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Biological Nitrogen Fixation and Pod Yield of Groundnut (*Arachis hypogaea* L.) as Influenced by a Salt-affected Alfisol at Kadawa, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author AIG designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author HM managed the literature searches, analyses of the study performed the spectroscopy analysis. Authors AAY and AIG managed the experimental process. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The cost of nitrogen (N) fertilizers continues to rise besides the fertilizer's role as a potential pollutant. Amelioration and/or improvement in the fertility of poor soils using such inorganic fertilizers prove less feasible as such. The *rhizobium*-legume symbiosis is, therefore, suggested as an alternative to solving the soil N fertility problem. Biological nitrogen fixation (BNF) can be an important means for a continued and sustainable productivity of N-demanding agricultural crops. Most legumes are very sensitive to saline condition yet the rhizobia they house, due to adaptation of some strains to saline conditions, are not. Assessment of groundnut for BNF on saline soils cannot be overestimated, especially as more farmers are coming into irrigated agriculture. Besides, there is little to no reported work on the subject, particularly on the groundnut genotypes under study. A screen house trial was conducted in 2012 at the Department of Soil Science, Ahmadu Bello University, Zaria, Nigeria. The study aimed at determining the symbiotic nitrogen fixation of some groundnut genotypes grown on a salt-affected soil. The treatments consisted of six groundnut genotypes (SAMNUT 10, 11, 21, 22, 23 and 24) and two soil types (saline and non-saline). A non-nodulating groundnut genotype (ICGL-5) was used as a reference crop. The treatments were laid in a completely

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randomized design (CRD) with three replications. Indices of nitrogen fixation and yield were recorded. Effective and total nodule numbers were highest in SAMNUT 24 (19 plant^{-1}) and 22 (34 plant^{-1}). Although there was no statistical difference between the genotypes in terms of N_2-fixed, highest amount of dinitrogen fixed was recorded for SAMNUT 21 (2, 500 mg N plant^{-1}) and the least for SAMNUT 11 (930 mg N plant^{-1}). SAMNUT 23 had the highest pod yield and SAMNUT 11 the least. Soil salinity did not affect N_2 fixation statistically although the normal (non-saline) soil tends to positively influence most other parameters. Further studies on roles of other biochemical factors would assist in understanding the phenomena more.

Keywords: Biological nitrogen fixation; dinitrogen; genotype; salinity.

1. INTRODUCTION

Biological nitrogen fixation (BNF) is the process whereby atmospheric nitrogen (N=N) is reduced to ammonia in the presence of nitrogenase. Nitrogenase is a biological catalyst found naturally only in certain microorganisms such as the symbiotic *Rhizobium* and *Frankia*, or the free-living *Azospirillum* and *Azotobacter* [1]. BNF is brought about both by free-living soil microorganisms and by symbiotic associations of microorganisms with higher plants [2].

The leguminous plants fix atmospheric nitrogen by working symbiotically with special bacteria, called rhizobia, which live in the root nodules [3,4]. Rhizobia infect root hairs of the leguminous plants and produce the nodules. The nodules become home for the bacteria where they obtain energy from the host plant and fix free nitrogen from the atmosphere and process it into combined nitrogen [3]. In return, the plant receives the fixed N from the nodules and produces food and forage protein [5,6].

The domesticated groundnut is an amphidiploid or allotetraploid, meaning that it has two sets of chromosomes from two different species. The wild ancestors of groundnut were thought to be *A. durances and A. ipaensis,* a view recently confirmed by direct comparison of the groundnut's chromosomes with those of several putative ancestors [7]. Archaeologists have thus far dated the oldest specimen to about 7, 600 years found in Peru [8]. The plant was later spread worldwide by European traders. Although groundnut was mainly a garden crop for much of the colonial period of North America, it was mostly used as animal feed stock until the 1930s [9].

Saline soils are often referred to as "white alkali" because of the white salt crust that forms on the soil surface. Saline soils are characterized by an EC >4, Exchangeable Sodium Percentage (ESP) <15, and pH <8.5. Although posing a serious threat [10], saline soils can still be easily reclaimed by application of sufficient water to promote leaching of salts beyond the root zone.

