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NODULATION AND NITROGEN FIXATION RESPONSE OF INOCULATED SOYBEAN [*Glycine max*(L.) Merrill] VARIETIES TO INORGANIC NITROGEN IN THE SEMI-DECIDUOUS FOREST ZONE OF GHANA

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ABSTRACT

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A field experiment was conducted at the Plantation Section of the Department of Crop and Soil Sciences, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana, in 2015 major rainy season to evaluate the nodulation and nitrogen fixing potentials of soybean. Four varieties of soybean (Sonqu-panqu, Quashie, Songda and Salintuya-1) inoculated at a rate of 10g of inoculant per 1kg of soybean seeds and two levels of starter N (30 and 60 kg/ha) were laid out in a randomized complete block design and replicated four times. Recommended agronomic practices were carried out. Number of nodules per plant, percentage effective nodules, nodule dry weight, haulm N, seed N, total fixed N and percentage nitrogen from the atmosphere were measured. Results of the study indicated that all parameters were significantly affected by varieties and inorganic N. Under inoculation alone, Sonqu-panqu significantly produced the highest number of nodules. At 30 kg/ha N, Quashie and Sonqu-panqu produced the highest nodules. At 60 kg/ha N, Sonqu-panqu varietal effect was significantly higher than all other varietal effects in terms of number of nodules. Under inoculation alone, Sonqu-panqu, Salintuya-1 and Songda Salintuya-1 varieties supported the highest total fixed N, while Quashie and Songda varieties produced the highest fixed N at 30 kg/ha N. At 60 kg/ha N, the highest total fixed N was produced by Quashie and Sonqu-panqu varieties. Significant interaction of inoculated soybean and inorganic N exist in all parameters indicating different BNF efficiencies of the inoculated soybean varieties in combination with different rates of inorganic N.

INTRODUCTION

Soybean is one of the most economically important beans, which is considered as the one

of the grain legumes that has the potentials to improve the livelihood of the African populace.

The major reasons behind the importance of soybeans lies in the fact that it contains high protein (which is good in essential amino acid balance), lipids and many biologically active compounds and other micronutrients (Colibar *et al.*, 2009) and grown in almost every part of the world. It is good for food (soymilk, soy-cheese, condiment, Tom Brown and many infant and weaning foods). Based on proximate analysis of 100g seeds, soybean contains 446 kcal of energy, 36.30 protein, 19.94 oil and 9.3 fibre. It also contains minerals such as Ca, Fe, Mg, P, K, Na and Zn and vitamins such as vitamin C, thiamin, riboflavin, niacin, vitamin B-6, vitamin A and E (USDA/NNDSRR28, 2015). As a source of an excellent vegetable oil, it has been estimated that soybean seeds contain about 20% oil on a dry matter basis, and this is 85% unsaturated and cholesterol-free. Soybean cake is an excellent livestock feed, especially for poultry, while the haulms provide good feed for sheep and goats. In addition, very few insects cause damage to soybeans and the level of damage may not be of economic importance to warrant control measures (Dugje *et al.*, 2009). It can also be grown in between trees to improve the fertility of the plantation fields (Shurtleff and Aoyagi, 2009).

One of the major problems with soybean is the yield gap within and between countries because of differing environmental conditions. This can be closed by improving crop management practices that will address specific site constraints and enhance the productivity of already existing grain legumes (Baijukya *et al.*, 2013). Maximum yield and utilization can be achieved by selecting among different varieties to obtain the most suitable variety based on the soil and climatic conditions of the local environment (Mapuwei *et al.*, 2014). Notwithstanding, other management practices may need to be employed to further improve

yields. Artificial inoculation of legumes with appropriate strain of rhizobium can be another effective way of increasing growth and yield thereby closing the yield gap (Tairo and Ndakidemi, 2013). Improved yields of some legumes have been reported with inoculation (Albareda *et al.*, 2009). Although yield increment has been reported due to use of inoculants, there is still potential for further yield increase when additional avenues are explored (Mweetwa *et al.*, 2014). It has been shown that supplementary fertilization during early growth can lead to improved performance (Cheema and Ahmad, 2000; Ruark, 2009).

