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Nodulation and Symbiotic Nitrogen Fixation by Groundnut (*Arachis hypogaea* L) Genotypes as Influenced by Inorganic Nitrogen Fertilizer in the Northern Guinea Savanna of Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author ABU designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors EI and JIM managed the literature searches, author MT managed the experimental process and performed the statistical analyses. All authors approved the final manuscript.

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ABSTRACT

Evaluation of existing groundnut genotypes for nitrogen fixation may be useful as selection criteria for high nitrogen fixation capacity. The objective of this study was to determine extent of effective nodulation and symbiotic nitrogen fixation by groundnut (*Arachis hypogaea L*) genotypes. The treatments consisted of ten groundnut genotypes (SAMNUT 24, SAMNUT 22, ARRORSICGX-SM 00017/5/P_{15}/P_2, SAMNUT 10, 6AT, ICIAR 7B, ARRORSICGX 000201/5/P_4P_{10}, SAMNUT 21, SAMNUT 23 and SAMNUT 14) and two rates of nitrogen fertilizer (0 and 30 kg/ha) and were laid out in a split plot design with three replications. There was significant variation in nodulation in most of the selected variables in the two years of the trial with 2011 statistically out-performing 2012. ARRORS-ICGX 000201/5/P_4P_{10} (29.11 kg N/ha) and SAMNUT 23 (22.27 kg N/ha) were the best

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genotypes in terms of biological nitrogen fixation, nodule number, and nodule dry weight in both 2011 and 2012. Application of 30 kg N ha^{-1} significantly increased nitrogen fixation and Ndfa but significantly reduced nodule number and weight. ARRORS-ICGX 000201/5/P_4P_{10} (2739 kg/ha) and SAMNUT 22 (3346 kg/ha) performed best even without the addition of the starter dose of nitrogen fertilizer signifying their ability to reduce the cost of production by saving cost on inorganic fertilizer. Three distinct categories based on the amount of biologically fixed nitrogen and pod yield were assumed. ARROS-ICGX000201/5/P_4P_{10} and SAMNUT 22 were high fixing and high yielding; 6AT was high fixing and low yielding; while SAMNUT 21 and ICIAR 7B are low fixing. Similarly, considering the drastic declined in the number of effective nodulation of the genotypes with increased dosage of nitrogen fertilizer, downward review of the current recommendation will be necessary to enhance the efficiency of nitrogen fixation of groundnut.

Keywords: Starter N; nodulation; BNF; nitrogen derive from atmosphere (Ndfa); yield.

1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the leguminous crops that can fix atmospheric nitrogen by symbiotic relationship with cowpea-type rhizobium which are found in tropical soils. Nitrogen fixing ability in groundnut varies widely, depending on genotype [1]. Increase in BNF in groundnut might translate into increased yield of the legume since it utilizes large quantities of nitrogen to produce protein, as well as provide nitrogen in the soil thus allowing a grain crop to be grown in rotation with it particularly when the residues are incorporated into the soil [2]. Groundnut fixing nitrogen at the rate of 21 to 206 kg/ha N $year^{-1}$ has been reported [3].

Despite its ability to fix N_2, groundnut may suffer a period of N starvation under field conditions especially when soil N is very low until nodules begin to function [4]. Similarly, the plant may continue to suffer N deficiency, if effective strains of rhizobium are absent in the soil. In these conditions, complementation with low mineral nitrogen otherwise known as starter N might be justified. In the Nigerian savannas 20-30 kg/ha N has been recommended [5]. However, 30-40 kg/ha N or 30 kg/ha N or less has also been recommended to be applied at planting [6,5]. However, farmers in the agro-ecological zone do not usually comply with this recommendation due to high cost or unavailability of mineral fertilizers; thus, BNF is expected to be sub-optimal. This may drastically affect soil N balance as well as crop yields such that groundnut production cannot be sustained over a long period of time. Current studies on biological nitrogen fixation by grain legumes have been restricted to cowpea and soybeans as reflected in the work conducted by several researchers [4,3]. However, most researches on BNF fixing ability of groundnut in the northern guinea savannah of Nigeria have been restricted to few (3-4) groundnut genotypes thereby creating an information gap on the classification of the remaining groundnut genotypes currently in used by farmers in the zone. Therefore the objective of this study is to determined nodulation and symbiotic N fixation as influenced by the application of inorganic nitrogen fertiliser in the Northern Guinea Savanna of Nigeria.

2. MATERIALS AND METHODS

2.1 Site Description

The experiment was conducted on one of the experimental fields of the Institute for Agricultural Research (I.A.R) Samaru located at an altitude of 686 m above sea level, latitude 11º11'008´´N and longitude 7º36'52.1" E in the Northern Guinea Savanna of Nigeria (NGS). The NGS is characterised by a monomodal rainfall pattern. Total annual rainfall was 1207 mm and 1333 mm for 2011 and 2012 respectively and an average minimum temperatures of 18ºC and 19ºC and maximum temperatures of 35ºC and 34ºC in 2011 and 2012 respectively. The monthly rainfall distribution chart is shown in Fig. 1. Soils at the experimental area are classified as Typic Haplustalf (Alfisols) according to the USDA soil taxonomy [7] and Acrisols according to FAO-UNESCO [8]. The soil is low in inherent fertility: organic matter, cation exchange capacity and dominated by low activity clays [9,10].

2.2 Field Layout, Treatment and Experimental Design

The experimental area was marked out from the field, ploughed, disc-harrowed and ridged at an inter-row spacing distance of 0.75 m. The various treatments consisting of ten genotypes of groundnut; SAMNUT 24, SAMNUT 22, ARRORS ICGX-SM 00017/5/P_{15}/P_2, SAMNUT 10, ICIAR 7B, 6AT, ARRORS ICGX 000201/5/P_4P_{10}, SAMNUT 21, SAMNUT 23, SAMNUT 14, and

