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IMPROVEMENT OF NODULATION AND NITROGEN FIXATION OF GROUNDNUT (*Arachishypogaea* L.) USING PASTURE AND GRAINLEGUME RHIZOBIA ISOLATES INDIFFERENT SOILMANAGEMENT.

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Abstract

A greenhouse experiment was conducted to examine the effect of pasture (*Centrosema pubescence, Centrosemapascorum, Mycrotlomauniflorum, and Mucuna pureness*) and legume (*Vigna unguiculata-2, Vigna subterranean-2, and Arachishypogaea*) rhizobia isolates on nodulation and nitrogen fixation of groundnut (Samnut 24) under two different soil types i.e. cultivated soil (CS) and fallowed soil (FS). Fresh nodules were obtained, crushed and rhizobia isolates extracted which were later inoculated via liquid inoculation to groundnut. All agronomic practices were followed and harvesting was done at eight week after planting. Results revealed that response of groundnut to both pasture and legume rhizobia inoculation was significantly higher ($p< 0.0001$) in CS than in FS in all the parameters measured with the exception of root dry weight, this may be due to the fact that the experiment was conducted in pot which limit root growth. Significant differences were observed between the isolates in terms of number of branches, number of pegs, number of pods, root length, nodule number, and phosphorus uptake,with one isolates or the other performing better than the un-inoculated control. Significant soil versus isolate interactions was also observed in terms of plant height, number of branches, root length, root dry weight, and phosphorus uptake. Correlation analysis showed that nodule number significantly correlated with shoot dry weight, number of branches, number of pods, number of pegs, nodule dry weight, dry matter yield, and ultimately nitrogen fixation (r = 0.66, 0.76, 0.64, 0.74, 0.92, 0.66, and 0.54 respectively).

Keywords: Groundnut, Soil-Type, Pasture/legume Rhizobia, Inoculation

Introduction

In an attempt to solve the issue of food security, bio-fertilizers (Rhizobia inoculation) play an important role in increasing crop yield and nutrient content of a soil by enhancing growth and yield of leguminous crops (Ahmad *et al.*, 2013; Khaitov *et al.*, 2016) as well as non-leguminous crops (Ziaf *et al.*, 2016). Legumes being the third largest family of flowering plant, play an important role economically and ecologically in natural and agricultural systems (Sprent *et al.*, 2017) by forming symbiosis relationship with rhizobia in their root nodules. The nodules of the legumes contain two types of bacteria, the rhizobia and endophytic bacteria (Velázquez *et al.*, 2017). Endophytic bacteria function in plants is still poorly known (Velázquez *et al.*, 2017), while rhizobia play many roles in plant such as biological nitrogen fixation (Jordan, 1982). Rhizobia also have the ability to secrete siderophores, phytohormones, and solubilize insoluble phosphates(Vargas *et al.*, 2017) thereby increasing the availability of phosphorus to both host and non-host plant. It also elicit plant defence reactions against phytopathogens(Vargas *et al.*, 2017), thereby decreasing the amount of pesticide use.Theyalso have ability to help host plant reduce effect of environmental stress, including drought (Oliveira *et al.*, 2017).

Groundnut (*Arachishypogaea*L.) isa leguminous crop that fixes nitrogen, but are still limited in their growth by the amount of nitrogen available, it therefore requires the supply of mineral nitrogen in fertilizer or requires the aid of its symbiotic nodule forming bacterial partners, rhizobia, for N_2 fixation. Result revealed that rhizobia inoculation of groundnut increases the number of shoots of groundnut and soil N.P.K. (Yusif *et al.*, 2016), and also help the plant to increase number of nodulesand nitrogen fixing ability (Voravit and Somabhi, 1989). This resulted in saving cost of nitrogen fertilization (Hungria and Campo, 2005) through reduced use of expensive synthetic fertilizers and pesticides in agricultural practices (Vargas *et al.*, 2017).

Even though there are well documented researches on beneficial effect of rhizobia inoculation, there is dearth of information regarding response of groundnut to pasture rhizobium isolates and responses of the isolates under different land uses. Therefore this paper tries to look at how to improve nodulation and nitrogen fixation of groundnut (*Arachishypogaea L.*) using pasture and grain legume rhizobia isolates under continuous cultivation and fallowed soil.

