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Genotype Effects of Cowpea and Soybean on Nodulation, N_2-fixation and N Balance in the Northern Guinea Savanna of Nigeria

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Abstract: This study was designed to evaluate N_2-fixation and N balance of improved cowpea and soybean genotypes in the NGS of Nigeria. Field experiments were conducted in 2003 and 2004 to assess nodulation, N_2 fixation and N contribution of two cowpea (IT96D-274 and SAMPEA-2) and soybean (SAMSOY-2 and TGx 1448-2E) on a leached ferruginous tropical soil (Haplustalf). The legume genotypes and a reference maize crop (Oba Super 2) were planted in randomized complete block design with three replications. The N difference method was used in estimating symbiotic N_2 fixation while the N contribution was estimated by the difference between N fixed and N exported in the grain during harvest. Although nodule number did not differ significantly among genotypes, the weight was significantly higher in soybean than cowpea. Significant difference in N_2 fixation was only observed between cowpea genotypes and it was attributed to the differences in maturity period. TGx 1448-2E derived on average 49.8 kg N ha^{-1} or 37% of plant total N from fixation compared to IT96D-724 with 15.8 kg N ha^{-1} or 19%. In both years, N balance ranged from -30 to 9 kg N ha^{-1} depending on the genotype. With the exception of SAMPEA-7 in 2003, all genotypes led to a net negative contribution to soil N and a positive N balance was only obtained when the nitrogen harvest index was less than the proportion of Ndfa. The results show that reasonable maize yield may not be obtained following these grain legumes without supplementary N added to the soil.

Key words: N_2 fixation, N balance, grain legumes, genotypes, cowpea, soybean

INTRODUCTION

Grain legumes are important component of the cropping system of the Northern Guinea savanna (NGS) of Nigeria. Cowpea features prominently in the cereal-based cropping system and is mostly grown in intercrop with maize, millet or sorghum. Although, soybean is a relatively new crop, its production and utilization has expanded approximately 10-fold in Nigeria over the past 10-15 years (Sanginga, 2003). Like other leguminous crops, they are considered by farmers to stabilize crop yields and serve as sources of income and protein for their families. In addition to these, agronomists generally consider them as a cheap, clean and renewable source of nitrogen (N) for the non-N-fixing crop component of the cropping system such that farming practices that favor the more economically viable and environmentally prudent atmospheric N (N_2) fixation will benefit both agriculture and the environment (Vance, 2001). Farmers in the NGS of Nigeria might not have fully benefited from the contribution of N_2 fixation by grain legumes due to the use of plant varieties limited in their ability to fix N_2 in symbiosis, and edaphic constraints that include soil acidification, drought, and shortage of specific nutrients (Graham and Vance, 2003; Mariangela and Vargas, 2000). Several genotypes of cowpea are found on farmers' fields across the entire savanna agro-ecology while soybeans are found mainly in the Guinean savanna. However, majority of the genotypes were obtained from unreliable sources.

The Institute for Agricultural Research (IAR), Samaru and the International Institute of Tropical Agriculture (IITA) have introduced improved high yielding cowpea and soybean genotypes to replace the farmers' varieties. However, recent research findings have shown inter- and intra specific differences in the amount of N_2 fixed by grain legumes. Several factors have been responsible for these variations and they include cultivar differences in

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nodule number and mass, speed of nodulation, lateral root nodulation, post flowering, N accumulation, acetylene reduction activity, allantoic acid production, and nodule enzyme production and function. However, not all these traits are universally useful in selection for high N_2 fixation. For example, N_2 fixation levels are correlated with time to flowering, but in the northern USA at least, the use of cultivars with a longer pre-flowering period is only useful if overall time to maturity can be held constant, therefore, nodule mass was considered as a more useful indicator of symbiotic potential (Graham *et al.*, 2004). Therefore, there is need to select cultivars with superior ability for N_2 fixation best matched to a particular environment (Okogun *et al.*, 2005). In addition, even though many reports have indicated that the cultivation of legumes results in the enrichment of soil N, this has often been dependent on the proportion of the legume's N that is fixed and N harvest index. For such a beneficial residual effect to occur, it is expected that the amount of fixed N_2 returned by the legumes to the soil must be greater than the amount of soil N in the harvested grain (Giller, 2001). The pioneer study on nitrogen fixation and N balance of recent IITA soybean genotypes was conducted in the southern Guinea savanna of Nigeria (Sanginga *et al.*, 1997; Sanginga *et al.*, 2002). The study did not involve genotypes commonly cultivated in the NGS. Similarly, very little information is available on N balance of popular cowpea genotypes in the NGS of Nigeria. This study was therefore designed to evaluate N_2-fixation and N balance of improved cowpea and soybean genotypes in the NGS of Nigeria.