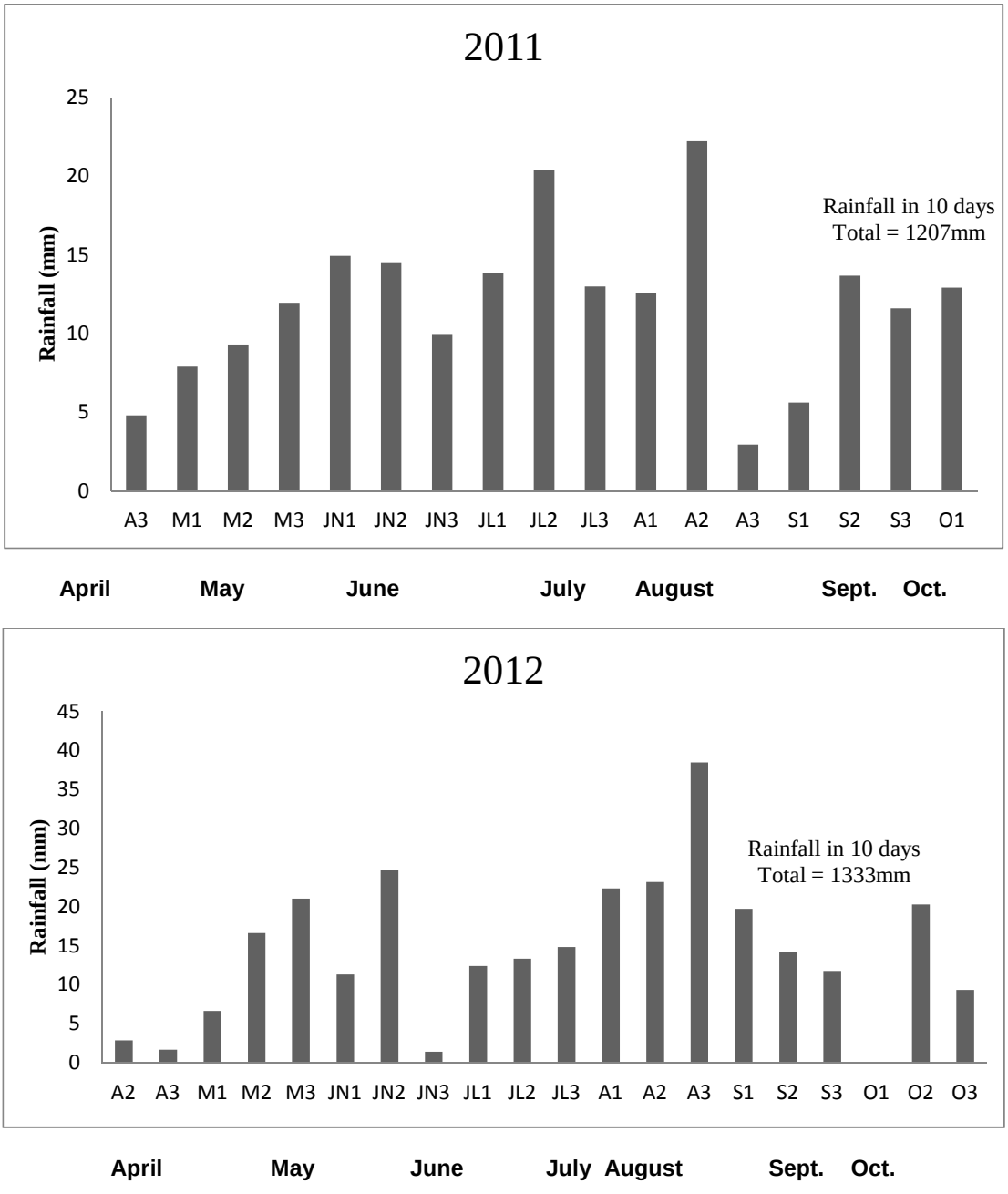

Fig. 1. Rainfall patterns in Samaru in 2011 and 2012

two rate of nitrogen fertilizer (0 kg ha^{-1} and 30 kg ha^{-1}) were arranged in a split plot design. (One non nodulated groundnut genotypes ICGL 5 was also included to determined symbiotic nitrogen fixation by N- difference method) Nitrogen rates was selected to represent the main plots while the sub plots consisted of ten groundnut genotypes replicated three times giving a total treatment combination of 60 Basal application of 20 kg/ha K as Muriate of Potash (60% K_20), 20 kg/ha P as Single Superphosphate (18% P_2O_5). One third of 30 kg/ha N of Urea (46% N) was applied two weeks after planting (2 WAP); while the remaining part (two third) was applied eight weeks after planting (8 WAP). The fertilizers were applied by banding about 5 cm away from the seed. One groundnut seed of each of genotype was sown by hand at a spacing of 20 cm by 75 cm inter and intra row spacing respectively. Each plot

size measure 3 m by 3 m and 1 m and 2 m where demarcated between sub plots and replications, respectively. The total plot area was 34 by 65 m^2.

2.3 Soil Analysis

Initial soil sampling was done at a depth of 0 -15 cm for physico-chemical analysis of the inherent nutrient status. An auger was used to collect a total of 20 soil samples bulked to form composite sample from which sub sample was taken for the analysis. The collected soil samples were air-dried, sieved using 2-mm mesh sieve and bagged with polythene bags in readiness for the following physico-chemical laboratory analysis; Particle size distribution was determined by the hydrometer method, as described by Gee and Or, [11], using distilled water and calgon (sodium hexametaphosphate) as dispersing agents while textural class was obtained from the USDA soil textural triangle; Soil pH was determined electrometrically in a soil to solution ratio of 1:2.5 [12]. Total nitrogen was estimated by micro-Kjeldahl digestion method [13]; Organic carbon was measured by the method described by Olsen and Sommers, [14]. Available phosphorus was estimated by the Bray 1 method [14]. Exchangeable Ca^{2+}, Mg^{2+}, K^+ and Na^+ were extracted with 1N ammonium acetate buffered at pH 7.0 [15]. Exchangeable Ca^{2+} and Mg^{2+} were determined by EDTA complexometric titration while exchangeable K^+ and Na^+ were estimated by flame photometry [16] Exchangeable acidity was determined by titration method [17]. The effective cation exchange capacity (ECEC) was estimated by summation method of all the exchangeable acidity and exchangeable bases. The extractable micro nutrients such as zinc (Zn), iron (Fe), manganese (Mn), and copper (Cu) in the soil sample were reacted (extracted) with 5 ml of HNO_3, 15 ml of concentrated H_2SO_4 and 0.3 ml of $HClO_4$. The mixture was digested in a fume cupboard, heating continued until a dense white fume appeared which was then ingested for 15minutes, set aside to cool and diluted with distilled water. The mixture was filtered through acid washed Whattman No.44 filter paper into a 50 ml volumetric flask and diluted to mark volume. The sample solution was then aspirated into the Atomic Absorption Spectroscopic machine at intervals. The AAS model used was Elmer 403 AAS iCE 3500 double beam Series which is connected to a computer monitor embedded with software. The software creates a calibration curve and automatically determines the concentration of each of the element in the samples depending on the availability of a cathode lamp.

