NODULATION AND NITROGEN FIXATION IN SIX GRAIN LEGUMES

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Uninoculated cowpea plants contain only 10-44 nodules per plant (1,4,9,10). Unusually high nodule counts were obtained by Tewari (11) working in eastern Nigeria. Inoculated cowpea contains 10-86 nodules per plant (5,9,10). The efficiency of cowpea nodules has not been critically examined.

Nodulation of the soybean, an introduced legume, is even more of a problem under field conditions (1). The number of nodules per soybean plant varies from 0 to over 50 depending on the nitrogen status of the soil, rhizobium strain, and plant type (1,3,7). Some of the grain legumes grown at IITA are inoculated with commercial inoculants whereas other receive combined nitrogen. Nodulation patterns and nitrogen fixation in most of these legumes are not well known. As part of the on-going program to select appropriate strains of Rhizobium for these legumes, an experiment was conducted to determine the effect of ammonium nitrogen and commercial inoculants on the nodulation and nitrogen fixation in six grain legumes.

MATERIALS AND METHODS

Apomu soil, pH 5.7, containing 12.0 ppm ammonium-N and 4.6 ppm nitrate-N was placed in 25 cm earthenware pots. Ten seeds were planted per pot and the seedlings were later thinned to six. The legumes were cowpea, (Pale Green), soybean (Bossier), lima bean (IP1 191), jack bean (ICe 1), winged bean (IPt 2), and pigeon pea (CMe 3D). The treatments were (a) no addition, (b) 100 ppm ammonium sulfate-N, (c) an appropriate commercial inoculant from the Nitragin Co., USA, or (d) both nitrogen and inoculant. Pots were kept in the screenhouse and watered twice daily. Temperatures in the screenhouse frequently reached 37-38C.

After seven weeks, ten plants per treatment were carefully harvested for nodule counts and dry matter determination. Nitrogenase activity was measured by the acetylene reduction technique. Duplicate plants from each treatment were cut at the soil line and the roots containing nodules were placed in vials. Acetylene was added to 10% (v/v) and vials were left in the screenhouse for 30 min. Gas samples were removed for analysis by the procedure of Dobereiner, Day, and Dart (3) except that the column temperature of the gas chromatograph was 70C.

RESULTS

All six legumes responded favourably to nitrogen fertilization (Table 1). Fertilization increased plant height, nodules per plant, and the average weight of each nodule. The average weight of a nodule of the untreated winged bean was more than that of a nitrogen-treated nodule.

Inoculation increased the heights of soybean, lima bean, and winged bean plants. Nodule numbers of inoculated plants were generally fewer and weights less than those of nitrogen-treated plants.

When the legumes received both nitrogen and inoculant, plant heights were equal to or greater than those of plants receiving nitrogen alone. It is striking to note that almost additive responses were obtained in winged bean and pigeon pea. The weights of nodules were slightly increased; nodule numbers were generally greatest in this treatment and insome instances there was an additive effect of nitrogen and inoculant.

Although not shown by the data in Table 1, the soybean and winged bean inoculants produced plants which were luxuriant. These legumes, when treated with nitrogen alone showed nitrogen deficiency. All untreated plants except jack bean also showed nitrogen deficiency.

The findings on nitrogenase activity paralleled those on nodulation (Table 2.) The discrepancy between nodule and plant activities may have resulted from low proportion of effective nodules. Thus, although cowpea plants grown on nitrogen showed greater activity than untreated plants, the activity per unit weight of nodules was the same. Moreover, although the activity of cowpea plants treated with inoculant and nitrogen was higher than that of nitrogen-treated plants, the activity per unit weight of nodules was less. This suggests that the proportion of effective nodules from plants treated with nitrogen and inoculant was less than that of those treated with nitrogen alone. Nitrogen alone or in combination with inoculant increased nitrogenase activity of most of the legumes. The most dramatic increase, of 1,400 times, was of inoculated pigeon pea fertilized with nitrogen. Most of the inoculated plants were very low in activity when compared with untreated or nitrogen-treated plants.

The ethylene produced per plant per hour in each instance was converted to the corresponding amount of nitrogen that would be fixed per hectare per hour assuming the ratio of three suggested by Hardy, Burns and Holsten (8) for leguminous systems. The results are shown in Table 3. Nitrogen fixation may be appreciable only in soybean treated with nitrogen plus inoculant. The other treatments and other Rhizobium-legume symbioses were ineffective. Inoculated plants fixed the least amount of nitrogen.

DISCUSSION

Nodulation of cowpea in Apomu soil is in line with past work except that inoculation reduced nodulation. This latter finding may be due to the greater ineffectivity of inoculant strains compared with indigenous ones. However, it seems more likely that nodulation was reduced because root growth was limited, thus leading to a reduction in the total number of available potential infection sites. Rotimi (10) found that cowpeas inoculated with the Nitragin inoculant produced 33-48 nodules/plant. Kang (9) inoculated Pale Green cowpea with the Nitragin inoculant and found 23 nodules/plant. The addition of 80 ppm N to inoculated seeds yielded plants with 24 nodules each. Inoculation generally has not significantly increased nodulation. Moreover, the effectiveness of the nodules from plants grown in these locations has never been critically assessed.

Soybeans which to the authors' knowledge has never been grown in this soil, formed few but effective nodules. The finding that soybean treated with nitrogen alone was well nodulated may have been due to rhizobia being carried on the seeds. It is equally likely that cross-inoculation by indigenous cowpea rhizobia occurred. However, although these plants formed more nodules, they looked

nitrogen deficient, strongly suggesting that indigenous, ineffective rhizobia infected these plants.