Sodic soils are often referred to as "black alkali" or "slick spots" because of the dissolved organic matter in the soil solution. Sodic soils are characterized by an EC < 4, ESP > 15, and pH >8.5. The exchangeable sodium causes soil particles to disperse, resulting in decreased pore space within the soil and increased soil crusting. The loss of permeability due to less pore space can severely restrict water movement into the root zone resulting in plant stress from lack of water. Crusting can severely affect seedling emergence. Reclamation of sodic soils involves the application of gypsum or sulphur, leaching of salts, special tillage operations or a combination of these measures.

Saline-sodic soils have properties of both saline and sodic conditions and are, therefore, characterized by the following: EC > 4, ESP > 15, and pH < 8.5. Properties of saline-sodic soils are generally similar to those of saline soils; however, “black alkali” sodic conditions can be a problem if excess soluble salts are leached without addressing the excess sodium. Reclamation of saline-sodic soils is the same as that of sodic soils to ensure that excess salts and sodium are removed [11].

Two main ways to characterize sodium status of a saline soil are exchangeable sodium percentage (ESP) and sodium adsorption ratio (SAR). The former identifies the degree to which the exchange complex is saturated with sodium. The latter is the second and a more easily measured property that is becoming even more widely used than ESP. It gives information on the comparative concentration of Na^{+}, Ca^{2+} and Mg^{2+} in soil solutions. The SAR of a soil extract takes into consideration that the adverse effect of sodium is moderated by the presence of calcium and magnesium ions.

Assessment for BNF of such pulse legumes as groundnut, especially when grown on saline soils cannot be over-measured [12], especially as more farmers are now coming into irrigated agriculture. There is also little to no reported works available on the subject, more particularly on the groundnut genotypes under study. More so, the percentage shells/pods produced by groundnut in Nigeria has alarmingly gone down over the years [13]. This is, mainly, as a result of the ever-increasing cost of production, usually due to the exhaustive cost of production arising from such inputs as fertilizers [14] and, in some cases, salinity problems [15]. The average pod yield, for example, is now at 980 kg ha^{-1} as against 1, 690 kg ha^{-1} of the world’s average [16]. This study had the objective of determining the symbiotic nitrogen fixation potential of some groundnut genotypes grown on a salt-affected soil.

2. MATERIALS AND METHODS

2.1 Location

The experiment was conducted in a screen house (latitude 11º9’ 52’’ N and longitude 7º37’ 58’’ E) of the Department of Soil Science, Faculty of Agriculture/Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria, Nigeria. Zaria is located between latitude 11º 00’ and 11º 30’N and longitude 7º 30’ and 8º 00’E at an altitude of about 700m above sea level. Geology of the area is mainly older and younger granite (laterite/plinthite) with patches of biotite gneiss [17,18].

2.2 Soil Sampling, Preparation and Analysis

The soil samples were collected from IAR Irrigation Research Station at Kadawa. The salt-affected and normal (not salt-affected) soils were respectively collected from fields named F3.6 (latitude 11º38’39’’ N; longitude 8º26’07’ E’) and F3.4 latitude 11º38’44’’ N; longitude 8º26’01’’ E). The predominant soil type found in Kadawa is Regosols, with mainly sandy to clay loam texture, the vegetation is Sudan savannah with a rainfall range of 550 to 1000 mm per annum [19], and a mean monthly temperature of 27.8ºC [20].

The samples both for, pre-planting routine, analyses and pot experiment, were collected from 0 - 15 cm depth and sufficiently air dried. These were ground and sieved through a 2 mm and 5 mm mesh, respectively for the analyses and pot experiment. The latter was transferred into, 3 kg capacity, plastic pots.