2.4 Plant Analysis

Plant samples were collected at eight weeks after planting in both cropping seasons (2011 and 2012) for the determination of N-accumulation in the plant. Destructive sampling was carried out on four plants, two taken from each of the outer rows. The plant samples were separated into shoot and roots, washed with distilled water to removed adhering soils, placed in paper envelops and oven dried at 65^oC for 68 hours. After oven drying, shoots and roots were grounds and allowed to pass through a 0.5 mm mesh before analysis for total N concentration using the micro Kjedahl method [13]. Similarly, ten representative nodules where randomly selected from each genotype, dissected using a sharp razor blade to determine their effectiveness. Nodules with pink colours were classified as effective whereas those with colours other than pink were considered ineffective. The sample size of ten nodules was taken to represent 10% of the overall nodule number.

2.5 Calculations/Estimations

Nitrogen fixation was estimated using the N-difference methods thus:

(i.e N content of nodulated - non-nodulated groundnut genotypes);
while percent N**-derived from atmosphere (Ndfa)** was estimated as the ratio of total N-fix to the total plant N uptake. Computed as;
%Ndfa = NF/Total plant N uptake*100
N uptake=Shoot or root dry weight /100*Shoot or root % N.

2.6 Statistical Analysis

Individual analysis of variance was performed for each character in each year using the General Linear Model (GLM) procedure of SAS; [18]. The effects of the various treatments and their interactions were compared using standard error of difference (SED) and LSD was used for means separation.

3. RESULTS AND DISCUSSION

3.1 Experimental Soil Characteristics in 2011 and 2012

Some physical and chemical properties of the site before commencement of the study were determined before the establishment of the trials

in 2011 and 2012 and the results obtained are as shown in Table 1. The results showed that the texture of the soil was sandy-loam. This may be due to sorting of soil materials (such as decomposed roots, leaves etc) by biological activities, clay migration (Illuviation/elluviation) or erosion by run–off or a combination of these factors [19].

The soil reaction was slightly acidic (5.30 - 6.00) but did not pose any limitation to groundnut production. The organic carbon and total N were both low in the soil in both years. However, there was a slight increase of total nitrogen in 2012 than in 2011 which could probably be due to the residual nitrogen resulting from mineralisation of below ground residues of previous groundnut crop. Similarly, decrease in organic carbon (OC) in 2012 might be due to increase in soil N which enhanced soil organic matter decomposition by soil organisms. The available P content and the exchangeable bases fall within the medium class for Nigerian soils [5]. The experimental soil can be classified as moderately suitable for the cultivation of most arable crops according to the modified FAO suitability classification [20].

The exchangeable acidity and effective cation exchange capacity (ECEC) were all low in the soil. The extractable micronutrients (Zn, Fe, and Mn) were all high while the extractable Cu was medium in the soil [21]. Hence, the micronutrients were considered adequate and did not limit groundnut production [22]. Generally, the soils properties were typically characteristic of Alfisols of Northern Guinea Savanna of Nigeria as described by Odunze, [21] and the level of the various nutrient elements fell within the ranges described for Nigeria soils [22].

3.2 Effect of N Fertilizer and Genotypes on Nodulation of Groundnut

The number and weight of nodules are commonly used as the criteria of effective complementary interaction between legume and micro- symbionts; thereby correlate on the whole with the rate of atmospheric nitrogen fixation [23, 24]. The number of nodule differed significantly depending on the genotype in both 2011 and 2012 cropping seasons as shown in Table 2. The result of the combined analysis of variance show a significant difference in nodule number across the two years with higher nodule number found in 2011 over 2012. Similarly, a highly significant difference was found among the groundnut genotypes in both years. In 2011 for instance, the highest nodule number (greater than 100 per plant) was observed in SAMNUT 22 and SAMNUT 24. These were followed by ARRORS ICGX-SM 00017/5/P_{15}/P_2 and

Table 1. Physical and chemical properties of the soil at the experimental site in 2011 and 2012

Soil properties	Level in soil		
	2011	2012	Average
Sand (%)	67.1	64.0	65.6
Silt (%)	19.8	20.0	19.9
Clay (%)	13.1	16.0	14.6
Textural class	Sandy-loam	Sandy-loam	Sandy-loam
pH 1:2.5 water	5.30	6.00	5.65
pH 1:2.5 $CaCl_2$	5.10	5.15	5.13
Organic Carbon (g kg^{-1})	5.37	2.28	3.83
Total Nitrogen (%)	0.15	0.19	0.17
Available P (mg kg^{-1})	11.00	8.32	9.66
Exchangeable Ca^{2+}(cmol/kg)	2.32	3.15	2.74
Exchangeable Mg^{2+} (cmol/kg)	0.40	1.01	0.71
Exchangeable K^{+}(cmol/kg)	0.20	0.16	0.18
Exchangeable acidity(cmol/kg)	0.18	0.12	0.15
ECEC (cmol/kg)	3.10	4.44	3.78
Extractable micronutrient (mg/kg)			
Zn	2.28	1.86	2.07
Cu	1.00	1.00	1.00
Mn	16.0	13.0	14.5
Fe	9.00	10.00	9.50

ECEC = Effective Cation Exchange Capacity

ARRORS ICGX 000201/5/P_4P_{10} while all the remaining genotypes had less than 100 nodules per plant. This trend was somehow maintained in 2012 where the best genotypes in terms of nodule number were ARRORS ICGX 000201/5/$P_4P_{10,}$ SAMNUT 24, SAMNUT 22 and SAMNUT 23. The least nodule number in both 2011 and 2012 was found in SAMNUT 21.

Application of 30 kg N/ha consistently and significantly reduced the number of nodules in all the genotypes relative to the control (0 kg N/ha) in both years. Generally, the significant difference observed in the N rates whereby high nodule number was observed in the control over the 30 kg N/ha is not surprising but confirming the result of numerous researches on nitrogen fertilizer and nodulation that concluded the suppressing effect of nitrogen fertilizer on nodulation of legumes. This is often the case particularly when applied in relatively higher quantity [24,25]. Meanwhile, the inhibitory effect of NO_3 on nodules has been recognised by Rhizobiologist and has been under investigation. Peoples et al. [26] and Takishima [27] in their separate findings attributed the effect of nitrogen on nodulation to the inhibition of the rhizobium infection process via the impairment of the recognition mechanism by nitrates. The result on the interaction between nitrogen and genotypes shows a significant ($P<0.05$) increase in nodule number of ARRORS ICGX 000201/5/P_4P_{10} even at 30 kg N/ha (Fig. 2).

