EVALUATION OF MEDIUM- MATURING SOYBEAN (*Glycine max* (L) Merrill) LINES FOR THEIR NITROGEN FIXATION POTENTIALS

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ABSTRACT
A field experiment was carried out to determine the nitrogen fixation ability of some soybean experimental lines and to determine the amount of residual nitrogen that could be made available to a succeeding arable crop. The study was undertaken at the Crops Research Institute, Fumesua, Kumasi. The experiment was laid out in a randomized complete block design with 3 replications. Each replication consisted of 10 plots which were randomly sown to a soybean line. The seeds were sown at a spacing of 75 cm between rows and 5 cm between plants. Data collected were nodule number per plant and nodule dry weight, number of pods per plant, number of seeds per pod, total seed yield, seed, residue and total nitrogen fixed by the soybean lines. The Total Nitrogen Difference method was used in determining the amount of N_2 fixed. The results showed that all the lines nodulated freely with the naturalized rhizobia in the soil. There were significant differences, however, in the nodulation abilities of the lines. Number of nodules was negatively correlated with nodule dry weight (r=- 0.45). The amount of nitrogen fixed was positively correlated with total seed yield (r= 0.65). Line GMX 92-16-2M produced the greatest number of nodules but did not fix the greatest amount of nitrogen. Line GMX 92-6-10M left the greatest amount of nitrogen (10 kg N ha^{-1}) in its residue. The results indicate that if farmers would grow this line, an amount of 10 kg N ha^{-1} would be made available to subsequent arable crop following harvesting.

INTRODUCTION
Soybean can utilize both biologically fixed nitrogen and chemically-manufactured nitrogen (N). However, with recent awareness of potential pollution of ground water by soil nitrates, much attention is being given to biological N fixation. In soybean the bacteria capable of fixing atmospheric N to usable forms live in symbiotic association with the roots. Like most tropical legumes, soybean can also be effectively nodulated by rhizobia of the cowpea miscellany, the *Bradyrhizobium* sp. The process whereby a rhizobium can nodulate another legume belonging to a different group is called promiscuous nodulation. Estimates of the amount of N fixed by soybean have varied from between 50 to 300 kg N

ha^{-1} year^{-1} (Weber 1966; Pal, 1989; Peoples and Gibson, 1989), although higher values had been obtained under specialized conditions.

Soybean production is expanding rapidly in Ghana. It is a source of dietary protein (40-42%), essential amino acids and minerals (Adu-Dapaah *et al.*, 2004). Soybean oil has low cholesterol. The soybean cake is an excellent source of protein for livestock feed (Mpepereki *et a* l., 2000). Much promotion about the nutritive value of the crop is being done in Ghana by the Ministry of Food and Agriculture (MOFA). As a result of this, research efforts must be intensified so as to present complete package to farmers and other stakeholders for sustainable soybean production and utilization. The Legumes Section of the Crops Research Institute, Fumesua, is in the process of releasing some soybean lines to farmers. Research into the nitrogen fixation potential at this stage is therefore very important since most soybean farmers are already too poor to afford chemical fertilizer. The objectives of this study were (i) to determine the potential for promiscuous nodulation and nitrogen fixation under field conditions and (ii) to estimate the residual nitrogen that would be made available to succeeding crops.

MATERIALS AND METHODS

The experiment was undertaken at the Crops Research Institute (CRI), Fumesua, situated in the moist semi-deciduous forest zone of Ghana. The soil type was Kumasi series, which is classified locally as ferric Acrisol or forest Ochrosols (FAO/UNESCO, 1990). The land was previously cropped to cowpea. The land was ploughed and disc-harrowed, pegged and laid out into 3 blocks, each block consisting of ten 5 x 2 m plots. The distance between plots was 1 metre and between blocks was 2 metres.

Ten medium-maturing soybean lines namely, *Anidaso*, GMX 92-6-10M, GMX 92-5-4E, GMX 92-16-4A, TGX 1841-2E, GMX 92-16-2M, TGX 1842-9E, TGX 1842-18E, TGX 1843-29E, and GMX 92-6-7M were used as treatments. The treatments were replicated three times. The maize variety, *Dorke*, was used as the reference crop (Martenson and Ljunggren, 1984). Seeds were planted at a depth of 3-5 cm. Thinning was done ten days after planting to two stands per hill. Initial weed control was done by spraying the field with Roundup at the rate of 300 ml per 15-litre knapsack sprayer before planting. Subsequent weed controls were carried out using a hoe during the third and eighth weeks after sowing.