Materials and Methods

Experimental site and Sampling

The experiment was conducted in a greenhouse at the Institute for Agricultural Research (IAR) station (latitude 11^{0}09.974′N and longitude 007^{0}37.968′E on the elevation of 698m) Zaria, Nigeria. Two bulk soil samples collected from IAR research fields were used for the experiments. The fields are Field S2 (N11^{0}10.541’ and E007^{0}36.590‘and 696m above sea level) and Field S13 (N11^{0}11.08‘and E007^{0}60.998ˋ and 701m above sea level) which are the Fallowed Soil (FS, More than 30 years of fallow) and the Cultivated Soil (CS, Cultivated annually) respectively. The soils were taken from five points in each field at 0-15cm depth, bulked, air-dried, sieved through 4mm mesh and weighed into PVC (3 litres) pots. The soils were mixed with nutrient solutions and left for 24 hours to equilibrate.

Soil and plant analysis

The physicochemical analyses of the experimental soils were determined thus: Soil pH by 1:1 soil/water ratio(IITA, 1982); Total organic carbon using Fourier Transform Near Infrared Reflectance Spectroscopy (Pearson *et al.*, 2014); Cation exchange capacity by saturation with 1N ammonium acetate and extraction of ammonium with 2M potassium chloride(Anderson and Ingram, 1994); Exchangeable acidity by titration method after extraction with 1N KCl(Anderson and Ingram, 1994); Effective CEC (ECEC) by summation of exchangeable cations; Soil texture by Gee method (Gee, 2005);available phosphorus by Bray-1 method(Sims *et al.*, 2009).

The plant samples were digested in a hot sulphuric acid solution with selenium catalyst(Novozamsky *et al.*, 1983). For the N analysis, the Berthalot (indophenol reaction) method was adapted from Searle(1984)); Nitrogen fixation was determined using N difference method (Hatfield *et al.*, 1974).

Isolates used for the Experiment

Tenrhizobia isolates which were isolated from nodules of *Centrosema pubescence, Centrosemapascorum, Mycrotlomauniflorum, Mucuna pureness, Vigna unguiculata-2, Vigna subterranean-2,* and*Arachishypogaea* and one standard strain (Biofix) were used for the experiment (Table 1). Standard method of Rhizobium isolation was followed(Somasegaran and Hoben, 1985).

Table 1: Inoculants used for the Experiment

Legume crop	**Code**
1.Centrosema pubescence	CPI01
2. Centrosemapascorum	CPI02
3.Mycrotloma uniflorum	MUI03
4. Mucuna pureness	MPI04
5.Vigna unguiculate	VUI05
6.Vigna unguiculate	VUI06
7.Vigna subterranean	VSI07
8.Vigna subterranean	VSI08
9.Arachis hypogaea	AHI09
10.Biofix	Standard

Greenhouse trial

The greenhouse experiment was inoculation trials with groundnut (Samnut 24) as test crop on two soils under two different management status; Cultivated and Fallowed. The treatments for each soil type include: Control (minus isolates, minus mineral N), Reference (minus isolates, plus mineral N) and ten (10) rhizobium isolates (plus isolates, minus mineral N) replicated three times, making a total of twelve treatments per soil type. The experimental design was a completely randomized design (CRD). Inoculation was done with the liquid inoculants using syringe around the rhizosphere after plant emergence. All agronomic practices were followed including taking measurements. Harvesting was done at eight weeks after planting. The shoots were cut at the soil surface using a sharp knife and were taken to the laboratory, weighed and then oven dried at 60^0C for 48 hours for the plant tissue N analysis. The roots were carefully washed in running tap water making sure that the nodules were not detached from the roots. The roots were then taken to the laboratory and the nodules were carefully removed. Number of noduleswere determined as well as their fresh and dry weights.

Statistical analysis

Analysis of variance (ANOVA) was used to investigate the effects of the measured parameters, where there is significant differences, Post-hoc Tukey tests was used for means separation. Correlation analysis was carried out to determine the linkages between the measured parameters.Rcommander(Fox and Andersen, 2005) in R Statistical software (Version 3.0.1, (R_Core_Team, 2014)) was used for all the analysis.

Results and Discussion

The physical and chemical properties of the two soils used for the experiments are shown in Table 2. The FS is sandy clay loam while CS is sandy loam in texture, both soils are mildly acidic in nature and the acidic nature of the two soils reflected the common nature and feature of Savannah soils (Jones and Wild, 1975).The organic carbon content of the two soils is very low which is much lower than critical level of 1.5g/kg (Okalebo *et al.*, 2002). The available P in FS is much lower than critical level of 7mg/kg,whereas the available P in CS is quite moderate(Enwezor *et al.*, 1990).Analysis of exchangeable cations shows that both soils were low(Marx *et al.*, 1999). The effective cation exchange capacity (ECEC) was higher in CS than in FS. In general, the CS can be described as medium fertility soil while FS low.