2.3 Treatments and Experimental Design

The treatments consisted of six (6) groundnut (SAMNUT 10, 11, 21, 22, 23, and 24) genotypes and two soil types, saline and normal. The genotypes were obtained from gene bank of groundnut unit of IAR and are, currently, among the most popularly cultivated amongst farmers in the Nigerian savanna agro-ecologies either as sole or intercropped with such cereals as maize, millet or sorghum [21]. SAMNUT 10 and 11 are classified as late maturing, SAMNUT 21 and 22 as early maturing and SAMNUT 23 and 24 as extra-early maturing genotypes [21]. A non-nodulating isoline of groundnut (ICGL-5) was used as a reference crop for calculating N_2 fixed by the six genotypes. N-difference method was employed for the estimation of the fixed N_2, as suggested by Evans and Taylor [22], using the following equation:

N_2 fixed (Q) = N yield (of N-Fixing crop) – N yield (of Reference Crop)
The ESP and SAR where, however, respectively calculated using the following expressions:

$$\text{ESP} = \frac{Exchangeable\ Sodium\ (cmol/kg)}{Cation\ exchange\ capacity\ (cmol/kg)} \times 100$$

$$\text{SAR} = \frac{[Na+]}{\sqrt{1/2([Ca^{2+}] + [Mg^{2+}])}}$$

Where $[Na^{+}]$, $[Ca^{2+}]$ and $[Mg^{2+}]$ are the concentrations (in Cmol kg^{-1}) of the sodium, calcium and magnesium in the soil solution respectively.

Three kg of the 5mm-sieved saline and normal soil samples were each weighed into different plastic pots, of 13.5cm length and 17.6 cm in diameter. The treatments were laid in a completely randomized design (CRD) replicated thrice.

2.4 Planting Watering and Weeding

Three seeds of each genotype were planted per pot and later thinned to one plant per pot at two weeks after planting. Macro and micro (fertilizer) nutrients were appropriately applied per pot, according to the Broughton and Dilworth [23] N-free Plant Nutrient Solution. The plants were watered daily appropriately. Weeding was done manually by hand picking and weeds left in the pots. The plants were harvested at eight weeks after planting.

2.5 Statistical Analysis

The data generated were subjected to analysis of variance (ANOVA) using the statistical analysis system (SAS) package [24]. Significantly different means were separated using the Fisher's least significant difference (LSD) at $P \leq 0.05$ [25]. Parameters with coefficient of variability (CV) exceeding the acceptable limit of 40 percent were transformed following the log transformation procedure.

3. RESULTS AND DISCUSSION

The result in Table 1 showed that the saline soil had a pH of 8.3. This pH, coupled with an EC of less than 4dSm^{-1}, confirms the salinity of the soil [26]. Also, the availability of most

micronutrients, with the exception of Mo, is reduced with increasing pH (data not shown). Such a deficiency can, however, limit nodulation and can therefore constrain BNF [27]. Likewise, soil pH values greater than 8.0 and an ESP above 15%, suggests the likelihood of soil structural and reclamation problems [28]. The sodium adsorption ratio (SAR), as also shown in Table 1, was 1.60 $Cmolkg^{-1}$ for the saline soil. This indicates that even the saline soil did not contain alarming amounts of sodium in its exchange sites, as it is not within the >13 - 15 range that is necessary before a soil can be termed sodic or saline-sodic [26]. The Electrical conductivity (EC) range for both soils is within 0-2 mmhos cm^{-1}. This means that the salinity effects are negligible except for the most sensitive plants [29].

Table 1. Soil physical and chemical properties of the experimental site

Parameters	Saline soil	Normal soil
Particle size distribution (%)		
Sand	73.68	67.68
Silt	14.00	10.00
Clay	26.32	22.32
Textural class	Sandy clay loam	Silt loam
Soil pH (H_2O) 1:2.25	7.1	8.5
EC (mmhos cm^{-1})	0.55	0.18
SAR ($Cmolkg^{-1}$)	1.60	0.22
ESP (%)	45.50	6.70

EC=electrical conductivity, SAR=sodium absorption ratio and ESP=exchangeable sodium percentage