Studies in Ecuador (7) have indicated that uninoculated soybean form 0-3 nodules/plant as compared with 23-31 nodules per plant inoculated with Nitragin. Inoculated plants had fewer nodules in the present study. These differences in results could be due to differences in soybean types, edaphic or environmental conditions, or rhizobial strains since Nitragin is a mixed-strain inoculant.

It seems that for the six legumes used in this experiment, the rhizobia were highly infective and quickly occupied all available infection sites. Unfortunately, nodulation was limited by root growth. Thus, the addition of nitrogen to inoculated plants increased the number of nodules.

None of the treatments significantly contributed to the nitrogen demands of the legumes examined. However, inoculation of soybean, winged bean, and pigeon pea gave promising results. It is likely that nitrogen fixation by soybean would have been sufficient to yield a good crop. It is also likely that the activity of inoculated winged bean and pigeon pea would have increased to a level where the demands of the plants would have been met, since these plants are perennials.

The low nitrogen status of the soil, high screenhouse temperature, poor survival of rhizobia and.or insufficient number of rhizobia per seed singly or several influenced nitrogen fixation. Nitrogen fixation was not depressed by combined nitrogen as had been expected (8). The established finding that nitrogenase activity is inhibited by combined nitrogen needs to be carefully examined in the highly leached, nutrient-poor soils of the humid tropics.

Temperature is a more critical environmental factor than inorganic nitrogen. Dart and Mercer (2) noted that growth and nodulation of cowpea were more affected by temperature than light or combined nitrogen. Galleti et al. (6) found temperature to influence nodulation very highly in the soybean; exotic strains of rhizobia were more sensitive to temperature than indigenous ones. The temperatures in the screenhouse were very high, frequently reaching over 37C during sunny days. The effect of temperature coupled with that of nitrogen led to a reduction in growth, nodulation and nitrogen fixation in inoculated legumes.

From the viewpoint of plant vigour, nodulation and nitrogen fixation, it is apparent that plants grown in Apomu soil would benefit significantly from inoculation. The addition of nitrogen to poor soils at planting would ensure establishment and well-rooted plants. This would lead to good nodulation and possibly nitrogen fixation, as observed here. Inoculation must be with Rhizobium spp. selected to withstand the high temperatures likely to occur in the soils.

Table 1. Plant height and nodule characteristics

Legume	Treatment	Height, cm	Nodules/Plant	Dry Nodule Weight, mg
COWPEA	NONE	20.0	8	0.71
	NITROGEN (N)	185.0	34	7.41
	INOCULANT(I)	24.0	4	1.38
	N + I	163.0	27	5.73
SOYBEAN	NONE	29.7	7	5.34
	NITROGEN	85.7	101	6.78
	INOCULANT	37.4	12	3.39
	N + I	81.0	60	7.56
LIMA BEAN	NONE	28.0	4	2.46
	NITROGEN	129.0	33	4.84
	INOCULANT	56.0	14	0.92
	N + I	125.7	27	5.76
JACK BEAN	NONE	67.9	9	12.58
	NITROGEN	180.0	25	17.59
	INOCULANT	56.0	28	2.59
	N + I	167.0	135	4.62
WINGED BEAN	NONE	30.4	<1	24.90
	NITROGEN	146.9	8	19.59
	INOCULANT	71.4	12	5.90
	N + I	203.0	23	13.13
PIGEON PEA	NONE	35.4	3	1.75
	NITROGEN	101.5	16	5.48
	INOCULANT	39.4	5	0.97
	N + I	124.0	10	17.53

Table 2. Nitrogenase activity

Legume	Treatment	nmoles C_2H_2 reduced/mg fresh nodule/hr	µmoles C_2H_2 reduced/plant/hr
COWPEA	NONE	16.00	1.33
	NITROGEN	16.98	35.99
	INOCULANT	6.08	0.53
	N + I	9.47	51.01
SOYBEAN	NONE	9.70	1.97
	NITROGEN	42.74	81.86
	INOCULANT	19.59	3.00
	N + I	23.80	100.76
LIMA BEAN	NONE	371.15	21.23
	NITROGEN	9.15	9.23
	INOCULANT	0.69	0.15
	N + I	6.87	6.93
JACK BEAN	NONE	26.32	14.01
	NITROGEN	91.48	182.04
	INOCULANT	6.00	5.67
	N + I	20.70	59.37
WINGED BEAN	NONE	13.28	2.84
	NITROGEN	31.27	23.22
	INOCULANT	3.02	2.12
	N + I	23.99	96.27
PIGEON PEA	NONE	6.26	0.50
	NITROGEN	17.67	25.74
	INOCULANT	0.49	0.04
	N + I	26.23	58.39

Table 3. Levels of nitrogen fixed: Extrapolation to field conditions

Legume	Treatment	g N/ha per hr*
COWPEA	NONE	1.49
	NITROGEN	40.31
	INOCULANT	0.60
	N + I	57.13
SOYBEAN	NONE	2.76
	NITROGEN	114.60
	INOCULANT	4.20
	N + I	141.06
LIMA BEAN	NONE	15.86
	NITROGEN	6.90
	INOCULANT	0.11
	N + I	5.18
JACK BEAN	NONE	2.62
	NITROGEN	33.98
	INOCULANT	1.06
	N + I	11.08
WINGED BEAN	NONE	0.53
	NITROGEN	4.33
	INOCULANT	0.40
	N + I	17.97
PIGEON PEA	NONE	0.03
	NITROGEN	1.60
	INOCULANT	0.01
	N + I	3.63

*Based on the following populations/ha: Pale green, 120,000; Bossier 150,000; Lima bean, 80,000; Jack bean, 20,000; Winged bean, 20,000; Pigeon pea, 6,667.

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