Table 2: Physical and chemical propertiesof the experimental soils

		Test Value	
Property	**Unit**	**CS**	**FS**
pH (H_2O)		5.52	5.82
Organic C	g/kg	0.062	0.046
Total Nitrogen	g/kg	0.95	0.12
Mehlich-3 P	mg/kg	9.95	2.80
Exchangeable Base Cation	Cmol$^{(+)}$/kg		
Ca		0.21	0.11
Mg		0.02	0.02
K		0.82	0.40
Na		0.26	0.28
Exchangeable Acidity	Cmol$^{(+)}$/kg	0.40	0.40
ECEC	Cmol$^{(+)}$/kg	1.71	1.21
Sand	%	43.28	43.28
Silt	%	28	26
Clay	%	28.72	30.72
Textural Class		Sandy loam	Sandy Clay loam

Effect of soil type and rhizobia isolates on groundnut (Table 3 and Table 4) reveals that, all the parameters observed performed higher in *CS* than *FS*with the exception of root dry weight which shows no significant statistical difference.This is due to planting in pots which restricted the root growth thereby resulting in same root weight (Mathers *et al.*, 2007).Higher value ofparameters observed in *CS*may bedue to its higher fertility status than *FS* notably as a result of much higher available P and the exchangeable cations (Table 2) and these results in increased nodulation with resultant N uptake leading to higher biomass accumulation and nitrogen fixation in the *CS* compare to *FS*. Phosphorus availability has been widely reported to have a significant positive effect on biomass production, N accumulation and nodulation(Marschner, 1995; Mapfumo *et al.*, 2005; Pang *et al.*, 2018). Increase in shoot dry weight, numberof nodules, nodule mass, nodule size and nodulation index werenoted with phosphorus availability (Somado *et al.*, 2003; Kuang *et al.*, 2005).Also, phosphorus is required for the normal functioning of nitrogen fixing bacteria (the micro symbiont) and thus has favourable effect on number of nodules and weight of the effective nodule formation on the root system(Mapfumo *et al.*, 2005). Thus, lower nodulation and N_2 fixation in *FS* were as a result of lower available P. Phosphorus deficiency can decrease nodulation and N_2 fixation (Tang *et al.*, 1992; Giller, 2001). In plants relying on N_2 fixation, P stimulates nodulation and N_2 fixation more than it stimulates plant growth(Gentili and Huss-Danell, 2003).

Table 3: Influence of soil type and rhizobia isolates on plant growth parameters in groundnut

Treatment	Plant Height (cm)	No. of Branches	No. of Peg	No of Pods	Root length (cm)
Soil					
Cultivated Soil	29.44^{a}	32.8^{a}	4.94^{a}	4.94^{a}	37.72^{a}
Fallow Soil	22.52^{b}	20.75^{b}	1.89^{b}	1.89^{b}	29.25^{b}
Mean	25.98	26.775	3.415	3.415	33.485
SE	0.50	0.60	0.42	0.25	1.16
Inoculant					
AHI09	28.33	26^{bc}	14.5^{a}	3.17^{b}	32.33^{abcd}
Biofix	26.27	26^{bc}	11.5^{abc}	2.83^{b}	36.50^{abc}
CPI01	24	26.33^{bc}	14.67^{bcd}	4.33^{ab}	29.50^{bcd}
CPI02	23.83	24.83^{c}	10.33^{c}	2.67^{b}	28.00^{cd}
Control	26.17	29^{ab}	12.33^{abc}	2.67^{b}	37.67^{ab}
MPI04	24.83	26^{bc}	11.33^{abc}	4.33^{ab}	34.00^{abcd}
MUI03	25.33	24.67^{c}	10.33^{c}	3.00^{b}	29.33^{bcd}
Nitrogen	27.17	31.5^{a}	14^{ab}	5.33^{a}	34.33^{abcd}
VSI07	25.5	25.17^{c}	10.67^{bc}	2.67^{b}	38.17^{ab}
VSI08	27.83	30.5^{ab}	14.5^{a}	4.17^{ab}	26.00^{d}
VUI05	24.83	25.33^{c}	11.5^{abc}	2.67^{b}	41.33^{a}
VUI06	27.33	26^{bc}	11.67^{abc}	3.17^{b}	34.67^{abcd}
Mean	25.95	26.78	12.28	3.42	33.49
SE	1.22	1.45	1.03	0.62	2.83
Soil x Inoculant	**	**	NS	NS	**