3.1 Effect of Genotype and Soil Type on Nodulation

3.1.1 Effect on effective nodule number

The result of the analysis of variance was not significant (P>0/05) for effective nodulation (Table 2). However, a physical observation shows that SAMNUT 24 (19), which was not significantly different from SAMNUT 22 and SAMNUT 11, was better than all other genotypes with the least value recorded in SAMNUT 23. There was, however, a highly significant (P<0.001) difference between the two soils. More effective nodules were recorded in the normal than the saline soil. This is in agreement with Van Hoorn et al. [30] who observed a similar trend in a study. They attributed their observation to high osmotic stress imposed by the salt in the soil. Genotype and soil interaction was not significant. SAMNUT 24 and, to some close extent, SAMNUT 11 and 22 can also be good N_2 fixers, as nodulation can also be a positive indicator for BNF, as observed by Redecker et al. [31] and Lekberg and Koide [32].

3.1.2 Effect on total nodule number

The effect of genotype was significantly higher (P<0.001) in the total nodule number. The soil type also highly significantly (P<0.001) contributed to the difference observed in the total nodule number. A significant (P≤0.05) interaction effect, between genotype and soil, was also observed to have influenced the parameter (Table 2). On the basis of individual genotypes, however, SAMNUT 10, 22 and 23 were statistically similar and, together, recorded the highest total nodule number. These were also at par with SAMNUT 11. SAMNUT 21 and 23 were also statistically similar, in terms of the parameter, and together recorded the least total nodule number. The best soil was the normal soil which significantly recorded an increased total nodule number over the saline soils (Table 2). The effect of

interaction is presented by Fig. 1. On this basis, therefore, SAMNUT 22 and 24 statistically recorded similar and the highest total nodule number. They were followed by, the statistically different, other genotypes in the order: SAMNUT 22=SAMNUT 24> SAMNUT 10 >SAMNUT 11>SAMNUT 23>SAMNUT 21. This suggests that SAMNUT 22 and 24, SAMNUT 10 and 11 and SAMNUT 23 and 21 were likely to respectively be tolerant, less tolerant and susceptible to salinity, in terms of nodulation. The potential for insensitivity to salinity of SAMNUT 22 and 24; and to some extent, SAMNUT 10 and 11 corroborates a study by Sprent [33] which indicated nodulation in groundnut to be insensitive to salinity probably due to a direct mode of rhizobial infection. This therefore indicates that these genotypes would be a good group of crops in a food security programme of a saline soil environment, besides the potential for N_2 fixation and pod yield of some of them.

It is also very interesting to observe that SAMNUT 21, which is least tolerant to salinity, fixed the highest N_2 (Table 2). This indicates the likelihood of the fact that the rhizobia housed in the "so scanty" root nodules of SAMNUT 21 are more resistant and/or tolerant to salinity, as also discovered by Elsheikh and Wood [34] in a study with soybean, than all other genotypes, and hence more promising, in terms of supplementing for chemical N fertilizers, than all other genotypes under study. Shamseldin and Werner [35] also observed a similar trend among some common bean (*Phaseolus vulgaris*) cultivars.