The number of nodules formed by promiscuous legume genotypes depends on the prevailing environmental conditions and the population of indigenous rhizobia during the process of nodulation [28,29,30]. Though low soil fertility has been reported by various authors to inhibit initiation of nodules, but our results show high nodule number at low soil nitrogen level (0 kg N/ha) relative to improve soil N (30 kg N/ha) condition which perhaps suggest that the N rate was still very high. The promotion of high nodule number at low soil N could be attributed to the considerable energy that the root must expend to move the nitrogen through the cell membranes from the soil into the roots. Once the nitrogen is inside the plant, more energy is needed to convert it to a form that can be metabolized by the plant. This additional energy is intended to be supply either by symbiotic nitrogen fixation particularly if the nodules are efficient or through external source via mineral N input. This stress condition will tend to induce the legumes to produce more nodules relative to when soil N is readily available [21]. However, the increase in nodulation by ARRORS ICGX 000201/5/P_4P_{10} at 30 kg N/ha could be attributed to the tolerance of the nitrogenase enzymes, mediated by a non – covalent inhibitory mechanism in the rhizobia present in the nodules [31], although not confirmed in our present study and may also be due to the fact that the 30 kg N/ha was not sufficient enough to suppress nodulation completely due to the relatively low soil nitrogen characterising the experimental site. This is true evidenced by the fact that lower nodulation was recorded in the second trial following groundnut rotation which could be as a result of the residual effect of BNF to soil nitrogen pool. Tahir, [24] attributed the decrease in nodulation at higher nitrogen dosage to a decrease in the activities of rhizobia.

Apart from the widely reported effect of nitrogen on nodulation, environmental stress could play a significant role on nodulation. Typical environmental stresses faced by legume nodules and their symbiotic partner (*Rhizobium*) may include photosynthate deprivation, water stress, salinity, temperature, heavy metals, and biocides [32]. A given stress may also have more than one effect: e.g., salinity may act as a water stress, which affects the photosynthetic rate, or may affect nodule metabolism directly. The most problematic environments for rhizobia are marginal lands with low rainfall, extremes of temperature, acidic soils of low nutrient status, and poor water-holding capacity [33]. Similar trend of results was reported for the nodule weight. This trend could be attributed to the high nodule number reported in these genotypes.

3.3 Effect of N Fertilizer and Genotype on Symbiotic N_2 Fixation of Groundnut

The amount of N_2 fixed by groundnut genotypes as determined by the nitrogen difference method ranged from 8.99 to 34.93 kg/ha in the 2011 planting season with a mean nitrogen fixation value of 20.71 kg/ha while in 2012, the genotypes fixed between 3.45 to 23.29 kg/ha with a mean BNF value of 11.24 kg/ha. ARRORS ICGX000201/5/P_4P_{10} fixed the highest N of 34.93 kg/ha which was consistent even in 2012; though at a lower rate of 23.29 kg/ha but it was significantly higher than SAMNUT 10 which fixed a meagre 8.99 kg/ha N in 2011 (Table 3).

Table 2. Effect of N fertilizer and genotypes on nodulation of groundnut

Genotype (G)	Nodule (Number plant^{-1})		Combined	Nodule fresh weight (mg plant^{-1})		Combined
	2011	2012		2011	2012	
SAMNUT 24	138.00	66.00	102.00	173.33	70.83	122.08
SAMNUT 22	140.00	52.00	96.00	150.00	74.17	112.09
ARRORS ICGX-SM 00017/5/P_{15}/P_2	114.00	48.00	81.00	158.33	70.83	114.58
SAMNUT 10	61.00	48.00	55.00	89.33	50.00	69.67
ICIAR 7B	90.00	33.00	62.00	115.33	25.00	70.17
6AT	78.00	48.00	63.00	100.33	46.25	73.29
ARRORS ICGX 000201/5/P_4P_{10}	113.00	71.00	92.00	169.17	92.93	131.05
SAMNUT 21	51.00	19.00	35.00	89.33	21.67	55.50
SAMNUT 23	84.00	53.00	69.00	124.00	50.42	87.21
SAMNUT 14	59.00	48.00	54.00	120.50	62.50	91.50
SE±	13.71**	8.67**	8.83	17.12*	11.90**	10.53
Nitrogen (N) (kg/ha)						
0	114.00	56.00	85.00	153.30	57.40	105.35
30	71.00	36.00	54.00	104.63	46.09	75.36
Mean	93.00	46.00	66.00	128.97	51.75	88.30
SE±	4.60**	4.02**	3.93	7.66*	5.52**	5.30
Interactions						
G*N	*	NS	NS	NS	NS	NS

*NS=Not significant at 5% level of probability, *Significant at 5% level of probability, **Significant at 1% level of probability*

