Ife Journal of Agriculture, 2018, Volume 30, Number 3

RESPONSE OF SOYBEAN (*Glycine max* (L.) Merrill) TO METHODS AND TIME OF RHIZOBIAL INOCULATION IN ABEOKUTA, SOUTHWESTERN NIGERIA

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ABSTRACT

This study investigated the effect of time and methods of rhizobial inoculation on growth and nodulation of soybean in the screenhouse. The experiment was a 4 x 2 factorial, arranged in completely randomized design with three replications. The treatments were 2 methods of rhizobia inoculation and 4 levels of time of application. The liquid (broth culture) and solid inoculants were applied at different time intervals, at sowing (0 week), 1, 2 and 3 weeks after planting (WAP), respectively. Observations were made on plant height, number of nodules (primary and secondary), root weight, shoot weight, number of pods, number of leaves, nodule dry weight, pod dry weight at 8 WAP and grain yield per plant at harvest. Application of liquid inoculant gave the highest value in almost all the parameters measured compared with solid inoculants. The liquid inoculant gave 100, 60 and 40.3% increase in secondary nodules, total nodule count and grain yield, respectively, over the solid inoculant. The time of inoculant application significantly ($p < 0.05$) affected shoot weight, number of pods, number of leaves and grain yield per plant of soybean. Generally, inoculation at sowing gave the best performance in most of the agronomic parameters measured with either solid or liquid inoculants. However, plants that received liquid inoculant gave highest number of pods and grain yield at 2 WAP which amounted to 46.7 and 70.6% increase, respectively, over inoculation at sowing. It is, therefore, concluded that application of liquid rhizobial inoculant at 2 WAP could enhance optimum production of soybean.

Keywords: Liquid inoculant, Rhizobial inoculation, Solid inoculant, Soybean.

INTRODUCTION

Soil nitrogen which is considered as the most limiting nutrient particularly in the tropics can be supplied to the soil when effective nodulation occurs as a result of inoculation of effective rhizobia strains. Inoculation of legume is a practice that has been carried out for a long time now, especially in the temperate region (Brockwell *et al*., 1985; Oad *et al*., 2002; Gold *et al*., 2012; Faridul Alam *et al.*, 2015); and a relatively new area yet to be fully explored by the low-income farmers that practice low or no input agriculture in Nigeria. Total adoption of this technique will cut on the cost expended on, and reduce the use of mineral fertilizer that has resulted in unacceptable levels of water pollution and eutrophication of lakes and rivers (Lupwayi *et al*., 2010).

Seed inoculation with rhizobium strain enhances nitrogen fixation in soybean, and several researches reported significant yield increase were obtained by inoculation of soybean with appropriate bacteria before sowing (Mpepereki, 2000; Ronner, 2016). Salih *et al*. (2015) reported that biological nitrogen fixation can supply nitrogen which may increase relative growth rate and yield of soybean. Significant increase in soybean growth parameters and grain yield due to inoculation of *Bradyrhizobium* isolates have been reported by several authors (Soe *et al*., 2010; Kala *et al*., 2011). Zuffo *et al*. (2015) reported increase in plant height and shoot dry weight of soybean as a result of *Bradyrhizobium japonicum* application. In addition, Mohamed and Hassan (2015) also found that inoculated plants produced higher nodule dry weight, grain yield, number of pods and number of seeds than the uninoculated plants. However, the type and period of inoculant application is very important during rhizobial inoculation. Thanni *et al*. (2016) also reported that different adhesive used as sticky agent determined the amount of rhizobial cells that came in contact with the seed.

The most common method for introducing rhizobia into the soil is by seed inoculation either directly or by first impregnating the rhizobium in a carrier (Roughley, 1970). The approach of introducing rhizobia by seed inoculation may not be very efficient as the soil is usually quite far-off from the location of infection foci on the seedling root and this is likely to be aggravated by inefficient natural transport of rhizobia. In view of this, the use of alternative means of inoculation emerged (Brockwell *et al*., 1980). It is also very important to screen and recommend a more effective method of inoculation to marginal farmers, especially in Nigeria, with low adoption of inoculation as an alternative to chemical fertilizer use. Moreover, reports are scanty on the use of liquid rhizobium inoculant for soybean production in Nigeria, especially in the southwestern region. Hence, this study was conducted to evaluate the effectiveness of *Bradyrhizobium japonicum* strain via broth or solid carriers at varying time of application on nodulation, growth and yield of soybean.