MATERIALS AND METHODS

Experimental site: The field experiments were conducted in 2003 and 2004 at Samaru. Samaru is located in the Northern Guinea savanna of Nigeria (Latitude 11°11'N and Longitude 7°38'E) and has a monomodal rainfall pattern with a mean annual rainfall of about 1011±161 mm concentrated almost entirely in the 5 months (May/June to September/October) of the cropping season (Oluwasemire and Alabi, 2004). The main soil sub-group is Typic Haplustalf.

Soil sampling and analysis: Twenty core soil samples (0-15 cm depth) were taken at random in the field and bulked to form composite. A sub-sample of the composite was air-dried, sieved through a 2 mm screen and analyzed for some chemical properties following standard procedure. The soil was loam in texture with the following properties: pH (Water), 5.4; organic carbon, 4.8 g kg^{-1}; total N, 0.40 g kg^{-1}; available P, 6.53 mg kg^{-1}; and exchangeable cations ($cmol^{+}$ kg^{-1}) of Mg^{2+}, 0.36; Ca^{2+}, 0.80; K^{+}, 0.15; and Na^{+}, 0.28. The native rhizobial population was estimated using a fresh soil sub-sample according to the most probable number (MPN) method (Vincent, 1970). The MPN estimate of rhizobia in the soil was about 31 cells g^{-1} soil well above the 20 cells g^{-1} soil that may respond to rhizobia inoculation (Singleton *et al.*, 1986; Sanginga *et al.*, 1996).

Seed sources and preparation: Seeds of cowpea (SAMPLE-7) and soybean genotypes (SAMSOY-2) were obtained from the Institute for Agricultural Research (IAR), Samaru, Zaria, Nigeria while seeds of cowpea (IT 96D-724) and soybean genotypes (TGx 1448-2E) were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. These seeds are currently in farmers' hands and are used for cultivation as sole or intercrops in multiple cropping systems. Maize genotype (Oba Super 2), a commercial hybrid was obtained from Premier Seeds Plc and was used as reference crop to estimate nitrogen fixation in the grain legumes by the difference method (Hardason and Danso, 1993). All seeds were surface sterilized with 95% ethanol for 30 sec and H_2O_2 for 3 min and then rinsed thoroughly with sterile water to avoid contamination with rhizobia.

Field experiment: The experimental area was marked out from the field, ploughed, disc-harrowed and ridged at an inter-row distance of 75 cm. The experimental design was a randomized complete block design with three replications. Each plot measured 4.5×12.0 m. A basal application of 20 kg N as urea (46% N), 17.5 kg P as Single super phosphate (18% P_2O_5) and 16.7 kg K as muriate of potash (60% K_2O) was made to soybean and cowpea genotypes. The fertilizers were applied at planting by side banding about 5 cm away and below the seed. Same amount of N was applied to maize but one-third of the quantity was applied at two weeks after planting (WAP) and the remaining two-third at six WAP. P and K were applied at planting by side banding at 26.2 kg P ha^{-1} and 50 kg K ha^{-1}. Two seeds each of the cowpea genotypes were hand sown at a spacing of 20 cm within row and the seedlings thinned to one per hill one week after emergence while soybean was planted at 50 cm within rows. Two maize seeds were planted at 25 cm within row and the seedlings thinned two WAP. All plots were weeded at about two and six WAP by hand and the cowpea sprayed with 20 ml L^{-1} solution of Karate 2.5 EC weekly as from 6 WAP to control insect pest attack.