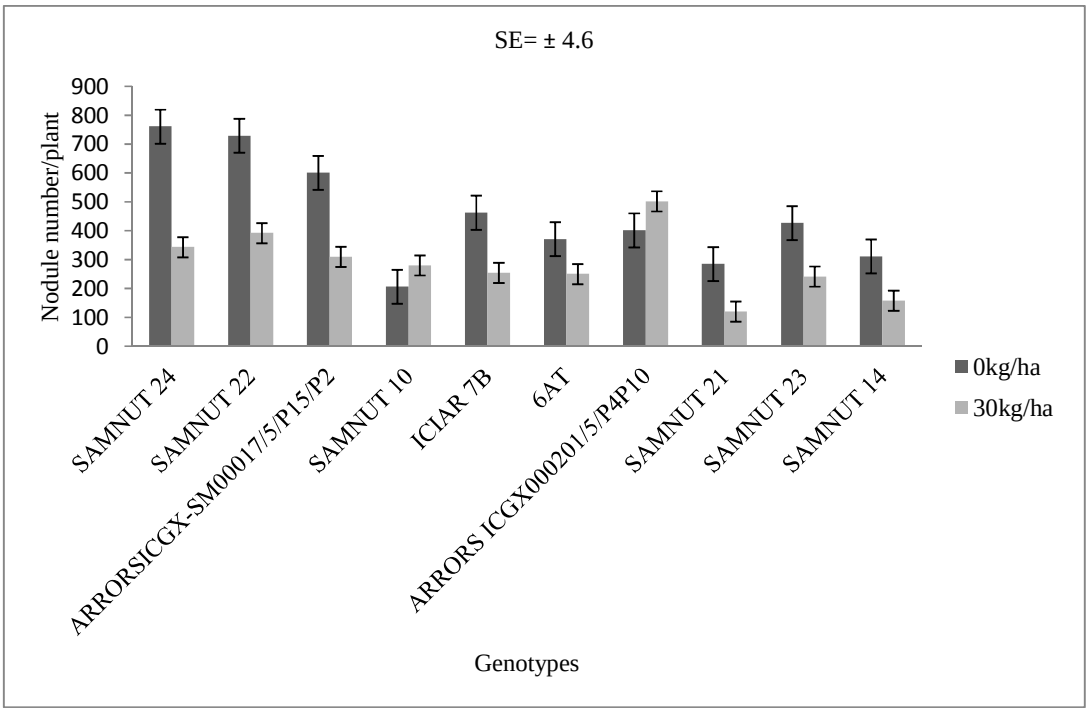

Fig. 2. Interaction of genotypes and nitrogen rates on nodule number of groundnut at 8WAP in 2011

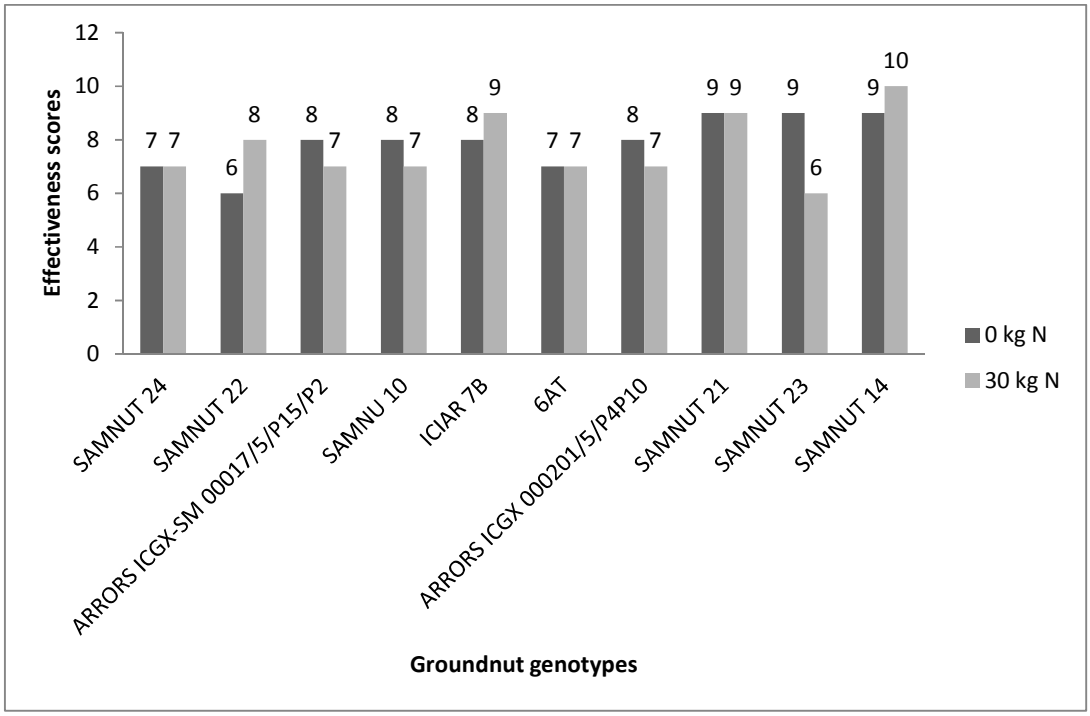

Fig. 3. Proportion of ineffective nodules in ten randomly selected nodules

Okito et al. [34] reported a mean BNF value of 40.9 kg/ha for groundnut in his latest finding which was less than the estimated value of 96 kg/ha N by Burris [35]; and both greater than the highest value of 34.93 kg/ha N recorded under this study which is greater than the 31.79 kg/ha N reported by Yakubu et al. [36] in south-eastern Nigeria. However, the amount of N fixed fall within the range of 32 to 134 kg/ha N estimated by Dakora and Keya [37]. Generally, there was no significant interaction between the genotype and N rate of application in 2011 but a significant difference however exist in 2012 season (Fig. 4). The higher values recorded by those researchers could be attributed to the prevalence of effective strain of rhizobium, conducive environmental conditions, compatibility of the indigenous rhizobium strain and the host legumes and/or a combination of these factors [24]. The higher amount of nitrogen fixation recorded by ARROR ICGX 0001/5/P_4P_{10} could be attributed to the higher and effective nodules found on the genotype compared to others as shown in Fig. 3. However, the highly significant difference among the genotype to nitrogen fixation confirmed results of Patterson and La Rue [38], and Hardason, [39] who earlier found significant variations in N_2 fixation among various maturity groups of soybean genotypes. These disparities in fixation could be attributed to the variation in the host plant characteristic which is control at a whole by the nitrogenase enzyme which is reserved only for prokaryotes and responsible for BNF. Similar trend was also observed for N rates with the application of 30 kg/ha N significantly outperforming the control in 2011 planting season but was not significant in 2012. This could be due to the fact that the application of 30 kg/ha N boosts nitrogen fixation by impacting positively on the activities of nitrogenase enzyme complex responsible for nitrogen fixation in legumes in agreement with previous researchers notably; Patterson and La Rue [38] and Hardason [39]. The high amount of N fixed in the first year over the second year could probably be due to the high amount of nitrate build up in the soil profile during the first season resulting in the suppression of nodule number in agreement with Wetselaar et al. [40] which has been shown to correlate positively with BNF [34] and/or probably due to the diversion of photosynthates toward assimilation of nitrates as a result of improved soil nitrogen which tend to equally suppress nodulation as noted by Peoples et al. [26].

The differences observed in the amount of N_2 fixed by the groundnut genotypes could be attributed to the number of days required to attain maturity. This was confirmed during the

course of our findings where higher amount of N was found in medium (90-110 days) maturing genotypes such as ARROR ICGX 0001/5/$P_4P_{10,}$ SAMNUT 21, SAMNUT 22, ICIAR 6AT and SAMNUT 14 all fixing within the range of 17.00 to 29.11 kg/ha N. The results of this finding corroborate with those obtained by Yusuf et al. [28].