Previous workers have studied the effect of inorganic dose of N among soybean varieties. However, most of N level used might have been too low. An improved approach of this study was to increase the N levels to investigate the effects of such higher levels of N on the soybean performance. This experiment was therefore, set up to determine the effect of inoculated soybean varieties and inorganic N on the nodulation and nitrogen fixation of some soybean varieties.

MATERIALS AND METHODS

The study was conducted at the Plantation Crops Section of the Faculty of Agriculture, KNUST during major season of 2015. Random samples of the soil of the experimental site were collected at the depths of 0–15 cm and 15–30 cm, following ploughing and harrowing of the field to determine initial fertility status of the soil. Four varieties of soybean (Salintuya-1, Songda, Sonqu-panqu and Quashie) were inoculated with legume fix inoculant at 10g/ 1kg of seeds and three levels of inorganic N fertilizer of 0, 30 and 60 kg N ha⁻¹. The inoculant was applied using the method of Woomer *et al.* (1994).

Maize variety (*Obaatampa*) was used as a reference crop to determine nitrogen fixation. Seeds were obtained from the Crops Research Institute of the Council for Scientific and Industrial Research. Treatments were arranged in a randomized complete design (RCBD) and replicated four times. Seeds were sown on 11th May 2015 at 2 seeds per hole at a spacing of 75 cm x 5 cm in plots measuring 2 m x 5 m. Thinning was done to one plant per hill two weeks after seedling emergence. Plots were weeded with a hoe twice at 4 and 8 weeks after planting (WAP).

DATA COLLECTION

Nodulation

Five plants were randomly selected from the second rows. These were carefully dug, washed

Total fixed N (kg/ha) = N uptake in legume - N uptake in reference crop

N uptake = dry matter x % N

$$\%Ndfa = \frac{N \text{ uptake in legume} - N \text{ uptake in reference crop}}{N \text{ uptake in legumes}} \times 100\%$$

(Adapted from Konlan *et al.*, 2013)

where

% Ndfa = percentage of nitrogen derived from atmosphere

Dry matter = dry matter yield (kg/ha)

% N = dry matter N (%)

RESULTS

Nodule Number

Results for nodule number of soybean are presented in Table 1. Significant ($P < 0.05$) interaction was recorded between inoculated soybean and inorganic N. Under inoculation alone, Sonqu-panqu significantly ($P < 0.05$) produced the highest number of nodules than all the other varieties except that of Quashie. Varietal effects of Quashie was also higher than those of Salintuya-1 and Songda varieties. At 30 kg/ha N, varietal effect of Quashie and Sonqu-panqu were similar, but either effect was

in a sieve to remove soil adhering materials. All nodules from each plant were removed and counted. Number of fixing nodules were determined by splitting the nodules open and counting those with pink colouration. Percent of effective nodules was calculated as the ratio of effective nodules to total number of nodules. The nodules were oven dried at 70°C for 48 hours and the weights recorded to obtain the nodule dry weight.

Estimation of Nitrogen Fixation

The Total Nitrogen Difference Method (TND) described by Hanssen (1994) was used to estimate the amount of nitrogen fixed by the different soybean varieties. Total N from the reference crop, maize (*Obaatampa*) variety was subtracted from that of the legume crop.

significantly higher than those of Salintuya-1 and Songda varieties. At 60 kg/ha N, Sonqu-panqu varietal effect was significantly higher than all other varietal effects. Nodule number of Quashie was also higher than for both Salintuya-1 and Songda varieties.

Percent Effective Nodules

Percent effective nodules of soybean varieties are presented in Table 2. Significant ($P < 0.05$) interaction was observed between the two factors.

Under inoculation alone, Salintuya-1 recorded significantly the highest effective nodules than all other varieties. The effect of Sonqu-panqu was the lowest, but this was similar to that of the Quashie. At both N rates, the Salintuya-1 varietal effect was significantly higher than all other varieties. Also at both N rates percent effective nodules in the Quashie was the lowest.

Nodule Dry Weight

Nodule dry weight as affected by inoculated soybean and inorganic N is presented in Table 3.

There was significant ($P < 0.05$) and inorganic N interaction on nodule dry weight. Under inoculation alone, Salintuya-1 varietal effect was significantly higher than those of Quashie and Songda only. The effect of Sonqu-panqu was also higher than that of Quashie only. At 30 and 60 kg/ha N application, Sonqu-panqu varietal effects were significantly ($P < 0.05$) higher than all the varietal effects. In both occasions, the Quashie varietal effect was significantly ($P < 0.05$) lower than all other varietal effects.