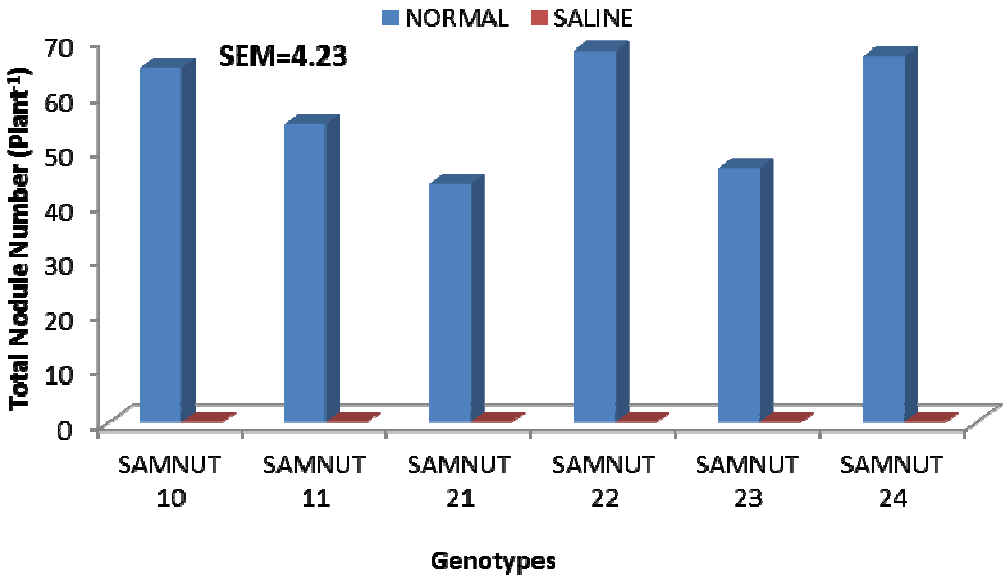

Fig. 1. Effect of genotype-soil interaction on total nodule number at 8 was

3.1.3 Effect on nodule dry weight

There was no significant difference between the genotypes in terms of their nodule dry weight (Table 3). However, significant (P≤0.05) difference was observed between the soil types. Highest nodule dry weight was recorded in genotypes grown on the normal soil (Table 3). The percentage difference between the soils, in terms of nodule dry weight, was 100%; the normal soil being higher. Although the genotype-soil interaction was also not significant in terms of the parameter, SAMNUT 23 physically recorded a higher nodule dry weight value than all the remaining genotypes and SAMNUT 21 was the least. The mean

nodule dry weight, however, still falls within the range recorded by Bala [36] in the same agro-ecological zone. The variation could be due to the differences existing among the genotypes in terms of their tolerance to salinity [33].

3.2 Effect of Genotype and Soil on Biological Nitrogen Fixation

There was no significant (P>0.05) difference among the genotypes with regards to nitrogen fixation; yet, SAMNUT 21 recorded the highest value of fixed N_2 while the least in the value, among all the genotypes, was SAMNUT 11 (Table 2). There was also no significant difference in the amount of nitrogen fixed by the genotypes grown on both the saline and non-saline soils. This is in contrary to the generally held view that salinity has a negative effect on rhizobium [37], on the host plant [38] and on the symbiotic relation [30]. The differences observed could be attributed to the differences in the amount of sodium present at the exchange sites, especially as the salinity observed in the soil is not that deleterious, [29] for less salinity-sensitive plants. Besides, the quantity of N_2 fixed (and proportion of total crop N derived from N_2-fixation) can (both) be influenced by such factors as cultivar [39].

Table 2. Effect of groundnut genotype and soil type on nodulation and N_2 fixed at 8 was

Treatment	Effective nodule. (plant^{-1})	Total nodule (plant^{-1})	Nodule dry weight (g plant^{-1})	N_2 fixed (mg plant^{-1})
Genotype (G)				
SAMNUT 10	13.67	32a	0.08	1760
SAMNUT 11	18.17	27ab	0.09	930
SAMNUT 21	12.17	21b	0.06	2500
SAMNUT 22	18.33	34a	0.10	1770
SAMNUT 23	12.00	23b	0.38	2100
SAMNUT 24	19.33	33a	0.08	1670
Means	15.61	29	0.13	1790
SE±	4.24	2.99	0.13	631
Soil (S)				
N	31.22a	57.00a	0.26a	1789
S	0.00b	0.00b	0.00b	1785
Means	15.61	29.00	0.13	1787
SE±	2.45	1.73	0.07	365
Interaction				
G*S	NS	*	NS	NS

*Means followed by dissimilar letters within the same treatment in a column are significantly different at 5% level of probability, NS=Not significant at 5% level of probability, * =significant at 5% level of probability*