It is a well establish fact that applied nitrogen significantly reduced BNF, but the fixation of high nitrogen even in the presence of apply nitrogen provides important criteria in selecting legumes genotypes most suitable in a mixed or multiple cropping systems [41,42]. Since most crops particularly cereals grown as companion with legumes will often require high dosage of apply nitrogen for their optimum yield expression.

Apart from most of the earlier mentioned physiological processes that influences BNF in groundnut, several environmental conditions are limiting factors to the growth and activity of the nitrogen fixing plants. In the rhizobium legume symbiosis, which is a nitrogen fixing system, the process of nitrogen fixation is strongly related to the physiological state of the host plant [43]. Unlike in most soil-plant system, the principle of limiting factor is equally at play in this regard. Therefore, a competitive and persistent rhizobial strain is not expected to express its full capacity for nitrogen fixation if limiting factors (e.g., salinity, unfavourable pH, nutrient deficiency, mineral toxicity, temperature extremes, insufficient or excessive soil moisture, inadequate photosynthesis, plant diseases, and grazing) impose limitations on the vigour of the host plant.

3.4 Proportion of N Derived from Atmosphere (%Ndfa) in 2011, 2012 and Combined

This is a measure of the proportion of N derived from BNF in plants [36]. The groundnut genotype showed wide variation in their proportion of Ndfa in both years. The result showed an average %Ndfa of 35.34 and 35.43 respectively in 2011 and 2012 trial respectively. The genotypes used did not have significant p<0.05) difference in their proportion of Ndfa in 2011. In 2012, however, the highest and lowest values were recorded for ARRORS ICGX000201/5/P_4P_{10} (53.17%) and ARRORS ICGX-SM 00017/5/P_{15}/P_2 (18.58%) respectively. However, a highly significant (p< 0.01) difference (p< 0.01) was observed in 2011where N was apply and the control with the application of 30 kg/ha N rates contributing more nitrogen from the BNF over the control (Table 3). Genotype * Nitrogen interaction was significant in 2012, but not in 2011 when most of the groundnut genotypes increased significantly, their proportion of nitrogen under the control compared to the 30 kg/ha N-rate (Fig. 5).

Table 3. Effects of N fertilizer and genotype on symbiotic N_2 fixation of groundnut and nitrogen derived from atmosphere

Genotype (G)	Nitrogen fixation (kg/ha)			% Ndfa		
	2011	2012	Combined	2011	2012	Combined
SAMNUT 24	14.13	3.45	8.78	27.11	16.36	21.74
SAMNUT 22	22.80	18.32	20.56	36.50	50.48	43.49
ARRORS ICGX-SM00017/5/P_{15}/P_2	21.72	8.77	15.25	38.93	30.76	34.83
SAMNUT 10	8.99	5.72	7.36	19.96	23.43	21.70
ICIAR 7B	10.20	8.65	9.43	22.68	32.62	27.65
6AT	26.87	13.03	19.95	43.09	41.59	42.34
ARRORS ICGX000201/5/P_4P_{10}	34.93	23.29	29.11	51.17	53.17	52.17
SAMNUT 21	11.91	8.04	9.98	27.83	28.01	27.92
SAMNUT 23	31.44	13.11	22.28	44.95	42.40	43.68
SAMNUT 14	24.17	10.03	17.10	41.12	35.41	38.27
SE±	12.55**	1.94**	2.28**	7.41^{NS}	4.06**	4.10
Nitrogen (kg/ha)						
0	10.06	11.56	10.81	23.19	35.35	29.35
30	31.37	10.92	21.15	47.48	35.50	41.49
Means	20.71	11.24	10.98	35.34	35.43	35.42
SE±	5.61**	0.87^{NS}	3.32**	3.32^{**}	1.81^{NS}	1.82**
Interactions						
G*N	NS	*	NS	NS	**	NS

*NS=Not significant at 5% level of probability, *Significant at 5% level of probability, **Significant at 1% level of probability*

