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Nitrogen-fixation Efficiency of Selected Cowpeas and Mung Beans (*Vigna* spp.) and *Rhizobia* spp. in Saline-Alkali Soils

The University of Arizona

Tucson, Arizona

Nitrogen-fixation Efficiency of Selected Cowpeas and Mung Beans (*Vigna* spp.) and *Rhizobia* spp. in Saline-Alkali Soils

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Millions of hectares of agricultural land are adversely affected by high temperatures and saline-alkali soils. Although not extensive, research has shown that symbiotic processes of legumes are reduced under these circumstances. When considering the magnitude of the problem and implications of salinity on agricultural productivity in developing countries, there is a paucity of information dealing with symbiotic processes in the arid and semi-arid tropics.

In many developing countries, including Ethiopia and Egypt, new lands are becoming available for agriculture. However, preliminary studies indicate that cowpeas and mung beans do not nodulate under these stress environments. A bidirectional approach to the resolution of the problem appears to be practical. Identification of superior adapted cowpea germplasm and rhizobia strains which perform consistently well under field conditions being our goal.

Objectives: Our primary goals were (1) to screen and identify *Vigna* spp (cowpea and mung beans) for Biological Nitrogen Fixation efficiency (BNF) and adaptibility to high temperatures and saline-alkali soils; (2) to isolate and purify native cowpea rhizobia strains and screen them for symbiotic efficiency; (3) to screen rhizobia strains from other areas for stress tolerance, and (4) to study BNF of selected cowpeas and mung beans under field conditions.

I. Field Screening of Cowpeas and Mung Beans for BNF

Sixty cowpea and mung bean lines were evaluated for heat and salt tolerance under field conditions at the University of Arizona Experimental

Farm at Safford, Arizona. Soil analysis of the field is shown in Table 1.

Considerable variability for adaptation was evident. Cowpeas (Table 2) appeared to be more tolerant of prevailing stresses than mung beans (Table 3). In order to assess BNF of indigenous rhizobia, seed were not inoculated at sowing. Plants were examined routinely from each plot for nodulation. After the final hand harvest, all surviving plants were dug-up and roots examined. No nodules were evident on any cowpea nor mung bean roots. Failure of plants to become nodulated indicated either a lack of indigenous strains or if rhizobia were present, the absence of a suitable environment for symbiosis to occur.

Agronomic characters were also routinely evaluated during the growing season. Plant height, plant type, stand counts, lime-induced Fe deficiency and sodium toxicity injury were noted. Considerable genetic variability for these characters was evident; due to field conditions and irrigation management, variability in salinity may be an important consideration. Therefore, in order to determine which cowpeas and mung beans would be suitable for use as host genotypes in growth chamber screening of rhizobia strains, soil samples were taken from the most promising plots (Table 4). Differences in Na, soluble salts, and ESP and SAR, in addition to yield, were used to select genotypes for further study.

Although the initial screening indicated that mung beans performed relatively well under high salt, field experiments in 1979 and 1980 resulted in a poor survival rate or 100% mortality in material selected

Table 1. Soil Analysis - University of Arizona Experimental Farm - Safford, AZ - Cowpea-mungbean BNF Experiment

pH[1]	EC X 10^3	Soluble salts-ppm	Na meq/L	K meq/L	ESP	N[2] ppm	P[3] ppm
7.9	4.9 mmhos	3620 ppm	37.6	0.8	14	44	5.6

[1] saturated paste

[2] water extract

[3] water extract

Table 2: Cowpeas Evaluated for Heat and Salt Tolerance and Nodulation at Safford, AZ - 1979.