3.3 Effects of Genotype and Soil on Peg and Pod

3.3.1 Effect on peg and pod numbers

From Table 3, we can see that there was no significant difference between the genotypes in terms of both peg and pod numbers. However, SAMNUT 23 was observed to be the best in, terms of both peg and pod. This clearly indicates the potential of SAMNUT 23 to high yields in such saline environments and, yet ultimately, ending up with a bumper harvest at the end of season. Effect of soil type was, however, significant ($P \leq 0.05$). The normal soil was the

best in terms of improved peg number but there was no significant (P>0.05) interaction between soil and genotype (Table 3). This result is also in total agreement and corroboration with Usman [40], who observed similar trend of results, while working with the same genotypes in the same agro-ecological zone. SAMNUT 11 recorded a few number of pegs, but none was transformed into pod, unlike SAMNUT 10 that transformed the few pegs produced into pods. This suggests SAMNUT 11 to relatively, be more soil-salt sensitive than the other genotypes under study.

Table 3. Effect of genotypes and soil on peg and pod numbers and pod weight

Treatment	Peg number (plant^{-1})	Pod number (plant^{-1})	Pod weight (g plant^{-1})
Genotype (G)			
SAMNUT 10	0.00b	1.67	0.17ab
SAMNUT 11	1.67ab	0.00	0.00b
SAMNUT 21	5.17ab	0.67	0.47ab
SAMNUT 22	2.50ab	0.19	0.00b
SAMNUT 23	7.17a	2.00	1.17a
SAMNUT 24	6.17a	1.00	0.04b
Means	3.78	0.92	0.31
SE±	2.099	0.783	0.318
Soil (S)			
N	5.17a	1.84a	0.61a
S	2.39b	0.00b	0.00b
Means	1.212	0.92	0.31
SE±	3.64	0.45	0.18
Interaction			
G*S	NS	NS	NS

Means followed by dissimilar letters within the same treatment in a column are significantly different at 5% level of probability, NS=Not significant at 5% level of probability

3.3.2 Effect on pod yield

Result on analysis of variance showed no significant (*P*>0.05) difference in pod weight (yield) among the genotypes. The highest and lowest physical pod weight values were, all the same, respectively recorded for SAMNUT 23 and SAMNUT 11, with the latter seemingly more salt-sensitive. SAMNUT 21 was however statistically similar to SAMNUT 23; and the lowest SAMNUT 11 was statistically similar to SAMNUT 22 and 24. The highest pod yield observed in SAMNUT 23 could be attributable to inherent genotypic trait. The high peg number observed for the genotype (Table 3) also suggests the possibility of more pods production. High pod yield was recorded in the normal than in the saline soil (Table 3). This buttressed the fact on the effect of salinity on groundnut yield in particular, as also observed by Motahari et al. [15] and the yield of many other crops [41] in general.

The result on pod yield is also in agreement with the outcome of a study by Van Hoorn et al. [30]. They equally observed similar trend of results which they also attributed to salt-sensitivity of the grain legumes, as salinity tends to hinder the pathway of biological nitrogen fixation in legumes. Pod formation was also found to be almost 100% poor in a study by Singh et al. [42] on an Indian soil. Usman [40] also observed similar trend of results. Effect of soil type was, however, significant (P≤0.05). The normal soil was proved to be more promising, in terms of pod yield, but there was no significant (P>0.05) interaction between the soil and genotypes (Table 3).

4. CONCLUSION

This study is sought to evaluate existing groundnut genotypes for symbiotic nitrogen fixation and yield, under saline soil condition, in the northern guinea savanna of Nigeria. SAMNUT 21 was observed to be the best genotype, amongst others, in terms of biological nitrogen fixation. On the other hand, SAMNUT 23 recorded the highest number of pegs and dry pod and nodule weights. Similarly, saline soil displayed a reduction effect on, virtually all, the parameters under study, but BNF, compared to the normal soil. SAMNUT 21 and 23 can therefore be tried closely, especially when programmes are aimed at substituting crops for highly priced mineral N fertilizers, groundnut yield, and consequently farmer income improvement, especially in areas with salt stressed soils. A more diverse research to isolate and identify the strains of rhizobia that are housed by the individual genotypes, so as to really appreciate the rationale behind the BNF potential of SAMNUT 21, amongst other genotypes, under saline condition would assist in various rhizobiological studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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