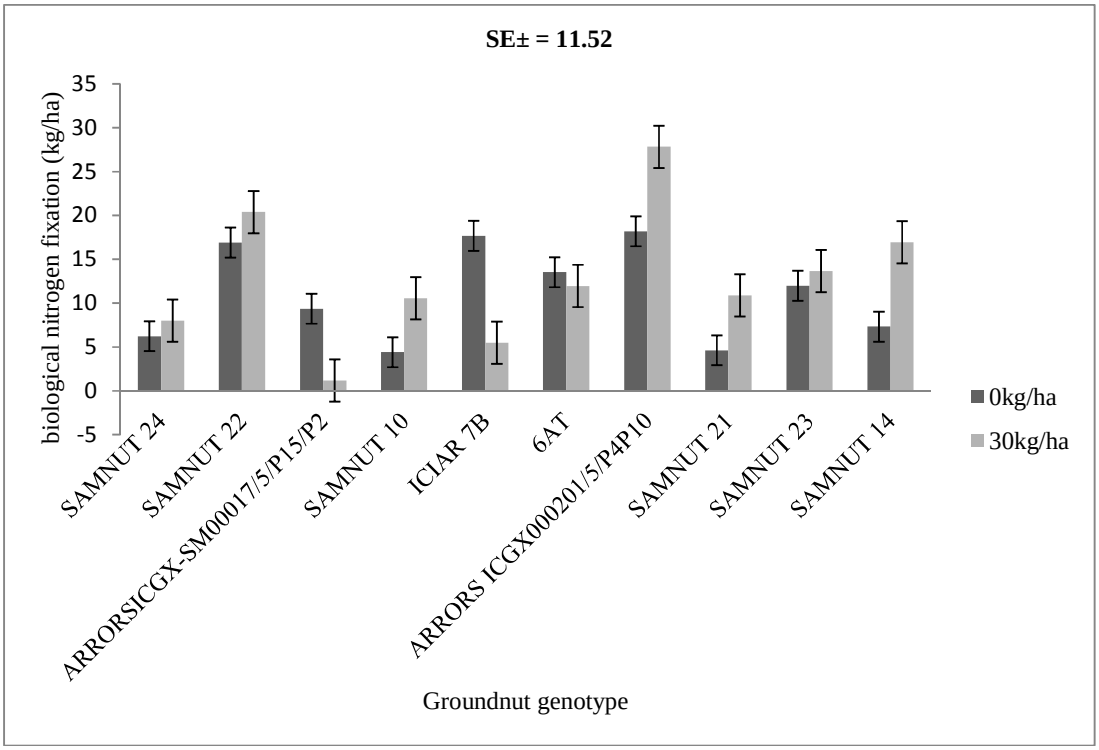

Fig. 4. Interaction between genotype and N-rates on BNF in 2012

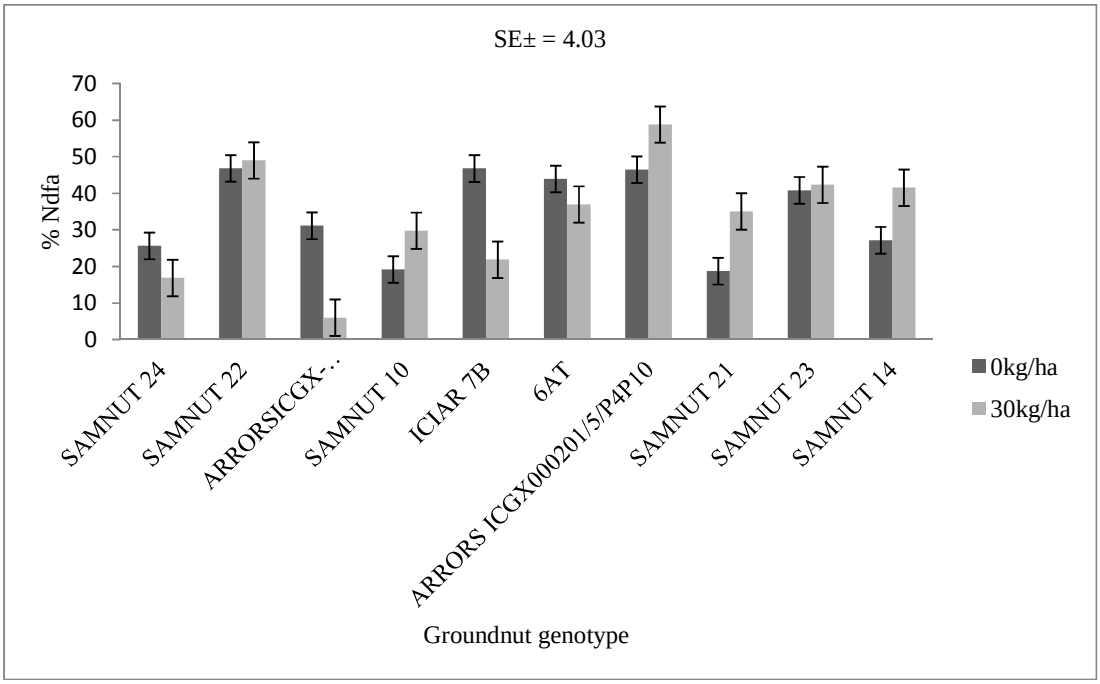

Fig. 5. Percentage Ndfa of groundnut genotypes in 2012 cropping season

The mean values of the proportion of Ndfa for both years were slightly lower than 52.4% recorded by Okito et al. [34] but fall within the range of 28-81% reported by Ganry [44] and Badiane and Gueye [45] in the moist Guinea Savanna of West Africa. The major contribution

of grain legumes to soil fertility lies in their ability to fix atmospheric nitrogen. Therefore genotype that derived high proportion of their N from fixation will be highly desirable especially in soils with low N status [28]. The result shows that less than half of the plant total N was derived from the atmosphere indicating that none of the genotype will be able to meet its N need for growth and development without the application of starter inorganic nitrogen at rates less than 30 kg N ha^{-1}. In conformity with the above hypothesis, the application of 30 kg/ha N as starter dose was still justified in order to enhance BNF in groundnut genotype. However, further investigation into more genotypes of groundnut in view of discovering genotype(s) with promising N fixing potential, without necessarily including starter dose of N fertilizer is highly desirable considering the high cost of mineral fertilizer and its negative impact on the environment.

Table 4. Effects of genotype and N fertilizer on pod yield

Treatment	Pod (kg/ha)		
Genotypes (G)	**2011**	**2012**	**Average**
SAMNUT 24	1108	1106	1107
SAMNUT 22	2179	2963	2571
ARRORSICGX SM 00017/5/P_{15}/P_2	2070	2738	2404
SAMNUT 10	1249	1433	1341
ICIAR 7B	1038	614	826
6AT	1088	674	881
ARRORSICGX 000201/5/P_4P_{10}	2801	2540	2670
SAMNUT 21	1025	2473	1749
SAMNUT 23	2122	1678	1900
SAMNUT 14	1138	669	904
ICGL5	479	525	502
Mean	1482	1547	1514
SE±	178**	266***	160**
N rates (kg/ha)			
0	1313	1619	1466
30	1650	1475	1563
Mean	1482	1547	1515
SE±	76**	113*	68.12^{NS}
Interaction G*N			
Significance	NS	**	NS

*NS=Not significant at 5% level of probability, *Significant at 5% level of probability, **Significant at 1% level of probability.*

3.5 Pod Yield in 2011, 2012 and Combined

The effect of genotype and nitrogen rates on pod yield showed a highly significant difference in both years (2011 and 2012) as shown in Table 4. However, higher pod yield was observed in 2012 than in 2011. Result of analysis of variance further showed that the highest pod yield was recorded in groundnut genotype SAMNUT 22 in 2012 which was not significantly different from ARRORS ICGX000201/5/P_4P_{10}, ARRORS 1CGX-SM00017/5/$P_{15}P_2$ and SAMNUT 21. However in 2011 ARRORS ICGX000201/5/P_4P_{10} was the best closely followed by SAMNUT 22 and SAMNUT 23 which was not statistically different from ARRORS ICGX-SM 00017/5/P_{15}/P_2 and the two genotypes maintained their consistency in high pod yield across the two year duration. Among the nodulating lines, ICIAR 7B and SAMNUT 14 consistently recorded the least yield in both the 2011 and 2012 season. Nitrogen rates effect was significant in both 2011 and 2012; with the 30 kg/ha N outperforming the control.

The increase in yield with inorganic nitrogen over the control could be due to the ability of fertilizer to provide growing plants with nutrients in readily available forms [46,47]. More so, Dashiell, et al. [48] reported similar trend with respect to the effect of nitrogen rates which he attributed to the low soil nitrogen at the experimental site which resulted to rapid response of the genotype to soil added nitrogen in conformity with Peoples and Crasswell [49] hypotheses that the benefit of nitrogen application are generally thought to be higher if nitrogen is a limiting factor; or deficient in the soil because of high nitrogen demands of high yielding cultivars. Genotype * Nitrogen interaction was not significant ($p<0.05$) in both 2011 and 2012, but physical observation shows that SAMNUT 22, ARRORS 1CGX-SM00017/5/$P_{15}P_2$, ARRORS ICGX000201/5/P_4P_{10} and SAMNUT 23 yield were slightly better in the control over the 30 kg/ha N rate in 2012 representing 32%, 14%, 15% and 17% increases respectively in the control over the 30 kg/ha N rate of application (Fig. 6).