Nodules were sampled once at flowering by carefully uprooting four consecutive plants from the border row of each plot. Nodules were detached from the roots, and those fallen in the soil were collected, washed and counted. The mean for each plot was recorded. Nodules were put in labeled envelops, oven-dried at 80°C for 48 hours, and their mean dry weights were recorded.

The following components of yield were measured. Five consecutive plants from the border row (as middle rows were reserved for yield data) of each plot were sampled from which the mean number of pods per plant, mean number of seeds per pod and mean seed weight were measured. The mean seed weight was obtained from weighing 100 random sample of seeds harvested from every plot.

Nitrogen fixation was determined by the Total Nitrogen Difference method (TND) which is based on comparisons of total N yield in a N_2-fixing crop, the soybean, and a non-fixing reference crop (Martenson and Ljunggren, 1984). At harvest 5 random soybean plants from each plot and the maize plants from each replication were taken, oven-dried at 80°C for 48 hours and ground. N content was determined using the Kjedahl's digestion method. Total N was determined for grains and residues separately. The amount of N fixed was calculated by subtracting N content in maize from that of the soybean.

Data Analysis

All data were analyzed with the Analysis of Variance (ANOVA) technique using the GEN-

STAT package and the Fisher's Protected Least Significant Difference (LSD) was used to determine treatments significant at 5% probability.

RESULTS AND DISCUSSION.

Nodulation effectiveness

Nodulation data are presented in Table 1. Line GMX 92-16-2M produced the highest number of nodules per plant, while line TGX 1842-9E produced the lowest nodule number. Nodule number for line GMX 92-16-2M was significantly higher ($p<0.05$) than those for all other lines. Additionally, line GMX 92-5-4E produced significantly higher nodule numbers than line TGX 1842-9E. All other treatments were however, not significantly different from one another. The highest nodule dry weight was recorded for line GMX-92-16-4A and was significantly higher ($p<0.05$) than those for lines TGX 1842-9E, GMX 92-6-7M and TGX 1841-2E. Nodule dry weight for line GMX 92-16-2M was higher ($p<0.05$) than those for lines GMX 92-6-7M and TGX 1842-9E.

Table 1: Mean nodule number and nodule weight per plant of ten soybean lines

Soybean line	Nodule number per plant	Nodule dry weight per plant (g)
ANIDASO	58.3	0.37
GMX 92-6-10M	64.0	0.47
GMX 92-5-4E	73.7	0.50
GMX 92-16-4A	66.3	0.60
TGX 1841-2E	48.7	0.33
GMX 92-16-2M	116.3	0.57
TGX 1842-9E	41.0	0.30
TGX 1842-18E	66.0	0.43
TGX 1843-29E	50.0	0.47
GMX 92-6-7M	48.3	0.30
Grand Mean	63.3	0.43
CV (%)	1.9	5.8
LSD (0.05)	31.3	0.24

Yield data

The highest plant stand at harvest was recorded by line TGX 1842-9E and this was significantly higher ($p<0.05$) than only for lines TGX 1843-29E and GMX 92-5-4E (Table 2). The lowest plant stand was recorded in line GMX 92-5-4E, but this was significantly lower than those of only lines TGX 1842-9E and Anidaso. All other treatment differences were not statistically significant. Line TGX 1842-18E recorded the highest value for 100-seed weight (Table 2), but this was significantly higher than that of only line GMX 92-16-2M which recorded the lowest value. All other treatment means were statistically similar at 5% level of significance.

Table 2: Mean plant stand, one-hundred seed weight and grain yield of ten soybean lines

Soybean line	Plant stand at harvest	100-seed weight (g)	Grain yield (tons per ha)
ANIDASO	149.3	13.8	1.72
GMX 92-6-10M	106.7	13.6	1.31
GMX 92-5-4E	89.0	13.6	1.53
GMX 92-16-4A	129.7	14.4	1.27
TGX 1841-2E	131.3	13.0	1.52
GMX 92-16-2M	130.0	11.4	1.81
TGX 1842-9E	153.3	14.9	1.61
TGX 1842-18E	132.7	15.2	1.92
TGX 1843-29E	99.3	14.9	1.61
GMX 92-6-7M	107.3	14.0	1.32
Grand Mean	122.9	13.9	1.55
CV (%)	6.2	1.2	6.5
LSD (0.05)	47.7	2.3	0.51

The highest grain yield was recorded by line TGX 1843-18E, while the lowest was produced by line GMX 92-16-4A (Table 2). Apart from lines GMX 92-6-7M, GMX 92-16-4A and GMX 92-6-10M that produced grain yields significantly lower than that of line TGX 1842-18E, all other treatment means were not significantly different.

