EVALUATION OF INDIGENOUS STRAINS OF RHIZOBIUM FOR LEUCAENA LEUCOCEPHALA (LAM.) DE WIT IN NIGERIA CONDITIONS

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ABSTRACT.

Experiments were conducted at the International Institute of Tropical Agriculture (I.I.T.A.) and at Fashola, Southwestern Nigeria, to identify, characterize and evaluate indigenous rhizobia nodulating Leucaena leucocephala (LAM.) de Wit.Most-Probable-Number indicated that leucaena rhizobia were few or absent in soils without previous history with leucaena cultivation. They were significantly higher in the field cropped to leucaen indicating that leucaena crops build up the population of compatible rhizobia in the root zone.

Rhizobia isolated from seven legumes (L. leucocephala, Tephrosia vogelii, Sesbania grandiflora, Si punctata, S.rostrata, Acacia albida and Vigna unguiculata) were tested for their N-fixing effectiveness with L. leucocephala in standard Leonard jars. Isolates from all plants except S. grandiflora and V. unguiculatata were able to form nodules with leucaena although a wide range of effectiveness was demonstrated. Based on this experiment, a group of 10 effective rhizobia were tested in pots. Only two rhizobia (IRc 1045 and IRc 1050) isolated from leucaena performed well and were further tested in the field. At I.I.T.A., only inoculated plants nodulated while at Fashola, all the plants produced nodules. At both locations, inoculation with Rhizobium IRc 1045 or IRc 1050 increased total N and dry matter of leucaena as compared to the uninoculated plants. This effect was statistically equal to the N treatment. In addition to their effectiveness, these strains were comptetitive and survived well in the field one year after their establishment.

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INTRODUCTION

Leucaena leucocephala has been described as a widely adapted crop of exceptional potential for the tropics (National Academy of Science: 1977). It can produce nutrition forage, firewood, timber and organic fertilizer rich in nitrogen.

However, there exists a number of soils where leucaena cannot establish well. Leucaena grows poorly in acid (HALLIDAY, 1981) and mineral deficient soils (National Academy of Sciences, 1977), and in some areas due to the absence of nodules plant roots (Ahmad and Ng, 1981; Diatloff, 1973; Mafuka, 1984).

A sufficient number of appropriate rhizobia in the rhizosphere of legumes is a prerequisite for adequate nodulation and nitrogen fixation However, published reports on the population sizes of rhizobia and nodulation of leucaena in Nigeria soils are lacking. Information on rhizobial nurbers in some soils and their ability to form nodules on the roots of leucaena plant would help workers to assess the need for inoculating leucaena.

The present study was undertaken to (i) monitor nodulation and growth of leucaena in relation to the population of native rhizobia, (ii) isolate and characterize the indigenous rhizobia nodulating Leucaena and (iii) select the best rhizobial strains in terms of effectiveness in N-fixing capability in symbiosis with leucaena.

MATERIALS AND METHODS.

1. Enumeration of rhizobia

Soil samples were collected at ten sites in Nigeria that had received no fertilizer for the past 18 months. These sites were selected to provide diversity in soil and cliratic factors (Table 1).

A soil auger of 2 cm diameter was used to collect soils at 30 cm depth. Ten cores collected at random were thoroughly mixed into composite samples and stored at 4°C until the analysis.

Leucaena rhizobia were enumerated by the most probable number and the plant infection method (Vincent, 1970). A 10-fold dilution series with five replicates per dilution was used. L. leucocephala var. K-28 was the host to enumerate leucaena rhizobia.

2. Nodulation, isolation and preliminary characterization of rhizobial isolates.

Prior to isolation, 7 legumes (L. leucocephala, Tephrosia vogelii, Sesbania grandiflora, S. punctata, S. rostrata, Acacia albida and Vigna unguiculata) were raised from surface sterilized seeds grown in pots containing soils from a field cropped to leucaena and from a secondary forest at I.I.T.A., and soil from a grassland at Fashola (70 Km North of I.I.T.A.). Physico-chemical properties of these soils and the population of indigenous leucaena rhizobia were determined prior to planting.

Soils were then air dried, sieved and transferred in five kilogram portions in plastic pots. A randomized complete block design was used with five replications and following treatments were applied : (i) no fertilizer, and (ii) fertilization with 250 mg P/pot as single supe phospate, 50 mg K/pot as muriate of potash and 5 ml of a complete frit of micronutrients (Bo 0.05 %; Mg 0.05 %; Zn 0.005 %; Mo 0.005 % and Cu 0.002 per pot.