MATERIALS AND METHODS

Study site and experimental design

The study was conducted in the screenhouse of the College of Plant Science and Crop Production, Federal University of Agriculture (FUNAAB), Abeokuta, Nigeria. The experiment was a 2 x 4 factorial, arranged in completely randomized design with three replications. The treatments were two types of rhizobium inoculants (solid and liquid inoculants) and four levels of application time (0, 1, 2, and 3 weeks after planting).

Soil sampling and analysis

The surface (0-15 cm) soil samples used for the pot experiment were collected from the research field of FUNAAB. The samples were bulked, air-dried, mixed thoroughly and passed through 2 mm sieve. Homogenized portion of the processed sample (< 2 mm) was taken to the laboratory for determination of soil physical and chemical properties, while each of 24 pots was filled with 5 kg soil. The soil pH was determined in soil:water suspension (ratio 1:1) using glass electrode pH meter. Soil particle size was determined using hydrometer method (Bouyoucous, 1951). Organic carbon was determined using the wet oxidation method of Walkley-Black (Walkley and Black, 1934). Total N was determined by the micro-Kjeldahl method (Bremner and Mulvaney, 1982). Available P was determined using Bray-1 method (Bray and Kurtz, 1945). Exchangeable bases (K, Na, Ca, and Mg) were extracted using 1N Neutral ammonium acetate and determined using flame photometer (K and Na) and atomic absorption spectrophotometer (Ca and Mg) (Sparks *et al*., 1996). Exchangeable acidity was extracted with 1N KCl and determined by titration with 0.05N NaOH.

Rhizobial inoculation and planting

Rhizobium broth culture sourced from IITA, Ibadan was impregnated into a finely ground lignite (carrier) passed through 0.5 mm sieve, after sterilizing in an oven for 1 hour at 160^0C. One hundred grams portions of the lignite carrier were weighed into small polythene bags and 25 ml of broth culture of rhizobia was added to each bag and sealed to make the solid inoculant. The carrier (lignite) was thereafter mixed thoroughly with broth culture and incubated for 5 days to allow for multiplication of rhizobia in the carrier. The rhizobium broth culture was used as the liquid inoculant. Soybean seeds (TGx 1448-2E) sourced from the Institute of Agricultural Research and Training, Moore Plantation, Ibadan, were mixed with the solid inoculant and planted two seeds per hole in the pots for the treatment application at 0 week after planting (WAP). Solid and liquid inoculants were thereafter applied to the soil around the root of each plant at 1, 2 and 3 WAP. Observations were made at 8 WAP on plant height, number of leaves, number of nodules, shoot dry matter yield and nodule dry weight. Pod dry weight and grain yield per plant were taken at harvest.

Statistical Analysis

All the data collected were subjected to analysis of variance (ANOVA) using Statistical Analysis System software (SAS, 2000) and the means were compared using Duncan's Multiple Range Test at $P < 0.05$.

RESULTS AND DISCUSSION

Characteristics of the Experimental Soil

The physical and chemical characteristics of the experimental soil are presented in Table 1. The soil was loamy sand in texture and slightly alkaline in reaction. Total nitrogen, available P and organic carbon of the experimental soil were very low (Okalebo *et al*., 2002). The soil condition is suitable for nitrogen fixation because low soil N stimulates the nitrogen fixing ability of legume plants. More nitrogen fixation and absence of response to inoculation had been reported for soils depleted (Turner *et al.*, 1984) and rich (Adeyeye *et al.*, 2017) in N, respectively.

Table 1: Physical and chemical properties of the experimental soil

Parameters	Value
Particle size	
Sand (g kg^{-1})	806
Silt (g kg^{-1})	146
Clay (g kg^{-1})	48
Textural class	Loamy sand
Organic carbon (%)	0.31
pH (H_20)	7.2
Total Nitrogen (%)	0.05
Available phosphorus (mg kg^{-1})	4.0
Exchangeable cations	
Ca (cmol kg^{-1})	6.78
Na (cmol kg^{-1})	0.24
Mg (cmol kg^{-1})	1.0
K (cmol kg^{-1})	0.28
Exchangeable acidity	0.8

Table 2: Effect of liquid inoculant application time on agronomic parameters of soybean at 8 WAP