Plant sampling and analysis: All plant sampling were confined to the four inner rows. Sampling was done at mid-podding and maturity. At the first sampling time, 8 plants were carefully dug out from the cowpea plots while all plants within 1.5 m^2 quadrat in the two central rows of the soybean plot were carefully dug out. To minimize damage to the root system, the soil around the plant was loosened using forks. Five plant samples were taken from the maize plot by cutting at the base of the stem. Plant samples were processed for number and dry weight of nodules and measurement of N_2-fixation. Harvested plant samples were chopped into 10-20 mm pieces and sub sampled, and about 500 g fresh weight were oven-dried at 70°C before grinding to pass through a 0.5 mm sieve. For the last harvest, plant samples were separated into reproductive (grains) and vegetative parts (shoots) before sampling. The grains were threshed from the pods and cobs and further dried in the sun until a moisture content of 12% was reached using a Dickey-john grain moisture tester. Plant shoots, nodules and straw were dried in the oven at 70°C to a constant weight before weighing. Total N in the grains, shoots and straws was determined by the Kjedahl procedure (Bremner and Mulvaney, 1982). The net contribution of N_2 fixation to the N balance of the soil was calculated as follows: Net N balance=Nf-Ns (Peoples and Craswell, 1992), where Nf is the amount of N_2 fixed and Ns represents the total N in the seeds.

Statistical analysis: Data were subjected to analysis of variance (ANOVA) using the GLM procedure of SAS (SAS, 1999). Where the F-ratios were found to be significant, treatment means were separated using the least significant difference (LSD) at 5% level of probability.

RESULTS AND DISCUSSION

Nodule number and weight: Both the legume genotypes nodulated with indigenous rhizobia in 2003 and 2004 but the number of nodules differed depending on the genotype. Both soybean genotypes and Sampea-7 were found to produce relatively higher number of nodules than IT 96D-724 (Table 1). This result was consistent for all the two years and combined. The number of nodules observed in this study falls within the range reported by some previous workers. Olufajo *et al.* (1988) reported a range of 3 – 34 nodules plant^{-1} for the same soybean genotypes while Okogun *et al.* (2005) reported an average of 44.3 nodules plant^{-1} for TGx 1448-2E and 61.1 for Samsoy-2 across 20 locations in the same agro-ecological zone. 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. Low soil moisture and high temperature could lead to poor nodulation even in the presence of high number of indigenous rhizobia. Low soil fertility especially Nitrogen (N) has also been reported to hamper initiation of nodules and onset of fixation but this constraint is removed through the application of 20 kg N ha^{-1} recommended as a starter dose in Nigeria. However, most farmers rely on native soil N or residual N applied to cereal to grow grain legumes. Generally, Biological Nitrogen Fixation (BNF) in legumes requires adequate levels of soil nutrients, such as P and micronutrients (Sanginga *et al.*, 1995). Since P was applied at recommended rate and levels of micronutrients were adequate except for copper (data not shown), variation in the values obtained in this study may be due to morphological characteristics of the genotypes.

Generally, soybean genotypes had significantly higher nodule biomass than cowpea in both years and when combined (Table 1). There was no significant difference in the nodule weight of both soybean genotypes in 2003 and/or 2004. However, TGx 1448-2E produced consistently higher nodule biomass than Samsoy-2 in both years. This result is in contrast to the findings of Okogun *et al.* (2005) who reported a significantly higher nodule biomass for Samsoy-2 than TGx 1448-2E on farmers' fields. The difference could be due to the prevalence of compatible indigenous rhizobia strains in the farmers' field where Samsoy-2 has been cultivated for several years. Soybean genotypes have been known to form higher nodule biomass than cowpea but this is mainly due to morphological differences in the crops. Sanginga *et al.* (2000) recorded an average

Table 1: Nodule number and nodule dry weight as influenced by cowpea and soybean genotypes in 2003, 2004 and combined

	Nodule No. plant^{-1}			Nodule dry weight mg plant^{-1}		
Genotype	2003	2004	Combined	2003	2004	Combined
IT 96D-724	11.30	7.40	10.90	97.0	120.0	108.0
Sampea-7	15.10	17.10	16.10	103.0	130.0	117.0
TGx 1448-2E	14.70	19.30	17.00	227.0	370.0	298.0
Samsoy-2	17.50	13.50	15.50	203.0	353.0	278.0
Mean	14.70	14.30	14.90	158.0	243.0	200.0
SEM	3.21	5.28	3.22	40.7	56.6	36.4
LSD	NS	NS	NS	122.1*	169.8*	109.2*