At 8 weeks after planting (WAP), shoots of these legumes were cut at the soil line and were oven-dried at 65°C for 48 h. Roots and nodules in each pot were removed carefully. Nodules were counted and weighed.

Isolation of rhizobia was performed as described by Vincent (1970) and single-colony isolates were maintained in Mc Cartney bottle on yeast extract mannitol agar (YMA). In order to characterize the isolates and to determine their generation time, cultures were grown in yeast extract mannitol broth (YMB) at 28°C on a reciprocal shaker. Samples were taken during the exponential growth phase and the viable counts of rhizobia performed. The level of intrinsic resistance to antibiotics were determined using YMA supplemented with 0.50; 100; 250 or 500 ug/ml of filter-sterilized streptomycin and spectinomycin. Compatibility of rhizobial isolates with leucaena was assessed by inoculating seedlings of leucaena in plastic pouches, and examining nodulation after 42 days.

3. Screening of rhizobial isolates.

Preliminary screening of 32 rhizobial isolates for effectiveness was done in leonard jar assemblies containing sterile, washed sand and N-deficient Jensen's solution (Vincent, 1970).

Ten rhizobial isolates selected from the Leonard jar trials were futher studied in potted soils collected on the previous sites at IITA, Ibadan and at Fashola. Soils were air-dried, sieved, weighed and fertilized as described above.

Leucaena seeds were scarified with concentrated H_2SO_4 for 30 min.; rinsed several times in sterile water and then inoculated with the appropriate strains. Inoculation was performed at sowing by pipetting 1 ml of broth culture (10^9 cells/ml) on the seed. Controls included uninoculated plants and those fertilized with 75 mg N/pot as urea. Each treatrent was replicated five times and randomized within blocks. Pots contained 5 Kg of soils. They were set in screenhouse and watered regularly. Plants were harvested at 10 WAP and the number of nodules and their dry weight assessed. In addition, plant dry weight, shoot total nitrogen and nitrogenase activity (Hardy et al., 1973) were recorded. Shoot dry weight was used to calculate the relative effectiveness defined as the dry weight of inoculated plants expressed as a percentage of the nitrogen control (Ahmad et al., 1981).

4. Field trial.

The two best strains selected from the pot experiment were then tested in the field at I.I.T.A. and Fashola. At Fashola, the field experiment was located in the same area from which soil was collected for the pot experiment. The I.I.T.A. trial was conducted on an Alfisol of the Iwo series with the following characteristics : pH (H_2O),6.0; clay, 7 %; sand, 85 %; silt, 5 %; organic C, 0.92 %; total N, 0.14 %; C.E.C., 4.00 meq/100 g of soil; Available P (Bray 1); 42.33 ppm and the number of rhizobia; 3.6×10^2/g of soil.

At both locations, the experimental design was a three replicated split-plot having three basic treatments : plants inoculated with the two rhizobial isolates, plants not inoculated, and plants not inoculated but fertilized with nitrogen at 150 Kg N/Ha urea applied in 3 equal doses. Mineral nutrients (phosphorus and micronutrients) were applied in subplots and their results have been described elsewhere (Sanginga et al., 1984).

The main plots measured 6 x 13.50 m with a spacing of 75 cm between rows and 20 cm within rows. Leucaena seeds were surface sterilized as described above and inoculated with peat inoculant containing either Rhizobium IRc 1045 or 1050. The number of rhizobia at planting was approximately 1 x 10^7/seed.

Five seeds per hill were hand-sown immediately after inoculation. Seedlings were thinned to two per hill and the plots were weeded as necessary.

At 12 WAP, 10 plants were harvested at random in three meter section within the second row of each replicate plot at both sites. Nodule number and dry weight, shoot dry weight, plant height, shoot nitrogen and phosphorus, and nitrogenase activity were assessed. The rhizobia in 40 nodules selected at random from the roots of ten other plants in each replicate plot were serotyped with antisera against IRc 1050 using the ELISA technique (Clark and Adams, 1977) and on the basis of the intrinsic resistance of IRc 1045 to 500 ug/ml of streptomycin (Schwinghamer and Dudman, 1973). At 24 WAP, plant height, shoot dry weight, total nitrogen and phosphorus contents were determined on 10 plants.