	Entry	Yield gm/plot	100 seed wt. gm		Entry	Yield gm/plot	100 seed wt. gm
1.	Brown Crowder	15.1	N.E.S.*	31.	P.I. 352964	60.0	--
2.	Burgundy	44.8	18.4	32.	P.I. 180494	31.0	5.8
3.	Pinkeye Purple Hull Bunch Cowpeas	--	--	33.	P.I. 352972	68.5	12.6
4.	Big Boy	175.6	29.4	34.	P.I. 257463	25.5	14.9
5.	Colossus	30.5	22.5	35.	P.I. 293918	383.2	12.3
6.	Sixweeks Browneye Cowpeas	--	--	36.	P.I. 312206	52.5	11.1
7.	Speckled Purple Hull	474.2	20.5	37.	P.I. 312210	15.4	10.1
8.	Calhoun	57.0	19.9	38.	P.I. 183251	82.2	22.5
9.	Calico	17.0	12.0	39.	P.I. 189374	77.2	7.3
10.	Bluegoose	125.3	25.7	40.	P.I. 292890	39.0	14.7
11.	P.I. 292900	9.8	8.2	41.	P.I. 290892	65.0	--
12.	P.I. 354469	259.0	11.8	42.	P.I. 292[illegible]06	239.5	11.6
13.	P.I. 179126	50.5	18.2	43.	P.I. 250238	33.0	4.5
14.	P.I. 292903	121.0	12.9	44.	P.I. 205240	14.5	5.2
15.	P.I. 353383	71.7	10.4	45.	P.I. 352770	--	--
16.	P.I. 175329	21.8	9.4	46.	P.I. 292913	10.0	N.E.S.
17.	P.I. 352987	252.1	9.9	47.	P.I. 208845	.3	N.E.S.
18.	P.I. 292907	4.0	N.E.S.	48.	P.I. 205527	3.0	--
19.	P.I. 353300	160.2	12.7	49.	P.I. 211109	402.0	10.7
20.	P.I. 353380	502.0	12.8	50.	P.I. 211641	429.0	17.4
21.	P.I. 293481	139.8	12.2	51.	P.I. 211642	453.0	12.5
22.	P.I. 179554	60.2	14.6	52.	P.I. 218122	93.6	4.7
23.	P.I. 352905	28.8	13.8	53.	P.I. 211110	56.0	12.3
24.	P.I. 124609	21.0	14.5	54.	P.I. 250416	30.5	12.8
25.	P.I. 292899	15.5	11.2	55.	P.I. 229796	49.0	--
26.	P.I. 353270	4.3	N.E.S.	56.	P.I. 229734	10.8	--
27.	P.I. 292909	7.0	N.E.S.	57.	P.I. 353011	215.5	7.7
28.	P.I. 292908	7.8	6.4	58.	P.I. 352945	17.0	11.8
29.	P.I. 353332	327.0	10.3	59.	P.I. 352931	74.0	11.5
30.	P.I. 271257	125.0	12.1	60.	P.I. 353010	--	--

* N.E.S. = Not Enough Seed

Table 3: Mung Bean Accessions Evaluated for Heat and Salt Tolerance and Nodulation at the University of Arizona Experimental Farm, Safford, AZ - 1979.

	Entry	Yield gm/plot	100 seed wt. gm		Entry	Yield gm/plot	100 seed wt. gm
1.	V-1381*	14.4	10.0	31.	P.I. 376956	163.6	5.1
2.	V-1944	11.5	8.1	32.	P.I. 376961	285.7	3.8
3.	V-1968	45.2	4.4	33.	P.I. 376963	362.0	3.8
4.	V-2007	6.2	--	34.	P.I. 376954	263.0	3.4
5.	V-2010	61.0	6.6	35.	P.I. 376965	342.0	3.2
6.	V-2184	1.4	--	36.	P.I. 376966	11.8	3.2
7.	V-2773	110.9	4.2	37.	P.I. 276696	--	--
8.	V-2984	--	--	38.	P.I. 376996	3.5	N.E.S.
9.	V-3404	27.0	7.8	39.	P.I. 376923	19.0	6.9
10.	V-3476	1.0	N.E.S.	40.	P.I. 377093	66.2	3.2
11.	P.I. 376925	--	--	41.	P.I. 377102	144.2	3.3
12.	P I. 376856	1.5	N.E.S.	42.	P.I. 377005	280.0	3.1
13.	P.I. 377003	15.5	3.4	43.	P.I. 377004	490.0	3.1
14.	P.I. 164336	104.6	5.1	44.	P.I. 377006	18.6	2.1
15.	P.I. 377860	29.0	8.3	45.	P.I. 377000	183.5	3.8
16.	P.I. 376951	1.5	N.E.S.	46.	P.I. 376995	--	--
17.	P.I. 376952	93.4	3.7	47.	P.I. 376993	--	--
18.	P.I. 377011	48.8	5.1	48.	P.I. 374149	--	--
19.	P.I. 179952	105.6	5.1	49.	P.I. 377007	--	--
20.	P.I. 207504	1.5	N.E.S.	50.	P.I. 377056	44.5	3.3
21.	P.I. 212614	--	--	51.	P.I. 376999	315.0	4.2
22.	P.I. 219613	27.2	3.3	52.	P.I. 377140	40.0	3.2
23.	P.I. 217953	1.5	N.E.S.	53.	P.I. 376989	277.5	3.4
24.	P.I. 223523	17.5	3.8	54.	P.I. 377044	44.8	3.5
25.	P.I. 219699	66.0	3.8	55.	P.I. 377045	40.2	3.2
26.	P.I. 227754	--	--	56.	P.I. 377042	34.4	4.6
27.	P.I. 176850	108.0	4.1	57.	P.I. 377039	8.0	3.0
28.	P.I. 376855	106.0	4.4	58.	P.I. 376998	6.0	4.6
29.	P.I. 376849	11.5	4.7	59.	P.I. 164301	5.0	2.2
30.	P.I. 246130	259.6	4.6	60.	P.I. 377102	--	--

