚C to constant weight for dry weight measurement. The roots were carefully washed in water making sure that the nodules were not detached from the roots. The roots were then taken to the lab where the nodules were carefully removed. Nodule number was determined as well as their dry weight. The roots were then oven dried at 60C to determine their dry weights.

Statistical analysis

All data collected were subjected to analysis of variance (ANOVA) Using SAS software 9.4. Differences between means were separated using Duncan multiple range test (DMRT). Correlation analyses were used to compare relationships between variables.

RESULTS

The figures presented showed comparison between the commercial inoculants, tested strains and the control. Here, the control is used as the benchmark for the comparison. It is therefore considered that those strains that surpassed control statistically are candidate strains for commercial production.

Analysis of nodule number following inoculation showed that there was highly significant ($P<0.001$) difference among the isolates, commercial inoculants and the controls (Figs 1a to 1d). Each figure compares ten different strains alongside controls and standard commercial inoculants.

From Fig 1a, it showed that 70% of the strains have higher nodule number than the control. However, only 30% were found to be statistically ($P<0.01$) higher than the control. These were notably SAMFIX 036, SAMFIX 074 and SAMFIX 113. Also, Fig. 1b showed that 70% of the tested strains were higher than control but only SAMFIX 221 1d showed that 70% and 80% of the inoculants respectively had higher nodule number. SAMFIX 306 (Fig. 1c), SAMFIX 369 and SAMFIX 381 (Fig. 1d) were found to be significantly ($P<0.01$) higher than control. While 60% of the Fig. 1e recorded higher nodule number, none of them were statistically different from the control. Generally, 70% of the strains tested recorded higher nodule number compared to the control. However, only 14% were found to be statistically higher than the control.

Analysis of nodule dry weight has shown that 74% of the tested strains recorded higher nodule number than control (Table 2). However when statistically analyzed, only 26% recorded significantly (P<0.01) higher nodule dry weight.

Analysis of the dry matter yield has shown that only 18% of the tested strains recorded higher dry matter yield (Table 2). However, only SAMFIX 221 recorded a significantly (P< 0.05) higher dry matter yield compared to control.