Table 1. Number of nodules as affected by inoculated soybean and inorganic N in 2015 major rainy season.

Inoculated soybean	Number of nodules		
	N rates in kg/ha		
	0	30	60
Quashie	68	51	44
Salintuya-1	50	34	21
Songda	54	29	35
Sonqu-panqu	76	58	58
LSD (0.05)	8.5		
CV (%)	12.2		

Table 2. Percent effective nodules as affected by inoculated soybean and Inorganic N in 2015 major rainy season.

Inoculated soybean	Percent effective nodules		
	N rates in kg/ha		
	0	30	60
Quashie	72.50	49.50	48.50
Salintuya-1	84.00	74.00	76.00
Songda	75.75	60.00	59.50
Sonqu-panqu	69.00	61.00	61.00
LSD (0.05)	6.03		
CV (%)	6.4		

Table 3 Nodule dry weight as affected by inoculated soybean and inorganic N in 2015 major rainy season.

Inoculated soybean	Nodule dry weight (g)		
	N rates in kg/ha		
	0	30	60
Quashie	0.45	0.31	0.30
Salintuya-1	0.53	0.46	0.48
Songda	0.47	0.38	0.37
Sonqu-panqu	0.52	0.55	0.64
LSD (0.05)	0.05		
CV (%)	7.2		

Haulm N

Haulm N result is presented in Table 4. The effect of interaction was significant ($P < 0.05$). Under inoculation alone, Songda and Salintuya-1 were similar and produced the highest haulm N; however, the effect of Salintuya-1 was not different from that of Quashie and Sonqu-panqu. Following application of 30 kg/ha N, Quashie and Songda varietal effect was the highest while the effect of Salintuya-1 and Sonqu-panqu was the least. At 60 kg/ha N, the effect of Quashie produced the highest haulm N. The effect of Salintuya-1 and Sonqu-panqu varieties was similar and higher than the effect of Songda. Songda varietal effect was the least.

Seed N

The results for seed N of soybean are presented in Table 5. There was significant ($P < 0.05$) interaction between the two factors. Under inoculation alone, Sonqu-panqu varietal effect was the highest. The effects of Quashie and Salintuya-1 varieties were similar and significantly higher than that of Songda. Following application of 30 kg/ha N, the highest seed N was produced by Sonqu-panqu. The effect of Quashie was higher than that of

Songda and Salintuya-1 varieties, while Salintuya-1 produced the least. At 60 kg/ha N, the effect of Sonqu-panqu was also the highest. The effects of Quashie was higher than that of Salintuya-1. The effect of Songda was the least.

Fixed N

The results for fixed N are presented in Table 6. There was significant ($P < 0.05$) interaction between the two factors. Under inoculation alone, Sonqu-panqu, Salintuya-1 and Songda varietal effects were similar and the highest, while Quashie, which was not significantly different from Salintuya-1 and Songda varieties, produced the least. Following application of 30 kg/ha N, Quashie and Songda varieties produced the highest fixed N. At 60 kg/ha N, the highest effect was produced by Quashie and Sonqu-panqu varieties followed by Salintuya-1, while Songda varietal effect was the least.

Percent Nitrogen Derived from the Atmosphere

The results for percent N derived from the atmosphere are presented in Table 7. There was significant ($P < 0.05$) interaction between the two factors.

Under inoculation alone, there was no significant difference between the varieties. Following application of 30 kg/ha N, Quashie and Songda varieties were similar and produced the highest effect. The effect of

Sonqu-panqu was higher than that of Salintuya-1. At 60 kg/ha N, Quashie and Sonqu-panqu produced the highest effect, while Salintuya-1 was significantly ($P < 0.05$) higher than Songda.