In order to assess the effect of inoculation one year after planting leucaena, 5 Kg of soils were collected at random at 30 cm depth in all plots. Following air drying, the soil was sieved, potted and arranged in a randomized complete block design with five replications. Four surface sterilized leucaena seeds were sown per pot. Plants were harvested at 10 WAP and data were recorded as in the previous experiments.

RESULTS AND DISCUSSION.

Rhizobia capable of nodulating leucaena were absent in six soils (Table 1). These data show that there is likely some geographical selection on adaptation to stress in these soils. The detrimental effects of physical and chemical stress on rhizobia in the tropics are well documented (Boonkerd and Weaver, 1982; Hartel and Alexander, 1984; Osa-Afiana and Alexander, 1982). The lack of leucaena rhizobia in Zaria soil may be explained by the effect of drought and high temperatures. Inability of leucaena to nodulate in Onne, Iseini and Ntije soils seems to be correlated with their low pH. In fact, the host plant is poorly adapted to acid soils (Ahmad and Ng, 1981; Halliday, 1981).

Data presented in table 1 and 2 show that numbers of rhizobia and nodulation of leucaena were high in the soil collected from a field cropped to leucaena. Several investigators have observed marked increases of native or introduced rhizobia in rhizospehere soils of various legumes (Bushby, 1984; Mulongoy et al., 1982; Robert and Schmidt, 1983). Our data substantiate the selective stimulation of rhizobial growth in the rhizosphere of legumes, and indicate that leucaena crops build up the population of compatible rhizobia in the root zone. Low rhizobial numbers at Fashola and at I.I.T.A. secondary forest (Table 1) explain partly poor nodulation of leucaena in these soils, (Table 2). Because there were usually fewer than 1.000 rhizobia per g of soil at the two sites; theses locations should be suitable for leucaena inoculation as little competition with the resident population is expected.

The results indicate better leucaena growth in I.I.T.A. soils as compared to that in Fashola soil (Table 2). This could be attributed to higher nutrient status of I.I.T.A. soils (Table 3). Growth of leucaena was closely correlated with clay content ($r = 0.98$) , organic carbon ($r = 0.92$), total N ($r = 0.82$) and phosphorus ($r = 0.99$). Fertilization with P, K and micronutrients improved leucaena growth in all soils but plants in Fashola soil remained smaller (Table 2). This can probably be explained by the low level of nitrogen in Fashola soil (5 times less than in I.I.T.A. soil cropped to leucaena) and suggest that leucaena in this soil can respond either to nitrogen application and/or to inoculation.

Rhizobia isolated from the legumes studied were divided into two groups (Table 4). Twenty-six rhizobia from L. leucocephala, S. grandiflora, S. Rostrata and S. punctata were fast growing and acid producers. The other sixteen isolates obtained from T. vogelii, A. albida and V. unguiculata were slow growing and produced alkali in the media. The mean generation times of the fast-growing and slow-growing strains were less than 5 and more than 8 hours, respectively. Slow-growing organisms raised the initial pH of the defined medium while the fast-growing organisms lowered it. The results of the inoculation tests are contained in Table 4. Leucaena was infected both by fast and slow growing rhizobia, exceptly those from S. grandiflora and V. unguiculata. Our finding are in agreement with that of Dreyfus and Dommergues (1981) who noted in Senegal that leucaena was

nodulated not only by fast growing strains of Rhizobium (Halliday, 1981; Trinick, 1980) but also by slow growing ones. The results in Table 4 also show that rhizobia isolated from leucaena grown in the grassland soil from Fashola and in the secondary forest soil from I.I.T.A. showed resistance to the probably highest concentrations of streptomycin and spectinomycin. This probably suggest that antibiotic resistance is needed for rhizobial survival in these soils.

The symbiotic performance of the 32 isolates nodulating leucaena were not identical. Based on the response of leucaena to inoculation in Leonard jars, 10 rhizobial strains were the most effective ones (Table 5). They produced more shoot matter than uninoculated plants and were equal or more efficient than the nitrogen control treatment. Ineffectively nodulated plants were stunted and greatly retarded in growth, they showed signs of nitrogen deficiency (Figure 1). Evaluation of strains for effectiveness in aseptic conditions however, is only an initial phase of strain selection. Effective strains from Leonard jars should be then tested under natural conditions (Date, 1982).