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

Table 2: N_2-fixation and percent N derived from atmosphere (%Ndfa) as influenced by cowpea and soybean genotypes in 2003, 2004 and combined

	N_2-fixation (kg ha^{-1})			Ndfa (%)		
Genotype	2003	2004	Combined	2003	2004	Combined
IT 96D-724	17.70	13.90	15.80	21.30	17.60	19.40
Sampea-7	40.30	28.20	34.20	48.30	36.00	42.10
TGx 1448-2E	51.10	48.50	49.80	42.50	46.10	44.30
Samsoy-2	46.30	36.10	41.20	44.80	47.40	46.10
Mean	38.90	31.70	35.30	39.20	36.80	38.00
SEM	3.80	10.74	5.70	4.77	8.03	6.94
LSD	*	*	*	*	*	*

*Significant at the 5% level of probability

of 2.05 g $plant^{-1}$ nodule fresh weight for two soybean cultivars in a non sterile soil while Ssali and Keya (1980) recorded an average of 3.23 mg $plant^{-1}$ nodule dry weight for three cowpea cultivars. The results obtained in this study for nodule dry weight followed similar trend to that obtained for nodule number.

Nitrogen fixation and percent N derived from atmosphere: The amount of N_2 fixed by cowpea genotypes ranged from 13.9 to 40.3 kg ha^{-1} in 2003 and 2004 with Sampea-7 having consistently significantly higher values in both years (Table 2). Sanginga *et al.* (2000) also reported a range of 13.1 to 31.9 kg ha^{-1} for 8 genotypes in the derived savanna of West Africa. In the Table 2, combined analysis for both years showed that the amount of N_2 fixed by Sampea-7 was significantly higher than that of IT 96D-724. On the other hand, the amount of N_2 fixed did not differ significantly between soybean genotypes with S1 having slightly higher values in both years. Similarly, combined analysis over the years showed no significant difference within the soybean genotypes. Among all legume genotypes, TGx 1448-2E fixed significantly higher amount of N_2 than IT 96D-724 in 2003 and 2004. There was no significant difference in the amount fixed between Samsoy-2 and Sampea-7 in both years. The result also showed that Sampea-7 fixed significantly higher amount of N_2 than IT 96D-724 in 2003 and in the two years combined.

The differences observed in the amount of N_2 fixed by the legume genotypes could be attributed to the number of days required to attain maturity. Higher amounts of fixed N are found in longer duration genotypes (Carsky *et al*., 1997; Sanginga *et al*., 2002). The insignificant values observed between TGx 1448-2E and Samsoy-2 may be attributed to their relatively similar maturity duration of approximately 120 days (Sanginga, 2003; Olufajo *et al*., 1989) while Sampea-7 had longer maturity period than IT 96D-724. This was confirmed on the field by calculating the number of days it took for each genotype to reach physiological maturity.

The average amount of N_2 fixed by the soybean genotypes in two years was 41.2 kg ha $^{-1}$ for Samsoy-2 and 49.8 kg ha^{-1} for TGx 1448-2E. These values fall within the range of 10-58 kg ha^{-1} reported by Abaidoo *et al.* (2007) and 42-83 kg ha^{-1} reported by Sanginga *et al.* (1997), however, much lower than 114-188 kg ha^{-1} reported by Eaglesham *et al.* (1982). In addition to maturity period, the wide variations in the amount of N_2 fixed by different genotypes of the same crop depends on the N fixing capability of the genotype, the native fertility of the soil (Sanginga *et al*., 1997), the indigenous *Bradyrhizobia* sp. and methods of crop management (Okogun *et al*., 2005).

The grain legumes show wide variation in their proportion of plant N derived from N_2 fixation. The result showed that both soybean genotypes and Sampea-7 had significantly higher Ndfa% than IT 96D-724 in 2003. Highest value of 48% was recorded for Sampea-7 in 2003 and the lowest value of 18% for IT 96D-724 in 2004. On the other hand, Ndfa% by Samsoy-2 was slightly lower than S1 but not significant. The result followed similar trend as reported by Sanginga (2003) for the same genotypes at three locations in Nigeria (Table 2).