Table 4. Haulm N as affected by inoculated soybean and inorganic N at harvest in 2015 major rainy season

Inoculated soybean	Haulm N (kg/ha)		
	N rate (kg/ha)		
	0	30	60
Quashie	56.92	77.13	83.44
Salintuya-1	60.21	56.05	68.86
Songda	65.16	77.24	55.47
Sonqu-panqu	53.58	48.68	60.52
LSD (0.05)	8.06		
CV (%)	8.8		

Table 5. Seed N as affected by inoculated soybean and inorganic N at harvest in 2015 major rainy season

Inoculated	Seed N (kg/ha)		
	N rate (kg/ha)		
	0	30	60
Quashie	8.40	12.04	9.04
Salintuya-1	8.85	2.70	7.17
Songda	4.42	7.29	2.76
Sonqu-panqu	20.66	18.92	26.51
LSD(0.05)	0.87		
CV (%)	5.7		

Table 6. Fixed N as affected by inoculated soybean and inorganic N at harvest in 2015 major rainy season

Inoculated soybean	Fixed N (kg/ha)		
	N rate (kg/ha)		
	0	30	60
Quashie	65.33	89.16	92.49
Salintuya-1	69.06	58.74	76.03
Songda	69.58	84.54	58.23
Sonqu-panqu	74.24	67.60	87.03
LSD(0.05)	8.64		
CV (%)	8.1		

Table 7. Percent nitrogen derived from the atmosphere as affected by inoculated soybean and inorganic N at harvest in 2015 major rainy season

Inoculated soybean	Nitrogen derived from the atmosphere (%)		
	N rate (kg/ha)		
	0	30	60
Quashie	76.95	81.99	82.27
Salintuya-1	77.86	74.86	79.21
Songda	77.87	81.22	74.73
Sonqu-panqu	78.89	76.97	81.48
LSD(0.05)	2.08		
CV (%)	1.8		

DISCUSSION**Nodulation**

Significant interaction was recorded between inoculated soybean and inorganic N rates on nodule number, percentage effective nodules and nodule dry weight. This means that the soybean varieties responded differently in the treatments indicating differences in their symbiotic potentials and combined N use. Several authors (Sarkodie-Addo et al., 2006;

Konlan et al., 2013) reported such varietal differences in nodulation. Differences in nodular frequency among four soybean cultivars subjected to *Bradyrhizobium japonicum* and in combination with arbuscular mycorrhizal has been reported (Meghvansi et al., 2008). Different nodulation response of soybean varieties to different inoculations was also been reported (Soe et al., 2012).

Dogra and Dudeja (1993) observed that sensitivity of symbiosis in legumes to combined N is strongly dependent on the plant and *rhizobium* interaction not on the individual plant or microsymbiont.

Application of N adversely affected nodule numbers. Nodulation in all varieties decline with application of 30 kg N/ha. There was further decline in nodulation, except in Songda, at 60 kg N/ ha. The result agrees with the findings of many workers who reported decrease in number of nodules because of addition of inorganic dose of N (Mendes et al., 2003; Achakzai, 2007). Amba et al. (2013) also reported application of N a week after planting soybean, reduced number of nodules. Many authors attempted to explain this occurrence, but no single explanation was found sufficient.

Nitrogen Fixation

Results of haulm N, seed N and fixed N showed significant interaction of inoculated soybean with N rates. This means that the inoculated varieties had different N requirements for N fixation. For example, for inoculation alone, the highest haulm N was obtained by Songda, while seed N was highest in Sonqu-panqu. Differences in haulm and seed N of soybean varieties due to inoculation conforms to report of Mulongoy (2015) who reported that a strain of *rhizobium* can nodulate and fix different amount of nitrogen with a range of cultivars of the same plant species. Different responses of soybean

varietiesto inoculations on nitrogen fixation has also been reported (Soe et al., 2012)

Large quantities of fertilizer N inhibits N fixation, but low doses of fertilizer N can stimulate early growth of legumes and increase their overall N fixation (Mulongoy, 2015). However, some high yielding cultivars may require additional N to complement their growth and subsequently yield. Increased yield due to increased utilization of N has been reported (Bekele et al., 2016). There were inconsistencies on the response of inoculated soybean to combined N on nitrogen fixation in this study; while some varieties improved their BNF efficiency with combined N other varieties recorded reductions in such parameters. Such inconsistencies were because sensitivity of symbiosis in legumes to combined N is strongly dependent on the plant and *rhizobium* interaction not on the individual plant or microsymbiont (Dogra and Dudeja, 1993).

CONCLUSION

Differences in inoculation response of different varieties of soybean was exhibited. Generally, the effect of combined N rates differed among varieties of soybean, while N rates improved nitrogen fixation in some of the varieties, in other varieties, there was a reduction in such parameters. Such differences could have been due to differences in their host-*rhizobium* interactions.

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