*AVRDC accessions.

Table 4: Soil Analysis and Performance of Cowpeas and Mung Beans at Safford, AZ.

Entry	pH	EC X 10^3	Soluble Salts ppm	Na meq/L	ESP	SAR	N ppm	P ppm	K ppm	Yield gm/plot
Cowpeas										
Big Boy	7.6	4.97	3479	27.1	9.74	8.21	14.0	2.75	0.82	175.6
Speckled Purple Hull	7.6	4.92	3444	29.2	11.14	9.42	32.5	3.25	0.77	474.2
P.I. 352945	7.5	3.99	2793	34.1	22.83	21.05	26.75	2.25	0.56	17.0
P.I. 211110	7.6	2.44	1708	19.5	18.44	16.16	16.75	2.75	0.31	56.0
P.I. 211109	7.6	2.20	1540	18.1	15.76	13.61	17.50	2.50	0.38	402.0
P.I. 211641	7.6	2.35	1645	18.1	13.44	11.46	12.75	2.75	0.38	429.0
P.I. 293518	7.6	2.49	1743	18.8	13.21	11.19	18.25	10.5	0.44	383.2
P.I. 353332	7.6	3.28	2296	20.2	9.77	8.24	20.00	2.85	0.56	327.0
P.I. 292903	7.6	2.95	2066	16.7	7.89	6.73	40.50	2.75	0.51	121.0
Mung Beans										
P.I. 377000	7.6	2.16	1512	17.1	13.97	11.88	19.75	2.75	0.36	183.5
P.I. 376963	7.5	3.52	2464	19.5	8.45	7.12	18.75	3.25	0.72	362.0
P.I. 376965	7.5	2.63	1841	16.7	9.33	7.84	17.25	2.50	0.49	342.0
P.I. 246130	7.5	2.95	2065	16.7	7.92	6.73	21.75	2.50	0.51	259.6
P.I. 377011	7.6	3.19	2233	21.6	11.59	9.77	26.75	2.75	0.49	48.8
P.I. 376962	7.5	2.63	1841	15.3	7.85	6.68	20.75	2.0	0.49	108.0
P.I. 377004	7.6	2.46	1722	17.4	11.12	9.41	17.5	3.0	0.33	490.0
P.I. 376966	7.5	2.95	2066	18.1	9.28	7.80	15.5	2.75	0.67	11.8
P.I. 376954	7.5	2.63	1841	17.4	10.11	8.49	25.0	4.75	0.49	263.0
P.I. 376995	7.5	2.95	2065	23.7	16.76	14.54	38.75	2.75	0.46	--

previously. Although non-replicated observation nurseries were planted during the course of the study, no genotypes were identified as being salt-tolerant over three seasons. Mung beans were continually stunted, and showed varying degrees of sodium toxicity injury even if they survived.

An important consideration for future studies of BNF in stress environments, is that germplasm agronomically adapted to the stress for which rhizobia are being screened be utilized as host plants.