In this study, Rhizobium IRc 1045 and IRc 1050, two elite strains under sterile conditions performed also well in natural soils, but others did not. This was shown by their high shoot dry matter, nitrogenase activity and total plant N content (Table 6). Their symbiotic effectiveness was over 100 % relative to the N control. The relative effectiveness of IRc 1042, IRc 1046 and IRc 1048 was poorer than the uninoculated control. This emphasizes the interplay of both biological and non-biological factors in the natural environment in modifying the expected symbiotic response (Dart, 1974; Vincent, 1965).

The performance of IRc 1045 an IRc 1050 isolates were then tested in the field. At I.I.T.A., only inoculated plants nodulated and all the nodules were produced by inoculants strains (Table 7). The absence of nodules at this field confirms that leucaena has specific Rhizobium requirements (Halliday, 1981; Trinick, 1980) and can benefit from inoculation. Mafuka (1984) found similar results at I.I.T.A. as well as at Fashola. In the field at Fashola, nodules were found in all the treatments (Table 6). Seventy-five percent of the nodules from inoculated plants were produced by the introduced rhizobia. In inoculated plots with IRc 1045, 21 % nodules contained IRc 1050. In the uninoculated treatments, nodules were due partly (69 %) to Rhizobium 1050 used in a previous inoculation trial with leucaena and other legumes at this site.

Strain IRc 1045 isolated from Fashola performed better at I.I.T.A. and IRc 1050 isolated from I.I.T.A. was more effective at Fashola. Mulongoy et al. (1982) in a review on cowpea nodulation and response to inoculation also concluded that a strain of Rhizobium isolated at a particular location was not necessarily a better inoculant at that location than isolate from other environments. In both soils, shoot dry weight, total nitrogen and phosphorus contents were statistically equal in inoculated and N fertilized plants and the values were superior to the ones in uninoculated plots (Table 7). Plants in the latter treatments were stunted, lacked vigour and had yellow leaves at 24 WAP (Figure 2). Ahmad and Ng (1981), Diatloff (1983) and Mafuka (1984) reported that adequate nodulation and nitrogen fixation helps young leucaena plants to become established and to grow well. The results presented here show that the inoculum was effective and able to provide the plants with their requirements for nitrogen.

To assess the residual effect of inoculation and fertilization of leucaena, the persistence and the symbiotic effectiveness of introduced rhizobia was studied in pots containing soils from the above field experiments.

Soils from inoculated plots contained more rhizobia and promoted increased nodultation and shoot dry matter production (Table 8). Nodule typing indicated that most nodules were formed by the introduced strains. This indicate that Rhizobium IRc 1045 and IRc 1050 survived well, outcompeted ted the indigenous strains and were stimulated in the leucaena rhizosphere with nitrogen-fixing ability one year after their establishment. Thus we can assume that if adequate strains of rhizobia are introduced into a soil, the populations will survive, eventually, multiply over the years under continous leucaena cropping without additional inoculation. Inoculation constitue an evident advantage over nitrogen fertilization which is to be applied frequently for consistent high yields.

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Table 1: Number of leucaena rhizobia (MPN/g soil) and pH and % of soils from 10 selected sites in Nigeria

Soil Origin	Ecological Zone	PH (H_2O)	TOTAL N (%)	NUMBER of rhizobia (MPN / G soil)
IITA leucaena plot	transition forest savanna	5.30	0.18	7×10^4
IITA secondary forest	" "	5.90	0.10	3.1×10^2
Fashola grarsland	derived savanna	6.00	0.04	5.8×10^2
Annurakama	" "	5.10	0.18	0
NOKWA	guineen savanna	5.30	0.05	0
Nkalagu	" "	5.00	0.15	0
Zaria	sudan savanna	4.90	0.06	0
Onne	Forest	4.30	0.14	0
Iseini	"	4.40	0.06	10
Mtije	"	4.90	0.07	0

Table 2. Nodulation and growth of Leucaena leucocephala in pots containing soils fertilized or not with P, K and micronutrients, at 8 weeks after planting

Treatments	Soil Origin	Nodule number (No/plant)	Nodule dry weight (mg/plant)	Shoot dry weight (g/plant)	Height (cm)
No fertilizer	Leucaena fallow	27	79.00	1.5	35.40
	Secondary forest	10	63.60	1.5	31.40
	Grassland at Fashola	10	31.20	1.0	25.40
With fertilizer	Leucaena fallow	39	91.60	3.7	54.20
	Secondary forest	11	66.80	2.5	40.80
	Grassland at Fashola	12	65.40	1.6	35.60
LSD (5%) (1) for the same treatment		10	21.07	0.4	5.95
(2) for different treatments		7	14.90	0.3	4.21

