Assessment of Growth, Nodulation and Nitrogen Fixation in Different Varieties of Cowpea in A Humid Tropical Environment Using N-Difference Method

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***Abstract*—The study aimed at assessing the growth, nodulation and nitrogen fixation of different varieties of cowpea using nitrogen difference methods. A complete randomized design with seven treatments and ten replications were used. The trial was conducted in a green house using soil as growth medium. The treatment consists of six varieties of cowpea (IT93K-734, IT86D – 719, IT84S -2246-4, IT89KD-391, IT90K-227-2, RC1) and a rice variety (RP1045-25-2-1) as control. Seeds were planted and grown under green house condition. At four weeks stage of growth, leaf number, plant height, Nodule number, root and shoot dry matter production were determined. Biologically fixed nitrogen was determined using the N-difference method. Result showed variety IT89KD-391 had the highest number of nodules (66.20g/pot) while RCI had the lowest number of nodules (45.00 g/pot).The variety IT86D-719 gave the highest concentration of Soil N(4.9gkg^{-1}) and subsequently fixed the highest quantity of nitrogen (550.96kg ha^{-1}) more than other varieties. Varieties IT90K-277-2 and IT84S-2246-4 produced the highest shoot and root dry matter yield. Hence varieties IT90K-277-2 and IT84S-2246-4 could be considered along with variety IT86D-719 for amelioration of inherent low fertility of the soil through organic matter amendment. Further studies under field condition are recommended to confirm these findings.**

Keywords—Assessment, cowpea varieties, Humid tropical, N-difference method, Nitrogen fixation

1. INTRODUCTION

Legumes play a vital role in soil fertility maintenance through biological nitrogen fixation [1]. Of a total of 139 million tons biologically fixed N added to the system annually on a global scale, 25% comes from legumes [2]. The amounts of N_2 fixed and the N contribution from leguminous crops such as cowpea,

soyabean, pigeonpea are influenced by a number of environmental factors, including soil type, nutritional status of soil, species and varieties, water availability and temperature as well as soil and crop management [3]. Under favorable conditions, legume such as cowpea can add nitrogen equivalent between 64 and 131 Kg N ha^{-1} Yr [4] and 200 Kg N ha^{-1} of ammonium sulphate [5].

Cowpea cultivation has become an integral part of the farming systems [6] and is grown in mixtures with other crops in various combinations [7]. Cowpea is an important source of organic fertilizer [8], because it nodulates freely and fixes atmospheric nitrogen in the soil [9]. [10] been reported that indigenous varieties of cowpea contain 22-24% crude protein on dry weight basis while improved varieties contain 23-35% crude protein [11]. [12] noted that the combination of cow dung and NPK 20-10-10 on varieties of cowpea contributed to the differences in number of count obtained per plant of cowpea as well as the amount of N-fixed. However, [13] stressed the capability of bacteria in root nodules of cowpea to convert atmospheric nitrogen to nitrate for plant use. Nitrogen obtained from biological nitrogen fixation is especially important for the subsistence farmer as costs of N fertilizer continue to escalate. Enhanced nodulation tend to lead to increased N_2 fixation which, at the long run can substantially minimize the need for application of external inputs of mineral fertilizers in the production system to the benefits of millions of Africa's subsistence of farmers [14]. Growing cowpea as a green manure for incorporation in the soil has several beneficial effects. The most important is its increase in the available nitrogen content of the soil, resulting from the rapid decomposition of plant material with a relatively low C/N ratio. It is particularly beneficial for sandy soils that are low in organic matter where nitrogen applied could easily leached out [15].

In soils of Southeastern Nigeria, pressure due to population increase has led to the intensive use of land for agriculture, resulting to soil quality deterioration as well as soil organic matter losses, low acidity, low nutrient content, structure degradation and low crop yield [16]. The low crop yields are often attributed to low nitrogen content, low organic matter and available phosphorus [17] [18]. Low fertility tends to hinder optimum crop production which has been a problem encountered by most farmers in the area. Organic matter inputs through organic amendment such as incorporation of legumes (Cowpea) as green manure into the soil, to nutrient supply would represent a management strategy that would counteract depletion of organic matter in soils [19]. In addition to nutrient supply, there would be stimulation of microbial diversity and activity [20]. The study therefore was conducted to assess the growth, nodulation, and nitrogen fixation of different varieties of cowpea in a humid tropical environment using N-difference method.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted at Michael Okpara University of Agriculture Umudike lying between latitude 07^0 33'N and longitude 05^0 29' E with an elevation of 122m above sea level. The geological material from which the soil was formed is Coastal Plain Sand [21]. The area is characterized by bimodal rainfall with one peak in July and the second in September and an annual rainfall of 2175 mm. Temperature is high and ranges from $26\text{-}31^0$ C. Tropical rainforest is the dominant vegetation of the area. Farming is a major socio-economic activity and soil fertility regeneration is by bush fallow which is fast disappearing due to population pressure.