Nitrogen Fixation

Table 3 shows that line GMX 92-16-2M directed the highest amount of nitrogen into the seed while line GMX-92-6-10M directed the least. At 5% significant level, significant differences were observed among lines GMX 92-16-2M, GMX 92-6-10M and GMX 92-16-4A. All other treatment differences were not significant.

Residue nitrogen results showed that line GMX 92-6-10M left the greatest amount in the residue, which was significantly higher ($p<0.05$) than in all lines, except TGX 1842-9E. Residue N determined in Anidaso, GMX 92-5-4E and GMX 92-16-2M were statistically similar, but were all significantly lower than that determined in TGX 1842-9E. All other treatment means were not significantly different from one another.

The maximum amount of total plant N was fixed by line TGX 1842-18E (Table 3), and this was significantly higher than the amount fixed in lines GMX 92-16-4A and GMX 92-6-10M. The differences among other treatments were not significant.

Table 3: Mean seed nitrogen, residual N and total plant nitrogen of the soybean lines

Soybean line	Seed N (kg/ha)	Residue N (kg/ha)	Total plant N(kg/ha)
ANIDASO	86.8	1.3	88.1
GMX 92-6-10M	60.6	10.0	70.6
GMX 92-5-4E	74.0	2.8	76.8
GMX 92-16-4A	66.3	4.4	70.6
TGX 1841-2E	77.6	5.3	82.9
GMX 92-16-2M	96.1	3.8	99.9
TGX 1842-9E	86.8	8.3	95.1
TGX 1842-18E	95.0	5.5	100.5
TGX 1843-29E	75.1	4.7	79.9
GMX 92-6-7M	69.4	4.2	73.6
Grand Mean	78.8	5.0	83.8
CV (%)	1.9	15.3	2.4
LSD (0.05)	28.9	4.1	29.2

The results showed that all the soybean lines nodulated freely with the naturally-occurring Bradyrhizobia in the soil. Similar results were obtained by Mpepereki *et al.* (2000). Rhizobia of the cowpea miscellany have been known to nodulate most tropical and subtropical legumes. Indeed, they constitute the single greatest problem of inoculant application since they are naturally abundant in tropical soils and also very competitive. Evaluation of nodulation provides a useful tool to the measurement of nitrogen fixation, but there is often no simple relationship between nodule number, nodule weight and total nitrogen fixed (Slyvester-Brady and KipeNolt, 1988). Differential abilities in nodule numbers might be due to genotypic differences since soil and climatic variations were minimal.

Nodule dry weight was negatively correlated with nodule numbers ($r=-0.45$). Line GMX 92-16-2M which produced the greatest number of nodules per plant, did not record the greatest nodule dry weight. Also the greatest nodule dry weight was recorded in line GMX 92-16-4A although it was third in the nodule count. This could be due to the fact that lines that produced more nodules produced relatively small-sized nodules, whilst those that produced fewer nodules produced bigger well-filled nodules. Similar observations were reported by Addu (2003) and Sarkodie-Addo (1991). Indeed, Giller (2001) reported that the ability to form nodules is not enough to obtain an effective nitrogen fixation symbiosis. Tour (2003) observed that cowpea lines that supported the greatest amount of nitrogen fixation produced lower grain yield. However, Caldwell and Vest (1970) and Sarkodie-Addo (1991) observed a positive correlation between grain yield and nitrogen fixation in several legumes including soybean. In the present study, line TGX 1842-18E which fixed the greatest total plant nitrogen (Table 3) also produced the greatest total grain yield (Table 2)

Total N for lines GMX 92-16-4A and GMX 92-6-10M were least among the ten lines studied, however, the amount of N left in the residue was greatest in line GMX 92-6-10M. For grain legumes to play any important role in the maintenance of soil fertility for other crops in rotation, they must obviously leave behind more N from nitrogen fixation in their residue. Increases in the amount of legume N contributed through residual effects are generally possible only if grain yield is decreased. This can rarely be justified in economic terms (Schwenke *et al.*, 1998), but this is worthwhile especially where maintenance of soil fertility is the major objective.

CONCLUSION

Nodulation results indicated that all the lines nodulated freely with the natural cowpea bacteria, differences in nodulation abilities were attributed to genotypic variations. More soybean lines should be screened to identify lines with more residual N for succeeding crops; such lines should be incorporated into the soybean improvement programme in Ghana. This would reduce the cost of inorganic fertilizer and thereby reduce the cost of production. Under the conditions of the study, line TGX 1842-18E fixed the highest amount of total plant N. The amount of N fixed ranged from 70.6 to 100.5 kg ha^{-1} which is consistent with literature.

Line GMX 92-6-10M left the greatest amount of N in the residue, thus if farmers are to grow this line, an amount of 10 kg N ha^{-1} would be added to the soil annually following the incorporation of the residue.

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