NS not significant, ** Significant at P<0.01 and SE is the Standard error of difference of means, means followed by the same letter are statistically the same

Table 4: Influence of soil type and rhizobia isolates on yield components, nodulation and nitrogen fixation in groundnut

Treatment	Shoot dry weight (g/plant)	Root dry weight (g/plant)	Dry matter yield (g/plant)	Number of Nodule	Nodule dry weight (mg/plant)	Nitrogen fixation (kg/ha)
Soil						
Cultivated Soil	4.95^{a}	0.61^{a}	5.56^{a}	221.44^{a}	108.56^{a}	124.68^{a}
Fallow Soil	3.64^{b}	0.66^{a}	4.31^{b}	57.72^{b}	31.14^{b}	95.33^{b}
Mean	4.30	0.64	4.94	139.58	69.85	110.01
SE	0.13	0.038	0.13	6.05	4.43	4.03
Inoculant						
AHI09	4.53^{a}	0.55^{a}	5.08^{a}	116.83^{cd}	55.00^{a}	110.12^{a}
Biofix	4.72^{a}	0.52^{a}	5.24^{a}	132.67^{abcd}	79.00^{a}	120.40^{a}
CPI01	4.35^{a}	0.59^{a}	4.94^{a}	180.50^{a}	99.33^{a}	126.86^{a}
CPI02	3.72^{a}	0.68^{a}	4.40^{a}	134.50^{abcd}	66.00^{a}	99.63^{a}
Control	4.32^{a}	0.48^{a}	4.80^{a}	121.00^{bcd}	64.50^{a}	111.24^{a}
MPI04	3.75^{a}	0.86^{a}	4.61^{a}	167.33^{ab}	77.50^{a}	96.27^{a}
MUI03	4.15^{a}	0.81^{a}	4.96^{a}	160.83^{abc}	82.50^{a}	101.48^{a}
Nitrogen	4.88^{a}	0.49^{a}	5.37^{a}	106.33^{d}	48.50^{a}	123.70^{a}
VSI07	4.27^{a}	0.75^{a}	5.02^{a}	129.17^{bcd}	63.50^{a}	99.40^{a}
VSI08	4.92^{a}	0.60^{a}	5.52^{a}	166.50^{ab}	82.17^{a}	126.90^{a}
VUI05	3.67^{a}	0.68^{a}	4.34^{a}	132.83^{abcd}	65.00^{a}	95.54^{a}
VUI06	4.30^{a}	0.66^{a}	4.96^{a}	126.50^{bcd}	55.17^{a}	108.53^{a}
Mean	4.30	0.64	4.94	139.58	69.85	110.01
SE	0.31	0.09	0.33	14.82	10.86	9.88
Soil x Inoculant	NS	NS	NS	NS	**	NS

NS not significant, ** Significant at P<0.01 and SE is the Standard error of difference of means, means followed by the same letter are statistically the same

Analysis of the isolates (Table 3 and Table 4)revealed that variation in measured parameters were observed among the treatments in terms of number of branches, number of pegs, number of pods, root length and number ofnodules, while no statistical differences were observed in terms of plant height, dry matter yield, nodule dry weightand nitrogen fixation. This indicates that some isolates are higher in nodulation quality than others, because rhizobia strains of different origin vary in their symbiotic efficiency(Zaman-Allah *et al.*, 2007; Drevon *et al.*, 2015). Some rhizobia strains did not show significant difference in measured parameters compared with the uninoculated control, it is possible that rhizobia cells in these strains have low nodulating ability than the others, because the success of the isolatesto serve as inoculants depends on the number of viable bacteria available to participate in the infection process at the point of use(Catroux *et al.*, 2001; Bashan *et al.*, 2014). While for those isolates that performed better than the uninoculated control, the rhizobia isolates may have some genetic characters which may be responsible for improved micro (bacteria) and macro (host) symbiosis leading to higher competitive ability of the isolates over the indigenous rhizobia species. The interesting part of the result is with the number of nodules and nodule dry weight. A significant differencewasobserved between the isolates in terms of number ofnodules, while in terms of nodule dry weight, there was no significant differences.. This is to show that the content and amount of rhizobia in the root nodules is what matters and not the number of nodules.