2.2 Field Studies

The study was conducted in a green house at Michael Okpara University of Agriculture Umudike. The experiment was a completely randomized design with seven treatments and Ten replications. The treatments consisted of six varieties of cowpea namely IT 93K-734, IT86D-719, IT84S-2246-4, IT89KD-391, IT90K-227-2, RC1 and a rice variety RP 1045-25-2-1 which serve as a control. Varieties IT93K-734, IT86D-719, IT 84S-2246-4, were brought from IITA (Ibadan) while varieties IT 89 KD-391, IT 90K-277-2, RCI and RP 1045-25-2-1 control) were brought from the farm centre at Michael Okpara University of Agriculture Umudike. Soils were randomly collected from different points at a depth of 0-15 cm using an auger. The soil samples were bulked together to form a composite samples. The bulked samples were air-dried and sieved using a 2 mm sieve. Ten kilograms of sieved soils were weighed into different plastic containers. Soil samples were collected from each pot before planting and at harvest for laboratory analyses. Seed viability test was carried out in each variety before planting. Planting was done at the rate of 4 seeds /pot. Germination commenced four days after sowing. The seedlings were later thinned down to three plants per pot, two weeks after planting. No fertilizer was applied. The plants were watered throughout the duration of the experiment. Weeds were kept under control by hand weeding as necessary. The plants (cowpea varieties and non-fixing reference crop, rice) were kept in pots under daily observation in the green house for six weeks.

At four weeks stage of growth, data were collected on number of leaves per plant, plant height (cm) and number of nodules per pot. At six weeks after planting that is at flowering stage of growth, each plant were gently pulled out from the soil in each pot and placed on a sieve to avoid loss of nodules during cleaning. The roots were washed gently with water to remove the adhering soils. Nodules were then carefully removed from the root, counted and recorded. The harvested plants (cowpea and rice) were dried and subsequently oven-dried at a temperature of 65^0C for 48hours. The dry weight of shoot and root of each treatment were taken and milled using a milling machine. The total nitrogen in shoot, root and soil was determined using microkjeldahl method. The amount of nitrogen in the cowpea and rice (control) was calculated using N-difference method described by [22] below:

N fixed = N_{leg} cowpea – N_{ref} (rice) "equation 1"

Where N fixed = fixed nitrogen in the soil

N_{leg} cowpea = Amount of nitrogen (N uptake in mg/plant) in legume (cowpea)

N_{ref}(rice)= Amount of nitrogen (N uptake in mg/plant) in the reference crop (rice).

2.3 Laboratory Analyses

Particle size distribution was determined by hydrometer method [23]. Soil pH was determined in water using 1:2.5 Soil –liquid ratio [24]. Exchangeable cations (Ca, Mg, K and Na) were determined by 1 N Ammonium acetate extraction procedure [24]. Exchangeable potassium (K) and sodium (Na) in the extract were determined by flame photometer while exchangeable calcium (Ca) and Magnesium were determined using atomic absorption spectrophotometer. Exchangeable acidity was measured in 1N KCL [25]. Organic carbon was determined by the wet oxidation method [26]. Total Nitrogen was determined by the Kjeldahl digestion method [27]. Available phosphorus was determined by Bray 2 method [28].

2.4 Data Analysis

Data were subjected to Analysis of variance (ANOVA) using SAS statistical package version 9.1. Means were separated using fisher's least significant difference (FSLD) at $P < 0.05$. Correlation analysis was carried out to determine the relationship between the growth parameters and N-fixation in the soil.