II. Screening *Rhizobia* spp Strains for Heat and Salt Tolerance

a. Growth Pouch Study

Eight strains of cowpea and mung bean type *Rhizobia* were screened for heat and salt resistance (Table 5). Scientific Products Seed-Pack Growth Pouches were used. Salt concentrations were determined weekly and maintained by additions of sterilized nutrient solution, plus 4000 ppm salt, or deionized water. Pouches were filled with either 40 ml of nutrient solution or 40 ml nutrient solution plus 4000 ppm salt and then autoclaved for one-half hour at 121°C, and 15 psi. The 4000 ppm salt consisted of 1000 ppm each of NaCl, K_2SO_4, $MgSO_4 \cdot 7H_2O$ and $CaCl_2 \cdot 2H_2O$. Cowpea seeds, variety Blue Goose, and mung bean seeds, variety Berken, were surface sterilized (rinsed with 95% Ethanol, soaked three minutes in 0.2% acidified mercuric chloride, rinsed with five changes sterile water) and planted in pouched, two seeds per pouch. Seeds were inoculate by pipeting 0.2 ml of inoculum directly onto each seed. Inoculum was prepared by growing *Rhizobia* to log phase in yeast extract mannitol

Table 5. Origin and Inoculation Rates of *Rhizobia* Strains Used in Growth Pouch Studies.

Rhizobia Strain	Origin	# Organisms/Seed
#571	Rhodesia	$10^8 - 10^9$
#591	Rhodesia	$10^7 - 10^8$
#618	Rhodesia	$10^7 - 10^8$
#581	Rhodesia	$10^8 - 10^9$
#484	Rhodesia	$10^6 - 10^7$
C5	Argentina	$10^7 - 10^8$
WA-826	Argentina	$10^6 - 10^7$
CB-756	Australia	$10^6 - 10^7$

Broth. Half of the pouches were placed in a growth chamber at 27°C (Percival walk-in growth chamber model #PGW-9675). The remaining half were grown at 40°C in another chamber (Corporation West Environator model #E42SS). Respective night temperatures were 16 and 27°C, day-length was twelve hours. Each host-strain symbiosis plus uninoculated controls for each species was replicated four times. The number of Rhizobia spp. applied per seed was determined by plating the inoculant at various dilutions.

After four weeks, plants were removed from growth pouches and their roots were examined for the presence of nodules. Nodules were visually rated as active or not active, based on the presence or absence of pink coloration. Degree of nodulation was ranked as follows: no nodules, few or small-sized nodules, moderate or medium-sized nodules, and many or large-sized nodules. The relative nodulation ability of the strains was compared for high and low temperature and salt treatments. Strains CB756 and 591 were selected as most tolerant for cowpeas and CB756 and 484 for mung beans.

b. Leonard Jar Study

The two most promising cowpea and mung bean lines and the two most stress tolerant rhizobia strains from the Growth Pouch Study were used (Table 6) in the Leonard Jar Study.

Each host-symbiont combination was subjected to two temperature regimes: 27/16°C and 33/27°C, day/night, and two salinity levels; 1/5 strength nutrient solution + 1 salt level + 4000 ppm salt. Uninoculated controls were

Table 6. Cowpea and Mung Bean Accessions Selected on Basis of Yield and Soil Analyses and Selected *Rhizobia* Strains Used in Leonard Jar Studies.

Entry	Yield gm/plot	Soluble Salts-ppm (Field)	Rhizobia Strain	# Organisms/Seed 33C	# Organisms/Seed 27C
Cowpeas					
Speckled Purple Hull	474	3441	CB756	10^6	10^7
Big Boy	176	3479	# 591	10^6	10^7
Mung Beans					
P.I. 376963	362	2464	CB756	10^6	10^7
P.I. 246130	260	2065	# 484	10^6	10^7

grown for each variety and treatments were replicated four times. Seeds were planted three per jar and inoculated with one ml of log phase culture grown in YEMB. Uninoculated seeds were treated by dripping one ml of sterile water onto each seed. The number of Rhizobia per seed was calculated by plating at different dilutions.

Mung beans were harvested after eight weeks when 50% of the plants in the high temperature regime had flowered. Cowpeas were harvested after ten weeks even though they had not begun to flower in the high temperature regime because plants were beginning to trail, thus making the Leonard jars unstable. Both species matured at a faster rate in the lower temperature regime, but harvest times were kept at eight and ten weeks for comparison. Mung beans were well into pod fill by this time and cowpeas were at about 25% flowering. After severing roots from shoots at the cotyledon scars and running acetylene reduction on roots with nodules, shoots and roots were dried at 65°C for twenty-four hours. Nodules were subsequently removed, counted, and weighed. Dry weights were also obtained for root and shoot materials. Percent N and total N were determined using micro-Kjehldahl techniques for low temperature shoot material.