The improved yield in the second year over the first could be due to the high N-fixed resulting from legume-legume mono-cropping, increased moisture during the pod filling stage and according to Bogino et al.[50] higher plant population posed by the uniform establishment of the field. Physical observation however shows that genotype which flower earlier seem to record high pod yield in agreement with Ngo Nkot et al. [51] who reported similar trend while working in same agro-ecological zone with same genotypes of groundnut.

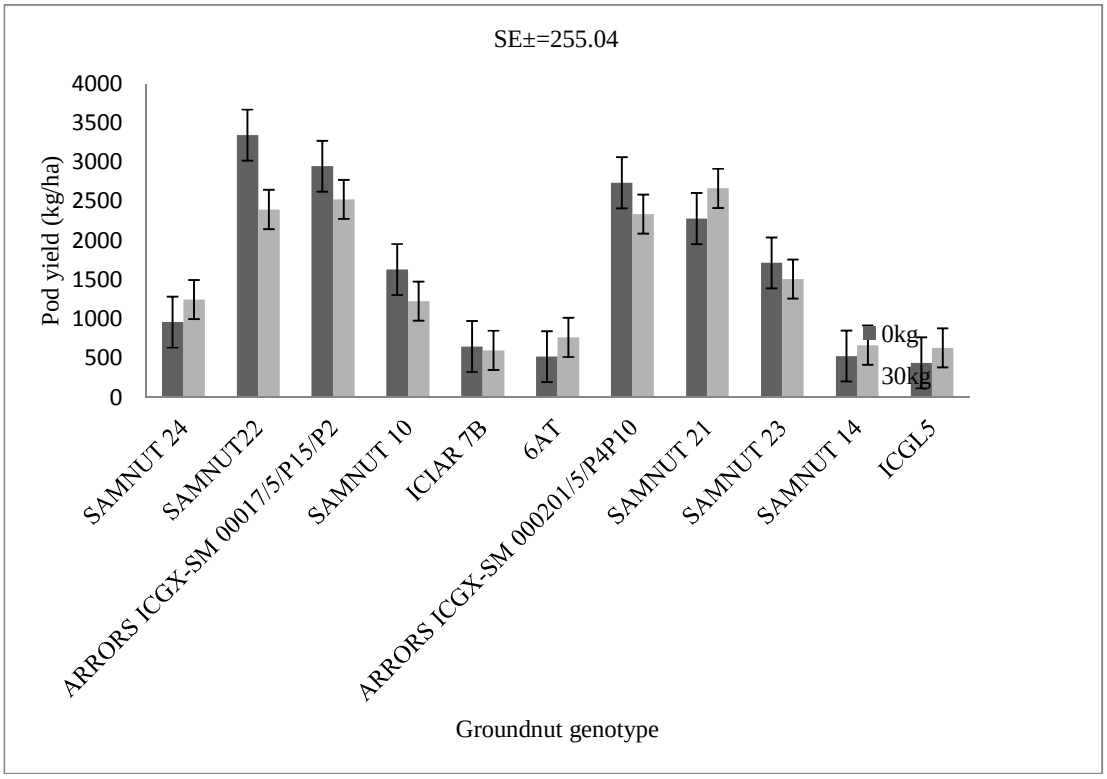

Fig. 6. Interactions between genotype and N rates on pod yield of groundnut in the 2012 trial

Environmental factors such as rainfall and temperature have been reported to have significant influence on groundnut yield. Thus the little variation in yield during the two year period could be attributed to the even distribution of rainfall during the flowering and pod filling stage which is considered critical to groundnut growth. Putnam et al. [52] from their maiden research at Minnesota indicted temperature and rainfall as the major environmental factors influencing the yield of groundnut. For instance, a peanut crop will not reach optimum maturity for a marketable yield to justify commercial production in areas with fewer heat units during the growing season. It is generally reported that little or no growth and development can occur at temperature below 20ºC and 30ºC [52]. Similarly, averaged yield increased from 1000 to 1450 kg/ha was obtained with increased moisture availability during the most critical growth period (flowering and pod filling) [52].

Similarly, results of some preliminary work by Ngo Nkot et al. [51] in different land use systems in Cameroun indicates that, soil infertility and low density of indigenous *Rhizobium* could be one of the causes of low pod filling of groundnut.

4. CONCLUSION

In conclusion, groundnut genotypes used in this study could be grouped into three distinct categories based on the amount of biologically fixed nitrogen and pod yield. (a) ARRORS ICGX 000201/5/P_4P_{10} and SAMNUT 23 are both high fixing and high yielding;(b) 6AT was high fixing but low yielding; while (c) SAMNUT 21 and ICAR 7B are low fixing and low yielding. The remaining genotypes could not be classified into any of these groups. The study also showed that application of 30 kg/ha N is adequate for increased BNF support to groundnut production in the Nigerian savannas without depleting soil N. This rate could also enhance the ability of certain genotypes to fix atmospheric nitrogen. Similarly, research finding suggest that future breeding effort should be targeted at improving the efficiency of nodulation and biomass yield to enhance profitable groundnut production in the Northern Guinea Savanna of Nigeria. Similarly, even distribution of rainfall in 2011 resulted in most of the variation observed in some of the agronomic and nitrogen fixing trait existing among groundnut genotypes.

5. RECOMMENDATION

Information generated from this study may be limited to the particular groundnut genotypes used and the specific environmental condition in which the parameters were measured. Nevertheless, experimental results clearly demonstrate that BNF efficiency can be successfully bred into adapted genotypes of groundnut; thus suggesting that future breeding effort be targeted at improving the efficiency of nodulation (number and mass) to achieve profitable output (Yield) of groundnut. Additional studies using various genotypes and environments however are essential to confirm the types of gene action governing the different measures of N-fixation in other groundnut genotypes and under different growth conditions and if possible their net nitrogen contribution should be ascertained by rotating the crop with a non legume maize/cassava. In addition, it may also be necessary to vary the nitrogen rates probably to a higher and lower dose to ascertain the nitrogen rate that could completely suppress or enhance nodulation of groundnut.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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