A significant interaction was observed between the soil and the isolates in terms of plant height, number of branches, root length, and nodule dry weight (Figure 1, 2,3 and 4. Interaction in plant height and nodule dry weight shows that more than 60%of the isolates performed better than uninoculated control in *CS* than *FS*, these confirm that even though there wasno significant difference between the isolates, *CS*performedbetter than *FS*, and this can be used to infer that different management practices couldhave different responses to rhizobia inoculation (Imran *et al.*, 2015). One of the beauty of the result is that in all the interactions, many isolates performed better than the uninoculated control indicating that pasture and legume rhizobia isolates were able to improvedimportant attributes of groundnut (Samnut24).

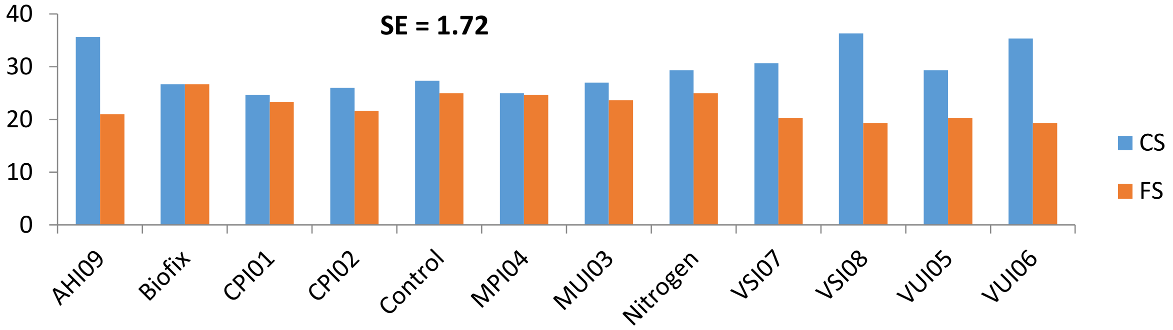

Figure 1: Interaction of soil type and pasture rhizobium isolates on groundnut plant height

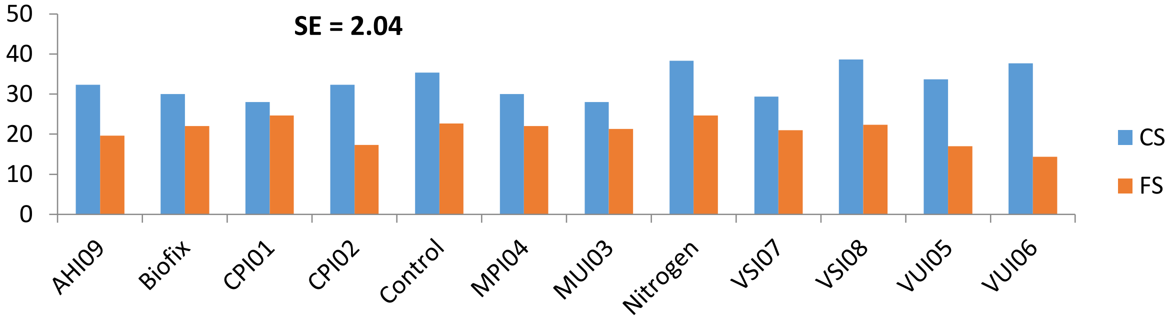

Figure 2: Interaction of soil type and pasture rhizobium isolates on groundnut number of branches

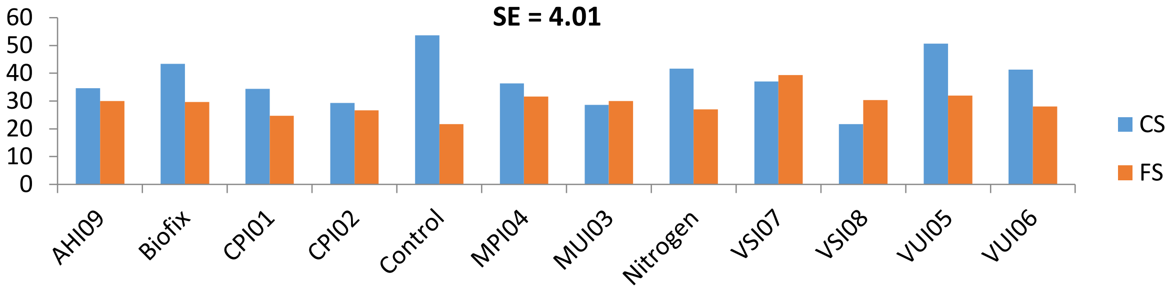

Figure 3: Interaction of soil type and pasture rhizobium isolates on groundnut root length