The wide variation in Ndfa% by grain legumes is not peculiar to certain crops only. The potential Ndfa% by soybean is in the range of 26-87 (Sanginga *et al*., 1997). Ganry (1992) and Badiane and Gueye (1992) reported a range of 28-81 for groundnut in the moist savanna of West Africa. The major contribution of grain legumes to soil fertility in cropping systems lies in their ability to fix atmospheric N. Therefore genotypes that derive high proportion of their N from fixation will be highly desirable especially in soils with low N status. However, the results indicated that less than half of the plant total N was derived from the atmosphere which implies that these genotypes used cannot meet all their N demand for growth and seed by only N_2 fixation. This indicates the need to adopt new strategies such as breeding new materials with high potential for N_2 fixation.

Grain N and N harvest index (NHI): Cowpea grains accumulated a narrow range of 30.8 to 32.7 kg N ha^{-1} over the two years (Table 3). Therefore, no significant difference was observed between the cowpea genotypes in 2003 and 2004. These grain N yields are lower than the range of 46-57 kg ha^{-1} reported by Eaglesham *et al.* (1982)

Table 3: Grain N and N harvest index as influenced by cowpea and soybean genotypes in 2003, 2004 and combined

	Grain N (kg ha^{-1})			N harvest index (%)		
Genotype	2003	2004	Combined	2003	2004	Combined
IT 96D-724	32.30	32.80	32.50	39.00	41.00	40.00
Sampea-7	30.80	32.60	31.70	37.00	41.00	39.00
TGx 1448-2E	65.70	78.30	72.00	55.00	51.00	53.00
Samsoy-2	53.70	56.20	55.00	52.00	49.00	50.00
Mean	45.60	50.00	47.80	46.00	46.00	48.00
SEM	4.20	7.94	4.49	3.00	3.10	2.10
LSD	12.60*	23.82*	13.47*	9.00*	9.30*	6.30*

*Significant at the 0.05 level of probability

but higher than 16.3-29.0 kg ha^{-1} reported by Sanginga *et al.* (2000). The difference could be attributed to differences in grain yields, as high yielding genotypes will produce correspondingly high N content in their dry matter. Awonaike *et al.* (1990) recorded 44.4 and 53.5 kg N ha^{-1} in grain of two cowpea genotypes that had grain yield of 2.4 and 3.2 t ha^{-1}. Soybean genotypes produced significantly higher grain N yield than cowpea genotypes in 2003 and 2004. TGx 1448-2E produced significantly higher grain N yield than the remaining genotypes. The average grain N observed for TGx 1448-2E in this study (72.0 kg N ha^{-1}) is similar to the values of 73.87 and 71.43 reported for TGx 1485-ID and TGx 1660-19F respectively (Bala *et al.*, 2003). Sanginga *et al.* (2002) also obtained 73 kg N ha^{-1} for TGx 1660-19F. Some authors have also confirmed higher N accumulation in soybean grains than cowpea grains (Eaglesham *et al.*, 1982; Carsky *et al.*, 2001).

The soybean genotypes had significantly higher NHI than the cowpea genotypes. The results followed similar trend in 2003 and 2004 (Table 3). The values obtained for soybean was very close to the mean of 55% reported for 5 uninoculated soybean genotypes (Sanginga *et al.*, 1997). The higher NHI recorded for soybean genotypes may be largely attributed to higher total N accumulation in the grains. Soybean genotypes accumulated larger proportion of their total N uptake in the grains on average 51% for both genotypes while both cowpea genotypes accumulated on average 38%.

Nitrogen balance: The importance of NHI depends on how it affects the soil N balance. The results obtained showed that the N balance of cowpea genotypes ranged between -18.9 and 9.4 kg N ha^{-1} with Sampea-7 having significantly higher value than IT 96D-724 in both years (Fig. 1). This range is very close to -10.6 to 11.1 kg N ha^{-1} reported for 8 cowpea genotypes (Sanginga *et al.*, 2000). In 2003, Sampea-7 which had a significantly lower NHI (37%) left a positive soil N balance while in 2004, the 2 genotypes had higher NHI which were statistically similar so that both genotypes led to a net negative N balance.