Seven variables; root, shoot and nodule weight, nodule mass, acetylene reduction, and total and %N; were analyzed using the Statistical Package for Social Sciences (1977). Four factors: plant cultivar, Rhizobia spp. strain, salt level and temperature and all their interactions were considered. Interactions were analyzed at the five percent level of significance using Least Significant Difference (LSD) or the Student-Newman-Kuel (SNK) tests of significance.

Cowpeas

No significant differences were found for mean nodule weight based on cultivar or *Rhizobia* spp strain. Heat and salt stress effects were not consistent. Analysis of salt by temperature interaction indicated that at low salt levels nodule weight decreased as temperature increased. However, at the high salt concentration, the reverse was true. The inconsistant response of nodule mass to heat and salt stress may be accounted for by the large but ineffective nodules on plants in the high salt, high temperature treatments (Table 7). Nodule numbers were not significantly affected by heat or salt stress.

Acetylene reduction was decreased by the addition of salt, regardless of cultivar, temperature or *Rhizobia* strain. The cultivar by temperature interaction was significant because for Speckled Purple Hull acetylene reduction decreased as temperature decreased, but for Big Boy acetylene reduction increased as temperature decreased. *Rhizobia* strains reacted differently with the cultivars which is shown in the variety by bacteria interaction. The only combination that was significantly higher in acetylene reduction was CB756 and Big Boy, when compared to CB756 and Speckled Purple Hull. In general, CB756 performed better with Big Boy and # 591 performed better with Speckled Purple Hull. Analysis of the three way salt by variety by temperature interaction gave three significant differences; Big Boy had significantly less acetylene reduction when salt was increased at the lower temperature, Big Boy had considerably less acetylene reduction when salt was increased at the lower salt level, and Speckled Purple Hull showed a significant decrease in

Table 7

Analysis of Variance for Cowpeas: Nodule Number, Nodule Weight and Acetylene Reduction

Source of Variation	Nodule Number			Nodule Weight			Acetylene Reduction		
	Sum of Squares	DF	Signif of F	Sum of Squares	DF	Signif of F	Sum of Squares	DF	Signif of F
Main Effects[1]	1024.413	4	.867	19286.356	4	.252	219.451	4	.001
V variety	2.168	1	.959	502.135	1	.705	11.497	1	.143
S salt	864.884	1	.309	13830.814	1	.052	202.190	1	.001
T temperature	176.663	1	.644	.684	1	.989	.841	1	.689
B Rhizobia strain	17.583	1	.884	5316.024	1	.222	.759	1	.704
2-Way Interactions	2469.415	6	.801	66263.895	6	.011	117.897	6	.004
V S	365.862	1	.507	14383.402	1	.048	18.239	1	.067
V T	188.174	1	.633	512.776	1	.702	39.129	1	.009
V B	199.017	1	.624	13191.288	1	.057	29.638	1	.021
S T	1493.557	1	.183	31497.298	1	.004	18.221	1	.067
S B	11.191	1	.907	670.995	1	.662	.230	1	.834
T B	107.238	1	.719	717.745	1	.651	5.048	1	.329
3-Way Interactions	3364.377	4	.402	13072.376	4	.447	90.238	4	.005
V S T	215.858	1	.610	1816.705	1	.472	63.845	1	.001
V S B	649.815	1	.377	9725.735	1	.101	16.943	1	.077
V T B	1779.030	1	.147	818.795	1	.629	1.180	1	.635
S T B	560.968	1	.411	409.310	1	.732	9.641	1	.179
4-Way Interactions	466.821	1	.453	24.890	1	.933	13.869	1	.109
V S T B	466.821	1	.453	24.890	1	.933	13.869	1	.109
Explained	7325.025	15	.859	98647.518	15	.050	441.454	15	.001
Error	35068.500	43		148658.583	43		222.338	43	
Total	42393.525	58		247306.102	58		663.792	58	

[1]On all analysis of variance, tables; 'V' indicates variety, 'S' indicates salt, 'T' indicates temperature, and 'B' indicates Rhizobia strain or control.

acetylene reduction when salt level was increased at the higher temperature. Although not statistically significant, Speckled Purple Hull performed better at higher temperature than Big Boy, based on visual observation and data trends.