Table 3 . Some physical and chemical properties of soils collected at IITA and Fashola

Soil Characteristics	Soil origin		
	Leucaena fallow IITA	Secondary forest IITA	Grassland Fashola
Sand (%)	85.00	85.00	83.00
Silt (%)	6.00	12.00	16.00
Clay (%)	9.00	3.00	1.00
pH (H_2O)	5.30	5.90	6.00
Organic C (%)	1.40	1.03	0.48
Total N (%)	0.18	0.10	0.04
Available P (ppm) Bray 1	8.30	9.30	3.80
NH_4-Acetate extractable cations (meq/100g)			
Ca	7.30	15.23	4.15
Mg	0.89	0.58	0.42
Mn	0.02	0.02	0.03
K	0.19	0.19	0.11
Na	0.06	0.07	0.03
Total acidity (meq/100g)	0.02	0.02	0.01

Table 4. Cultural characteristics of rhizobia isolated from some legumes and their compatibility with L. leucocephala

Soil origin	Host of isolation	No of isolates	Generation time (hr)	pH reaction	Nodulation on leucaena	Intrinsic resistance to antbiotics (ug/ml)
Leucaena fallow	L. leucocephala	2	5	H^+	+	500 spe
IITA	T. vogelii	2	8	OH^-	+	-
	S. grandiflora	2	5	H^+	-	-
	S. punctata	2	5	H^+	+	-
	S. rostrata	2	5	H^+	+	-
	A. albida	2	8	OH^-	+	-
	V. unguiculata	2	8	OH^-	-	-
Secondary forest	L. leucocephala	2	5	H^+	+	500 str, spe
IITA	T. vogelii	2	8	OH^-	+	-
	S. grandiflora	2	5	H^+	-	500 str
	S. punctata	2	5	H^+	+	-
	S. rostrata	2	5	H^+	+	250 str,100 spe
	A. albida	2	8	OH^-	+	-
	V. unguiculata	2	8	OH^-	-	-
Grassland	L. leucocephala	2	5	H^+	+	500 str, spe
Fashola	T. vogelii	2	8	OH^-	+	-
	S. grandiflora	2	5	H^+	-	-
	S. punctata	2	5	H^+	+	-
	S. rostrata	2	5	H^+	+	-
	A. albida	2	8	OH^-	+	-
	V. unguiculata	2	8	OH^-	-	-
IITA	L. leucocephala	1	5	H^+	+	50 str, spe
Hawaii	L. leucocephala	1	5	H^+	+	-

spe = spectomycin
str = streptomycin

Table 5. Effect of inoculation of leucaena with the best 10 Rhizobial isolates in Leonard Jar experiments at 6 weeks after planting.

Rhizobial Isolates	Host of isolation and origin	Shoot dry weight (mg/plant)	Symbiotic effectiveness (%)
Uninoculated		96	
N control		124	100
IRC 1041	Leucaena (IITA)	188	151
IRC 1042	Leucaena (IITA)	222	179
IRC 1043	S. rostrata(IITA)	157	127
IRC 1044	Leucaena (IITA)	164	132
IRC 1045	Leucaena (Pashola)	224	181
IRC 1046	Leucaena (Pashola)	154	124
IRC 1047	S. rostrata (Pashola)	175	141
IRC 1048	Leucaena (IITA)	148	119
IRC 1049	I. vogelii (Pashola)	169	136
IRC 1050	Leucaena (IITA)	195	157
LSD (5 %)		67	54

Table 6. Effect of pot inoculation with different rhizobia on nodulation, growth and nitrogenase activity of L. leucocephala (mean values for the soils from leucaena fallow plot, and a secondary forest at IITA and from a grassland at Fashola)

Treatments	Nodule per plant		Shoot dry weight (g/plant)	Height (cm)	Total N (mg/plant)	Nitrogenase activity (umoles/plant/hour)
	Number	Dry weight (mg)				
Uninoculated	7	36	3.07	63	104	6.0
Uninoculated + N	4	21	4.30	67	185	2.6
IRc 1041	39	62	3.43	58	111	4.9
IRc 1042	35	54	3.03	58	84	8.1
IRc 1043	32	61	3.09	57	97	10.9
IRc 1044	44	78	3.19	60	108	5.9
IRc 1045	32	95	4.58	64	146	21.5
IRc 1046	58	96	3.00	57	93	9.8
IRc 1047	32	83	3.46	50	107	9.3
IRc 1048	38	42	2.99	58	96	9.3
IRc 1049	42	59	3.53	59	111	8.3
IRc 1050	28	81	4.47	75	182	16.4
LSD (5%)	10	15	0.48	7	28	0.3