3. RESULTS AND DISCUSSION

3.1 The Physicochemical Properties of Soils of the Study Site

The results of the physico-chemical properties of soil in the study sites as shown in Table 1 indicated that the soils were sandy with coarse sand-sized fraction predominating over silt and clay. The sandiness could be attributed to coastal parent materials from which the soils are derived. Silt and clay contents were low. The soils are strongly acidic with pH value of 4.34, implying occupation of the soils exchange complex with aluminum cations. Exchangeable bases (Ca, Mg, K and Na) were low, suggesting possible influence of climate and land use. High rainfall amount, duration and intensity may have increased leaching of basic cations [29], leaving the acidic cations. Exchangeable acidity was fairly high (TEA=2.02 cmolkg^{-1}), indicating accumulation of aluminum at the exchange site and deficiency of calcium and magnesium in the soil. Organic matter was high (OM=3.03 gkg^{-1}) but lower than the critical level of 20 gkg^{-1} given by [30] for soil of the humid tropical region .Total N content was low, indicating high rate of mineralization in the soil. Available p was low (AV. P = 2 .90 mgkg $^{-1}$) probably due to low pH (4.34). At pH of 4 and 4.5, p is likely to be fixed by the oxide of Fe and Al [31] indicating reduction of phosphorus availability in soil [32]. Effective cation exchange capacity (ECEC) was generally low (ECEC = 4.26 cmolkg^{-1}) but was higher than the critical value of 4.00 cmolkg-1 recorded by [33].

Table 1: Physicochemical Properties of the Studied Soil

Sand	gkg^{-1}	704.0
Silt	gkg^{-1}	80.0
Clay	gkg^{-1}	216.0
pH ($H_2$0)		4.34
Exchangeable bases		
Ca	cmolkg^{-1}	1.04
Mg	cmolkg^{-1}	0.84
K	cmolg^{-1}	0.26
Na	cmolkg^{-1}	0.10
TEB	cmolg^{-1}	2.24
TEA	cmolkg^{-1}	2.02
ECEC	cmolkg^{-1}	4.26
BS	%	53.0
OM	gkg^{-1}	3.03
TN	gkg^{-1}	0.19
AV.P	mgkg^{-1}	1.90

Ca = Calcium, Mg = Magnesium, K = Potassium, Na = Sodium, TEB = Total exchangeable base, TEA = Total exchangeable acidity, ECEC= Effective cation exchange capacity, BS = base saturation, OM = Organic matter, TN = Total Nitrogen, AV.P = Available phosphorus

The low ECEC of this soil may be attributed to the fact that soils of Southeastern Nigeria are strongly weathered, have little or no content of weathered rock in their Sand and Silt fractions and have predominantly Kaolinite as their clay fraction [34]. Percentage base saturation was above 50% indicating high fertility.

3.2 Growth parameters and dry matter yield of different varieties of cowpea and rice (control) in the study site.

The growth parameters and dry matter yield of different varieties of cowpea and rice (control) given in Table 2 and Plate 1 showed that there were significant differences in the growth habit, nodulation and dry matter production of these plants. For the growth habit, varieties IT 86D-719, IT 84S-2246-4, IT89 KD-391, IT90K-277-2 and RCI were erect while IT93K-734 had a climbing feature (Plate 1). The shoots of IT93K-734, IT86D-719, IT84S-2246-4, IT89KD-391, IT90K-277-2 had green color while variety RCI had purple color. The calyx color of the varieties was green with the exception of IT86D-719 which had purple color. The leaves of RCI, IT90K277-2, were broad while varieties IT89KD-391, IT84S-2246- 4, IT86D-719, IT93K-734 and control (rice) had narrow leaves. However, the leaf number for the control (rice) (rice=38.00, $P<0.05$) was significantly higher compared to the leaf number of the cowpea varieties. Comparing the different varieties of cowpea in leaf number, variety IT84S-2246-4 produced the lowest number of leaves (17.70; $P<0.05$) compared to other varieties. Variety RCI produce higher number of leaves relative to IT86D-729 (17.70) and IT 93K-734 (21.20) **(**Table 2) Furthermore, the plant height of variety IT93K-734 was significantly higher than IT86D-719, IT84S-2246-4, IT89KD-391 and RCI ($P<0.04$) (Table 2). The variation in height of the cowpea varieties may be attributed to their growth habit along with environmental conditions and edaphic factors [35]. Variety IT93K-734 had a climbing habit (Plate 1) which was responsible for its higher height compared to other varieties.

Table 2: Growth Parameters and Dry matter Yield of different Varieties of Cowpea and Rice in the Studied Site.

Treatment	Leaf Number Per plant	Plant Height (cm)	Nodule Number/pot (g/pot)	Shoot dry matter (g/pot)	Root dry matter (g/pot)
IT93K-734	21.20	55.80	46.70	14.74	2.71
IT86D-719	17.70	29.30	51.30	15.49	2.45
IT84S-2246-4	23.50	29.40	55.33	14.94	3.84
IT89KD-391	22.60	28.30	66.20	15.33	3.08
IT90K-277-2	24.80	44.40	49.40	16.40	3 12
RCI	26.90	26.90	45.00	8.60	1.91
Control (rice)	38.00	62.10	-	15.90	9.12
F-LSD (0.05)	5.33	13.84	3.17	2.63	

Plate 1: Growth Parameters and Nodules of different varieties of cowpea and rice

However, the lowest plant height was observed in IT89KD-391 (28.30cm) probably due to the effects of shading from rice (control) and some other cowpea varieties.