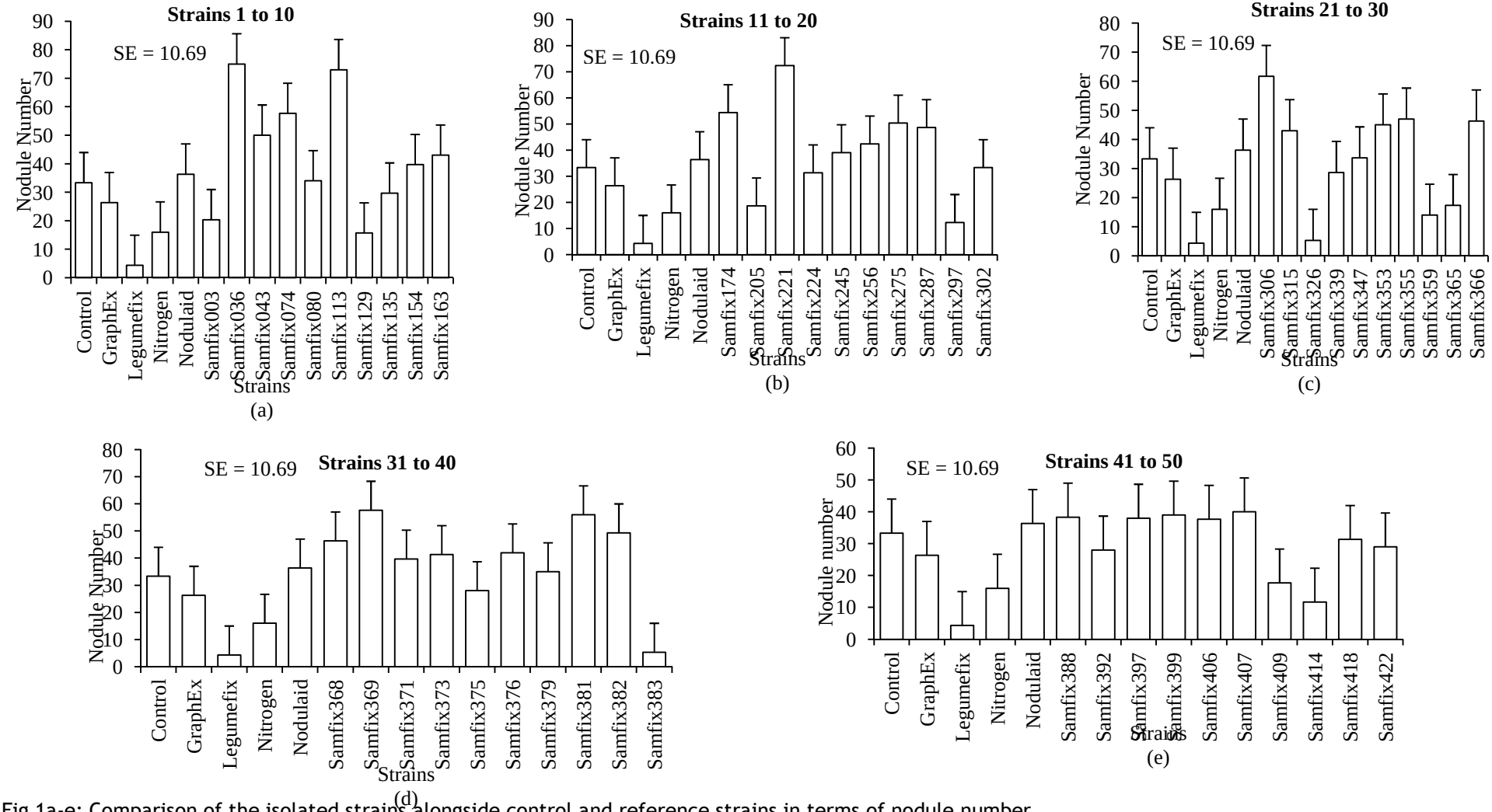

Fig 1a-e: Comparison of the isolated strains alongside control and reference strains in terms of nodule number

Table 2: Mean nodule dry weight and dry matter yield of soybean as affected by the isolated

S/N	Treatment	Nodule Dry Weight (mg)	Dry Matter Yield (g)	S/N	Treatment	Nodule Dry Weight (mg)	Dry Matter Yield (g)
1	Control	110.00	8.18	29	SAMFIX339	209.33	7.73
2	GraphEx	97.00	5.13	30	SAMFIX347	217.67	8.51
3	Legumefix	8.67	5.20	31	SAMFIX353	233.33	7.54
4	Nitrogen	39.33	7.24	32	SAMFIX355	300.33	8.04
5	Nodulaid	91.00	6.61	33	SAMFIX359	65.33	6.56
6	SAMFIX003	87.00	6.44	34	SAMFIX365	72.33	6.61
7	SAMFIX036	309.33	8.08	35	SAMFIX366	301.00	9.90
8	SAMFIX043	265.00	7.61	36	SAMFIX368	209.00	6.80
9	SAMFIX074	188.33	7.46	37	SAMFIX369	138.33	6.53
10	SAMFIX080	172.00	8.65	38	SAMFIX371	134.33	7.76
11	SAMFIX113	301.00	7.92	39	SAMFIX373	137.00	6.38
12	SAMFIX129	43.67	6.32	40	SAMFIX375	254.67	8.66
13	SAMFIX135	149.00	7.45	41	SAMFIX376	91.98	5.58
14	SAMFIX154	271.33	7.10	42	SAMFIX379	125.36	6.58
15	SAMFIX163	167.67	7.81	43	SAMFIX381	221.00	8.71
16	SAMFIX174	170.67	7.77	44	SAMFIX382	188.67	8.97
17	SAMFIX205	55.00	6.22	45	SAMFIX383	13.33	4.97
18	SAMFIX221	383.67	10.33	46	SAMFIX388	116.33	6.60
19	SAMFIX224	163.00	6.98	47	SAMFIX392	105.33	6.25
20	SAMFIX245	136.00	7.67	48	SAMFIX397	169.00	7.01
21	SAMFIX256	118.67	5.66	49	SAMFIX399	240.67	8.53
22	SAMFIX275	249.00	8.22	50	SAMFIX406	216.33	6.68
23	SAMFIX287	223.00	7.44	51	SAMFIX407	236.33	7.61
24	SAMFIX297	51.00	6.81	52	SAMFIX409	81.00	7.23
25	SAMFIX302	105.00	5.69	53	SAMFIX414	27.67	4.14
26	SAMFIX306	199.67	7.68	54	SAMFIX418	159.67	5.67
27	SAMFIX315	259.00	7.82	55	SAMFIX422	169.00	6.65
28	SAMFIX326	7.67	4.44				
		SE = 56.78	**SE = 0.97**			**SE = 56.78**	**SE = 0.97**

strains, controls and reference strains.