Time (WAP)	Plant Height (cm)	Primary Nodule	Secondary Nodule	Total Nodule Count	Root weight (g $plant^{-1}$)	Shoot dry matter weight (g $plant^{-1}$)	Number of pods	Number of leaves	Nodule dry weight (g $plant^{-1}$)	Pod dry weight (g $plant^{-1}$)	Grain yield (g $plant^{-1}$)
0	60.13	8	29	37^{a}	2.37	2.89^{a}	15^{b}	42^{a}	0.23	1.33	1.53^{b}
1	62.33	5	31	36^{a}	1.67	1.93^{ab}	8^{c}	25^{b}	0.18	2.39	1.05^{c}
2	62.98	1	26	27^{b}	0.80	1.26^{b}	22^{a}	18^{b}	0.13	3.48	2.61^{a}
3	73.20	3	20	23^{b}	0.84	1.91^{ab}	16^{b}	21^{b}	0.17	2.24	2.63^{a}
	NS	NS	NS		NS				NS	NS	

Means with different alphabets in the columns are significantly different ($P < 0.05$), NS = not significantly different, WAP = Weeks after planting

Table 3: Effect of solid inoculant application time on agronomic parameters of soybean at 8 WAP

Time (WAP)	Plant Height	Primary Nodules	Secondary Nodules	Total Nodule Count	Root weight (g $plant^{-1}$)	Shoot dry matter weight (g $plant^{-1}$)	Number of pods	Number of leaves	Nodule dry weight (g $plant^{-1}$)	Pod dry weight (g $plant^{-1}$)	Grain yield (g $plant^{-1}$)
0	44.02	8^{a}	10	18	0.81	1.30	14	19	0.11	5.23^{a}	1.85
1	58.40	2^{b}	6	8	0.88	1.98	22	20	0.07	5.71^{a}	1.67
2	62.77	10^{a}	14	15	0.75	2.04	17	24	0.10	3.83^{b}	0.78
3	54.02	8^{a}	11	19	0.90	1.39	13	17	0.17	4.83^{ab}	1.24
	NS		NS	NS	NS	NS	NS	NS	NS		NS

Means with different alphabets in the columns are significantly different ($P < 0.05$), NS = not significantly different, WAP = weeks after planting

Table 4: Effect of inoculation methods on agronomic parameters of soybean at 8WAP

Inoculant type	Plant Height (cm)	Primary Nodules	Secondary Nodules	Total Nodule Count	Root weight (g plant^{-1})	Shoot dry matter weight (g plant^{-1})	Number of pods	Number of leaves	Nodule dry weight (g plant^{-1})	Pod dry weight (g plant^{-1})	Grain yield (g plant^{-1})
Liquid inoculant	64.66	4.00	20^{a}	24^{a}	1.23^{a}	1.88^{a}	15	23^{a}	0.18	2.31	1.95^{a}
Solid inoculant	54.80	5.00	10^{b}	15^{b}	0.88^{b}	1.52^{b}	16	20^{b}	0.11	4.90	1.39^{b}
	NS	NS					NS		NS	NS	

Means with different alphabets in the columns are significantly different ($P < 0.05$), NS = not significantly different, WAP = weeks after planting.

Effect of liquid inoculant application time on agronomic parameters of soybean

Time of application of inoculants significantly ($P < 0.05$) affected shoot dry weight, total nodules count, number of pods, number of leaves and grain yield per plant (Table 2). Plants that received inoculant at sowing (0 WAP) had the highest number of leaves per plant, total nodule count and shoot dry weight compared with plants that received inoculation at other application times. The number of pods was highest in soybean plants that were inoculated at 2 WAP, and least in plants that were inoculated at the first week. However, there was no significant difference between number of pods in plant that received inoculation at 0 and 3 weeks of application. The highest grain yield was recorded at 2 and 3 WAP which had similar yield values while the application at 1 WAP had the least. The increase in number of pods and grain yield at 2 WAP was 46.7 and 70.6%, respectively, over inoculation at sowing (0 WAP). This result may be probably due to the opportunity presented by delayed introduction of the liquid inoculant until the root is well developed to immediately form symbiosis with the rhizobium as soon as it was introduced. Hence, the rhizobium was not exposed to symbiosis-limiting soil condition caused by acidity, alkalinity and other factors relating to soil structure (Subba Rao, 1988). Increase in the grain yield of soybean obtained in this study, as a result of rhizobium inoculant application has also been reported by several authors (DeJong and Roush, 2012; Salih *et al*., 2015; Mohamed and Hassan, 2015). Although, plants treated with liquid inoculant at sowing had higher total nodule count compared with others at different application times, this did not eventually translate into higher yield as evident by the number of pods and grain yield of soybean. This finding may be probably due to the fact that most of the nodules produced in plants inoculated at sowing were not efficient in nitrogen fixation.