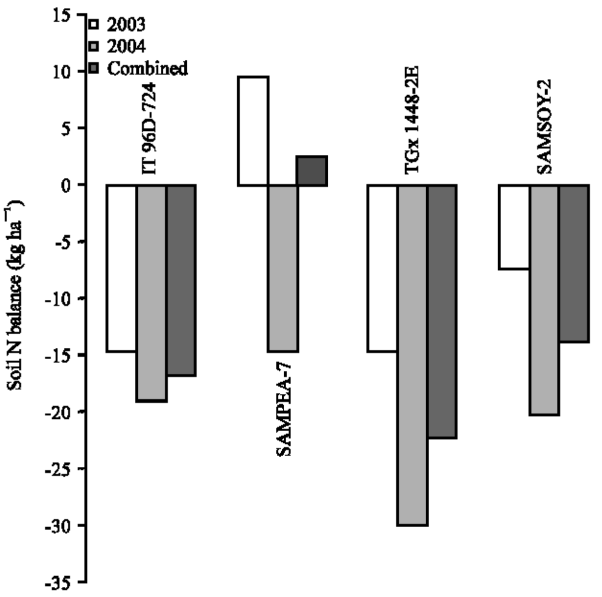

Fig. 1: Soil nitrogen balance of cowpea and soybean genotypes in 2003, 2004 and combined

Although both soybean genotypes contributed negatively to the net soil N balance in 2003 and 2004, Samsoy-2 had significantly higher value than TGx 1448-2E in both years. The negative N balances were as a result of high NHI values. The values obtained for TGx 1448-2E fall within the range (-46 to 20 kg N ha^{-1}) reported for some IITA promiscuous genotypes (Abaidoo *et al.*, 2007) but outside the range (-8 to 43 kg N ha^{-1}) observed for five promiscuous genotypes by Sanginga *et al.* (1997). Although Sanginga *et al.* (1997) used fewer numbers of genotypes than Abaidoo *et al.* (2007), the genotypes were inoculated and the inoculation was found to influence the N balance significantly with the inoculation improving the soil N balance.

Positive N balance may be attributed to the amount of biologically fixed N but for such a positive N balance to occur, it is expected that the amount of fixed N by the legumes to the soil must be greater than the amount of soil N in the harvested grain (Giller, 2001). This principle could only partially explain the results obtained in this study because it was not possible to distinguish the proportion of grain N derived from soil or fertilizer due to lack of facilities. It was however discovered that a positive soil N balance was only recorded where the proportion of Ndfa was greater than the NHI. It therefore suggests that all grain legumes with NHI greater than the proportion of Ndfa will lead to a negative contribution to the soil N balance. However, lower NHI may lead to lower grain yield, therefore to maximize net soil N balance, it is necessary to maximize the total crop N at grain harvest and percentage of N_2 fixed.

CONCLUSION

The actual contribution of grain legumes to soil N balance depends on the difference between the amount of fixed N_2 that is returned to the soil and the soil N in the harvested plant parts. In this study, we have tried to evaluate important traits affecting BNF in soybean and cowpea and calculated the soil N balance. Soybean genotypes had significantly higher nodule biomass than the cowpea genotypes and this was reflected in the amount of N_2 fixed. While there was no significant difference between the soybean genotypes, Sampea-7 fixed significantly higher amount of N_2 than IT 96D-724. In spite of the reasonable amount of N_2 fixed by the grain legumes, the results however indicated that less than half of the plant total N was derived from the atmosphere which implies that the these genotypes cannot meet all their N demand for growth and seed by only N_2 fixation. Therefore, further studies involving large number of genotypes are required to identify those that derive high proportion of their N from fixation as this will be highly desirable especially in soils with low N status. Similarly, positive soil N balance was only recorded where the proportion of Ndfa was greater than the NHI suggesting that all grain legumes with NHI greater than the proportion of Ndfa will lead to a negative contribution to the soil N balance. This will be a useful criterion for selecting grain legumes for high potential for N_2 fixation and positive N balance.

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