The Pearson correlation matrix was carried out to see if there were relationships between the measured variables (Table 3). It was found that nodule number was significantly (P< 0.0001) correlated with both the nodule dry weight and dry matter yield.

Table 3: Correlation matrix between nodule number dry weight and dry matter yield.

	Nodule number	Nodule Dry Weight	Shoot Dry Weight	Root Dry Weight	Dry Matter Yield
Nodule number	-				
Nodule Dry Weight	0.589***	-			
Shoot Dry Weight	0.336***	0.773***	-		
Root Dry Weight	0.072 ^{NS}	0.316***	0.519***	-	
Dry Matter Yield	0.291***	0.716***	0.962***	0.733***	-

*** = P<0.001; NS = Not Significant

DISCUSSION

Effectiveness of isolated strains of rhizobia is usually improved through screening of large number of rhizobial inoculants that have the capacity of acclimatizing themselves to specific soil environment to which they are applied. The result of the screening exercise showed that there were variations in nodulation and dry matter yield assessment. Some of the strains exhibited a higher nodulation and dry matter yield compared to the controls, reference strains and other isolates.

This shows that the nodulation potential of these strains and their competitive ability vary based on their origin i.e. where they were isolated from, the characteristics of the soils they were isolated from etc. This simply implies that some isolated strains are more efficient than the others in terms of symbiotic effectiveness. Rhizobia strains of different origin vary in their symbiotic efficiency (Zaman-Allah, 2007). Also, Fening and Danso (2002) reported that Bradyrhizobia number and effectiveness vary considerably among locations.

Among the isolated strains tested, SAMFIX 036, SAMFIX 074, SAMFIX 113, SAMFIX 306, SAMFIX 369, SAMFIX 381 and SAMFIX 221 were found to be outstanding. They demonstrated higher symbiotic efficiency than the other isolates, reference strains and controls. They can therefore be recommended as candidate strain for commercial production. Isolates of indigenous rhizobium have been demonstrated to produce successful results (Sessitsch *et al.,* 2002). A good example is *Rhizobium tropici* PRF81, isolated from Brazilian soil and recommended as a commercial inoculant for common bean (*Phaseolus vulgaris* L.) in Brazil since 1998 (Hungria and Vargas, 2000). Similarly, Drevon *et al*. (2001) isolated 49 indigenous rhizobia from Morocco and Tunisia and found that they are at least as efficient as commercial inoculant CIAT899 in symbiosis with local cultivar. The reference strains and some other isolates failed to improve the nodulation and dry matter of soybean. It is possible that rhizobial cells in these strains did not survive the storage process and therefore were in poor condition at the time of their application. The success of commercial inoculants is dependent on the number of viable bacteria available to participate in the infection process at the point of use (Catroux *et al.*, 2001). Hiltbold *et al*. (1980) reported that nodulation of soybean was directly related to number of rhizobia with no nodulation by the product supplying $<10^3$ rhizobia/seed and abundant by about 10^5 to 10^6 rhizobia/seed.

The analysis of the correlation coefficient matrix clearly revealed significant positive relationship between nodule number and dry matter yield (r = 0.291***) and the nodule dry weight with dry matter yield (r = 0.716***). This implies that nodule number and nodule dry weight can be used as criteria for prediction of inoculation response as opined by Nambiar (1985) and Wange (1989).

CONCLUSION

The study on evaluation of indigenous rhizobial isolates has shown that some of the indigenous rhizobial strains have higher symbiotic efficiency than others, which resulted in higher nodulation and dry biomass yield. Certain strains outperformed the commercial strains and controls statistically and therefore were recommended as best candidates for commercial production. However, further characterization and field evaluation across multiple locations are necessary to authenticate their veracity as commercial inoculants.

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