Table 1. Effect of inoculation with Rhizobium and fertilization with urea on nodulation and growth of L. leucocephala at IITA and Ibadan, Nigeria at 2% WAP

Treatments	Nodule No* per plant	Nodules* from inoculant strains (%)	Nodule* dry weight (mg/plant)	Shoot dry weight (g/plant)	Plant height (cm)	Plant N (kg/ha)	Nitrogenase activity (umoles/plant/hour)	Total phosphorus (kg/ha)
IBADAN								
Uninoculated	30	60	170	26	132	82	0.48	5.07
120 kg N/ha	36	60	87	72	174	232	0.11	15.22
Rhizobium IRc 1070	40	78	485	70	202	228	4.94	15.01
Rhizobium IRc 1065	15	94	174	68	169	209	2.54	12.85
IITA								
Uninoculated	0	0	0	51	152	174	0.00	14.62
120 kg N/ha	0	0	0	130	161	445	0.00	35.96
Rhizobium IRc 1070	17	100	198	103	170	398	2.65	26.91
Rhizobium IRc 1065	34	100	277	121	220	448	10.25	35.96
LSD (5%) Ibadan	11	ND	23	12	35	53	0.12	1.90
IITA	12	ND	25	22	24	66	0.17	0.60

* Nodule number and dry weight and percent of nodules from inoculant strains were recorded at 12 WAP

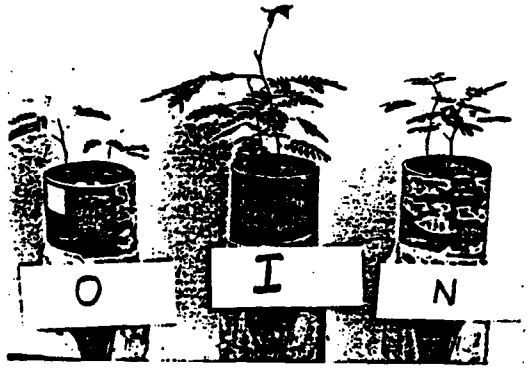
IITA and Yashola soils from previous inoculation trials at 10 WAP

il origin and vious treatment		nodule N° per plant	Nodules from inoculant (%)	shoot dry weight (g/plant)	Height (cm)	MPN leucaena rhizobia/g soil
SHOLA						
inoculated		32	82	1.08	48	11×10^3
izobium/Rc 1050		47	100	1.44	54	2.8×10^4
" /Rc 1045		33	98	2.21	64	3.5×10^4
IA						
inoculated		4	100	2.37	65	6.8×10^3
izobium/Rc 1050		18	100	3.35	79	16×10^4
/Rc 1045		23	100	3.86	87	16×10^4
D (5 %)	(1)	7.9	ND	0.46	7.9	ND
	(2)	8.6.	ND	0.54	8.6	ND

(1) Same treatment

(2) different treatment

ND Not determined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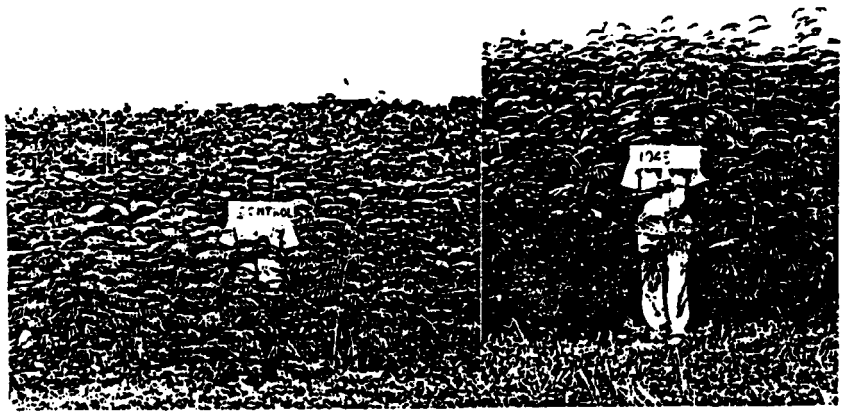
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