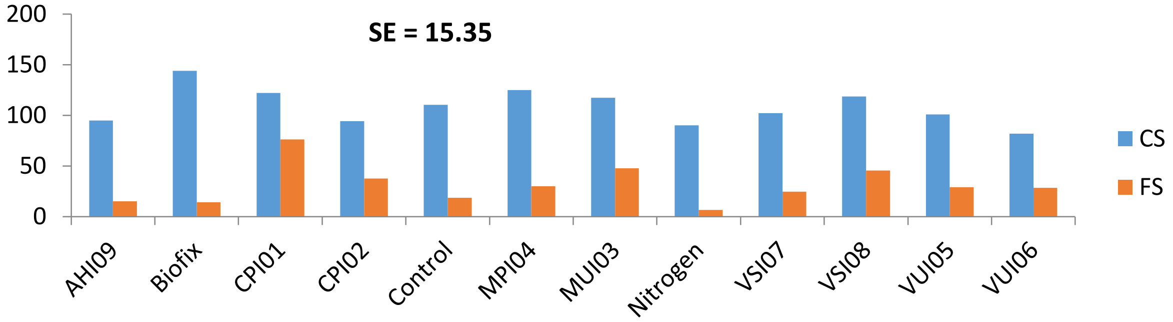

Figure 4: Interaction of soil type and pasture rhizobium isolates on groundnut nodule dry weight

Correlation Analyses (Table 5) shows that number of nodules significantly and positively correlated with shoot dry weight, number of branches, number of pod, number of peg, nodule dry weight, dry matter yield, and ultimately nitrogen fixation (r = 0.66, 0.76, 0.64, 0.74, 0.92, 0.66, and 0.54 respectively). This can be used to infer that significant increase in nodulation and nitrogen fixation of groundnut can be improved through inoculation with pasture and grain legume rhizobia isolates.

Table 5: Correlation analysis between the measured parameters

	Plant height	No. of Branches	No. of Peg	No. of Pod	Root length	No. of Nodule	Nodule dry weight	Shoot dry weight	Root dry weight	Dry matter yield	Nitrogen fixation
Plant height	1.000	0.733	0.582	0.560	0.184	0.592	0.467	0.772	-0.359	0.706	0.720
No. of Branches	0.733	1.000	0.675	0.730	0.404	0.760	0.657	0.762	-0.267	0.719	0.682
No. of Peg	0.582	0.675	1.000	0.658	0.310	0.635	0.624	0.637	-0.272	0.589	0.551
No. of Pod	0.560	0.730	0.658	1.000	0.281	0.748	0.719	0.663	-0.194	0.635	0.545
Root length	0.184	0.404	0.310	0.281	1.000	0.363	0.383	0.247	0.285	0.328	0.115
No. of Nodule	0.592	0.760	0.635	0.748	0.363	1.000	0.924	0.656	-0.046	0.666	0.542
Nodule dry weight	0.467	0.657	0.624	0.719	0.383	0.924	1.000	0.633	0.019	0.659	0.506
Shoot dry weight	0.772	0.762	0.637	0.663	0.247	0.656	0.633	1.000	-0.248	0.969	0.868
Root dry weight	-0.359	-0.267	-0.272	-0.194	0.285	-0.046	0.019	-0.248	1.000	-0.003	-0.320
Dry matter yield	0.706	0.719	0.589	0.635	0.328	0.666	0.659	0.969	-0.003	1.000	0.815
Nitrogen fixation	0.720	0.682	0.551	0.545	0.115	0.542	0.506	0.868	-0.320	0.815	1.000

Conclusion and Recommendation

In general, the response of groundnut (Samnut 24) to inoculation with pasture andgrain legume rhizobia isolates wasmore in CS than FS. This could be as a result of higher available P and the exchangeable cations in CS than FS. Phosphorus availability has been widely reported to have a significant positive effect on biomass productivity, N accumulation and Nodulation. Hence it is important to know the phosphorus status of a soil before embarking oninoculation exercise.

In conclusion,thestudy has shown that fertility status of soil has tremendous effect on the response of groundnut to inoculation. Therefore, soil fertility management strategy should be considered when embarking on inoculation exercise. The pasture rhizobia and grain legume rhizobia isolates were able to improve many important attributes of groundnut (Samnut24) and all the isolates are homologous rhizobia strains to groundnut, as they all formed nodules with groundnut. They can therefore be further evaluated under different soil condition, in the field and on different varieties to authenticate the veracity of their efficacy.

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