Also, the number of nodules per plot in Table 2 showed that significant difference existed between nodule number for the cowpea varieties studied (P<0.05). Variety IT89KD-391 had a significant higher number of nodules per pot (66.20 nodules per pot) compared to other varieties. The higher number of nodules may be related to varietal differences as a result of genetic composition [36]. The difference in the number of nodules observed among cowpea varieties in this study agrees with the result of earlier studies [37] [38]. Also, the lowest number of nodules per pot observed in RCI (45.00 nodule number per pot) may be related to the acidic nature of the soil [39]

[40]. [39] noted few rhizoid in soils greater than pH 4.2. [41] reported that N-fixation activities of beneficial organisms are inhibited in strongly acidic soils. Additionally, the low starter nitrogen (N) in this soil (low in nitrogen) may have resulted to the lowest nodulation in this variety (RCI). [42] also reported that when initial levels of available soil nitrogen were low, a period of nitrogen hunger can reduce nodulation. However, between RCI and IT93K-734; IT90K-277-2 and IT 93K-734, no significant difference was observed in nodule number per plot.

Furthermore, in the shoot dry matter production in Table 2, variety IT90k-277-2 produced the highest shoot dry matter (16.40 g/pot; P< 0.05) more than other varieties. While the lowest shoot dry matter was observed in RCI (8.60 g/pot; P< 0.05). Comparing the shoot dry matter production in rice (control) and cowpea varieties, a significant difference was observed between shoot dry matter production in control (rice) (15.90 g/pot) and RCI (8. 60 g/pot; P<0.05) compared to other cowpea varieties (Table 2).

Also, in Table 2 control (rice) gave the highest root dry matter production than the cowpea varieties. Similarly, when comparing the cowpea varieties for root dry matter production, variety IT 84S-2246-4 produced the highest root dry matter yield (3.84 g/pot), implying that where the above plant material is harvested for feeding animals, the variety (IT 84S-2246-4) hold good promise for improving soil organic matter through the contribution of the root system.

3.3 Concentration and Fixation of nitrogen in shoot, root and soil of different varieties of cowpea and rice

Table 3 and 4 shows the results of the concentration and fixation of nitrogen in shoot, root and soil between the different varieties of cowpea and control (rice). The concentration of N in shoot, root and soil was higher in cowpea varieties than in control (rice) (Table 3). However, between the cowpea varieties, the concentration of N in shoot was highest in variety IT89KD-391 (32.3 gkg^{-1}) and lowest in RCI (26.6 gkg^{-1}). The lowest concentration of N in shoot in RCI could be related to the growth performance which includes: Plant height, nodulation, shoot and root dry matter of this variety. Also, in the root system, the amount of nitrogen (N) accumulated was highest in two varieties of cowpea: IT90K-277-2 (N=24.2 gkg^{-1}) and IT 84S-2246-4 (N=24.1 gkg^{-1}) and lowest in IT 86D-819 (N=15.9 gkg^{-1}) (Table 3). Generally, when comparing the concentration of N in shoot and roots, the highest concentration of N was in shoot while the lowest was in the roots. This was expected as absorbed plant nutrients are first translocated to the leaves through the stem, the root usually has the least reserve [36]. Indeed, much of the variation in leaf photosynthetic capacity for different cultivars, age of leaves and growth conditions can be attributed directly to differences in leaf N content [43]. Also, the cowpea varieties gave higher soil N than the control (rice) (Table 3). Comparing the soil N among the cowpea varieties, it was observed that variety IT86D-719 produced the highest soil N (4.9 gkg^{-1}) possibly due to varietal difference as a result of genetic composition [36].