Effect of solid inoculant application time on agronomic parameters of soybean

The result indicates (Table 3) that the time of solid inoculant application evaluated did not significantly influence all of the agronomic parameters observed except primary nodules and pod dry weight. Plants that were inoculated at sowing, 1 and 3 WAP had significantly ($p < 0.05$) higher pod dry weight compared with plants inoculated at 2 WAP. This result is consistent with the report of Janagard and Ebadi-Segherbo (2016) who obtained higher pod dry weight per plant in the plants from seeds inoculated with Biosoy. All the other agronomic parameters were not significantly affected by the time of application of solid inoculants including grain yield. The lack of differences obtained in most parameters, especially the grain yield, with respect to time of solid inoculant application may be due to the fact that the placement of seed inoculant in the soil is usually relatively remote from the location of infection foci on the seeding roots. This circumstance is likely to be aggravated by inefficient natural transport of rhizobia (Brockwell *et al*., 1980).

Soybean growth and nodulation as influenced by methods of rhizobia inoculation

Inoculation methods significantly (P < 0.05) affected most of the agronomic parameters measured (Table 4). The results show that plant treated with liquid inoculant had consistently higher values of the measured parameters than those treated with the solid inoculants. For number of leaves, shoot dry matter weight, root dry weight and grain yield per plant, liquid inoculant-treated plants gave significantly (P < 0.05) higher values than the solid inoculant-treated plants. The percentage increase in number of leaves, shoot dry weight, root dry weight and grain yield of plant treated with liquid inoculant over those treated with solid inoculant was 15, 23.7, 39.8 and 40.3%, respectively.

Nodulation parameters evaluated (secondary and total nodule count) in this study were also significantly (P < 0.05) affected by the methods of inoculation. Plants treated with liquid inoculant consistently gave higher number of secondary (100% increase) and total nodules (60% increase) when compared with the ones inoculated with solid inoculant. These results may be due to the fact that liquid materials have a longer shelf life and are easier to use as reported by Delbert (2018). He reported further that some newer materials can maintain viability on untreated seed for sixty days or more, and for seven or more days when applied over some fungicides. The crops that received liquid inoculants produced more nodules which in turn translocated nitrogen from the lower parts to the growing tips and other part of the plant that includes the shoot and grain as obtained in this experiment. This result corroborates the report of Oad *et al.* (2002) and Oliveira *et al.* (2017) who found application of liquid inoculant to be viable and showing greater efficiency than that of solid/seed inoculation. Application of liquid inoculants has been reported to reduce the risks of losing viable bacteria by seed drilling equipment or when the seed coat is lifted out of the ground during germination of seeds treated with solid inoculants (Jauhri and Subba Rao, 1989). Brockwell (1977) reported that small-seeded legumes may also have an extra benefit from liquid in-furrow inoculation, by allowing the application of higher inoculum rates than is possible with seed inoculation. Lanier *et al*. (2005) also found that the pod yield of peanut was higher when inoculant was applied in-furrow using liquid inoculant compared with application to seeds through solid inoculant.

CONCLUSION

Generally, the liquid inoculants (rhizobium broth culture) performed best in most of the agronomic parameter measured in soybean. This was evident by the percentage increase in the shoot dry weight (23.7%) and grain yield (40.3%) of the liquid inoculant-treated plants over those treated with solid inoculant. Furthermore, application of inoculants at 2 WAP also performed best in most of the yield parameters measured, especially, the number of pods and grain yield per plant. The number of pods and grain yield of liquid inoculant-treated plants at 2 WAP gave 46.7 and 70.6% increase, respectively, over those treated at sowing (0 WAP). It is, therefore, concluded that liquid inoculant applied at 2 WAP could enhance optimum productivity of soybean in soil with similar properties.

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