Table 3: Concentration of Nitrogen (gkg^{-1})in Shoot, Root and Soil

Treatment	Shoot	Root	Soil
	gkg^{-1}		
IT93K-734	32.3	16.2	2.5
IT86D-719	29.6	15.9	4.9
IT84S-2246-4	32.3	24.1	2.2
IT89KD-391	35.1	22.6	2.3
IT90K-277-2	32.8	24.2	2.2
RCI	26.6	16.9	2.8
RP 1045-25-2-1 (Control)	19.2	8.9	2.1

Table 4: Fixed – N (kg/ha) in Shoot, Root and Soil

Treatment	Shoot	Root	Soil	Total
IT93K-734	1.03	0.11	80.00	81.14
IT86D-719	0.85	0.11	550.00	550.96
IT84S-2246-4	1.04	0.34	20.00	21.40
IT89KD-391	1.32	0.22	40.00	41.54
IT90K-277-2	1.19	0.26	20.00	21.50
RCI	1.13	0.23	130.00	131.40

Additionally, increase in nitrogenous activity after sowing may have contributed to the high soil N in this variety [44]. The lowest total N in soil was found between varieties IT84S-2246-4 (Soil N=2.2 gkg^{-1}); IT90K-277-2 (Soil N=2.2. gkg^{-1}) and IT 89KD -391 (Soil N = 2.3 gkg^{-1}) ("Table 3") and could be attributable to plant uptake, immobilization and volatilization [45] [46]. Nitrate-N is immobilized by soil flora or fauna [22]. Considering the whole nitrogen fixed by different varieties of cowpea in the soil (Table 4), variety IT86D-719 fixed the highest N in the soil (fixed N = 550.96 $kgha^{-1}$), followed by RCI (Fixed N = 131.40 kg ha^{-1}) and IT93K-734 (81.14 kg N ha^{-1}). One would have expected IT89KD-391 with its highest nodule number (nodulation) (66.20) to fix higher N more than other varieties. However, [43] reported that greater nodulation (greater nodule number) does not necessarily lead to greater nitrogen fixation. Therefore, the higher N – fixed in soil by cowpea variety (IT860-719) could be related to the genotype difference, rhizobium strain and differences in rooting characteristics of the cowpea varieties used in this study [12]. [47], asserted that the amount of nitrogen fixed by a legume crop varies widely due to legume genotype, rhizobium strain and the soil environment. Furthermore, the variety RP 1045-25-2-1 (control) did not contain much N in shoot, root and soil (Table 3), the Nodule number could not be determined and consequently, the fixation of nitrogen was not considerable. However, the low fixation of N in soil was found in varieties IT84S-2246-4 (Fixed-N = 21.40 kgha-1) and IT90K-277-2 (21.50 kg N ha-1) (Table 4) and may be related to soil pH, soil moisture, and shading probably from other varieties and control (rice). [48] noted that moisture stress can greatly reduce nitrogen fixation by reducing the number of bacteria (rhizobium) growth and activities of nodule already formed and restricting the production of new ones. The result of the correlation analysis in Table 5 showed that a significant negative relationship existed between fixed – N in soil and leaf number ($r = - 0.72^*$; $P< 0.05$).The lack of relationship between fixed – N in soil and other growth parameters (plant height, nodule number, shoot dry matter and root dry matter) indicated that fixation of nitrogen in this soil was characterized by genotype or varietal differences, rhizobium strain, and soil pH.

Table 5: Correlation between growth parameters and the distribution of fixed – N in Shoot, Root and Soil

Growth Parameters and Fixed – N	Leaf number/ pot	Nodule Number/ Pot	Plant height	Root Dry Matter	Shoot Dry Matter	Shoot	Root	Soil
Leaf Number/ Pot	1							
Nodule Number/ Pot	-0.01	1						
Plant height	-0.11	0.26	1					
Root dry matter	-0.04	-0.56*	0.06	1				
Shoot dry matter	-0.58*	0.04	0.35	0.67*	1			
Shoot	0.63*	0.30	-0.06	0.18	-0.05	1		
Root	0.67*	-0.51*	-0.38	0.61*	-0.06	0.44	1	
Soil	-0.72	0.17	-0.27	-0.47	0.03	-0.76	-0.63	1

* = Significant ,P< 0.05.

4. CONCLUSION

Soil of the study site is highly degraded and acidic. Sand sized fraction predominated over other particle size fractions. Cowpea varieties resulted in increased soil N in the study site. The variety IT86D-719 -719 gave the highest concentration of soil N (4.9 gkg^{-1}) and subsequently fixed the highest quantity of nitrogen (550.96 Kg N ha^{-1}). Varieties IT90K-277-2 and IT84S-2246-4 gave the highest shoot and root dry matter yield and may therefore be considered along with variety IT86D-719 for amelioration of the inherent low fertility of the degraded soils of Umudike through organic matter amendment such as incorporation of the these cowpea varieties as green manure to the soil. Further studies under field conditions are recommended to confirm these findings.

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