In general, shoot weight was reduced by increased salt and temperature (Table 8). Analysis of the salt by temperature interaction indicated that the only significant decreases occurred when temperature was increased at the low salt level and when salt was increased at the low temperature. Symbiotic combinations responded differently to salt stress as shown in the variety by salt by bacteria interaction. Big Boy accumulated more shoot weight at the low salt level when inoculated with CB756 versus # 591. Inoculated Big Boy plants had significantly more shoot weight than controls. Speckled Purple Hull did not respond differently to strain at either salt level. At the high salt level, inoculation did not produce more shoot weight than controls, possibly because contamination of controls was more prevalent for Speckled Purple Hull. Inoculation did produce higher shoot weights for Speckled Purple Hull at the low salt level, where no contamination of control occurred. The effects of cultivar in this three-way interaction were that at low salt levels the combination of Speckled Purple Hull and 591 accumulated more mean shoot weight than Big Boy and 591. However, at the high salt level Big Boy and 591 had more shoot weight. This effect was most pronounced at the lower temperature where the combination of Speckled Purple Hull and 591 accumulated relatively little shoot weight. The inconsistencies of these results make it difficult to make any conclusions based on the relative salt tolerance of the symbiotic systems.

Table 8

Cowpea Shoot[1] and Root[2] Weight as Influenced By *Rhizobia* Strain, Temperature and Salt Level[3]

Rhizobia Strain		33°C Shoot	33°C Root	27°C Shoot	27°C Root
		(mg/plant)			
a) Speckled Purple Hull					
Nutrient Solution	CB756	2268	2072	4638	1617
	591	4080	2279	4711	2011
	Control	1349	1377	354	681
Nutrient Solution + Salt	CB756	1235	1286	1001	503
	591	1503	1342	777	420
	Control	1164	1100	174	318
b) Big Boy					
Nutrient Solution	CB756	2748	1961	6215	2039
	591	1753	1400	4177	1997
	Control	368	1160	656	738
Nutrient Solution + Salt	CB756	1783	1068	1349	508
	591	1771	1289	1713	500
	Control	513	929	364	400

[1]No significant difference found for plant variety or *Rhizobia* strain. Significant interactions analyzed at the 5% level using LSD or SNK tests.

[2]No significant differences found for plant variety or *Rhizobia* strain. Significant interactions analyzed at the 5% level using LSD test.

[3]See Table 9 for significant interactions.

Root weight increased as temperature increased and decreased as salt increased. Analysis of the salt by temperature interaction showed that the only significant effect on root weight was the decrease caused by increased salt at low temperatures. No strain or variety effects were found (Table 9).

Increasing salt level tended to decrease total N. The salt by bacteria interaction showed that at high salt levels inoculation did not significantly increase total N, but that it did increase total N at low salt levels. Also at low salt levels Rhizobia strain # 591 produced significantly more total N when combined with Big Boy than did CB756. This was the only significant difference found when the salt by bacteria by variety interaction was analyzed.

In general, mean %N was decreased by increased salt and inoculation significantly increased %N. The variety by salt interaction showed that Speckled Purple Hull did better than Big Boy at high salt but a lack of a significant variety difference in total N and the fact that Speckled Purple Hull treatments in general had higher dry shoot weights prevents concluding that Speckled Purple Hull is more salt tolerant. Controls at the high salt level were found to have a higher %N content than those at the low salt level when the salt by bacteria interaction was analyzed, but this was due to contamination of controls at the high salt level and was not significant (Table 10, Table 11).

Conclusions

Results obtained from cowpea data are inconsistent with regard to plant variety and *Rhizobia* strain. Strain CB756 when combined with Big

Table 9

Analysis of Variance for Cowpeas; Shoot and Root Weight

Source of Variation	Shoot Weight: Sum of Squares	Shoot Weight: DF	Shoot Weight: Signif of F	Root Weight: Sum of Squares	Root Weight: DF	Root Weight: Signif of F
Main Effects	108147485.620	5	.001	18447978.858	5	.001
V	140386.674	1	.654	144054.618	1	.393
S	81574871.971	1	.001	16935388.385	1	.001
T	12951707.008	1	.001	2078459.990	1	.002
B	3233542.712	2	.107	187801.423	2	.618
2-Way Interactions	45805111.945	8	.001	3553182.615	8	.037
V S	1440197.100	1	.155	22646.404	1	.734
V T	1853519.254	1	.108	649522.362	1	.073
V B	4158687.899	2	.059	168051.052	2	.650
S T	25985190.253	1	.001	2249104.641	1	.001
S B	1392390.334	2	.372	11748.070	2	.970
T B	1227311.028	1	.188	36230.372	1	.667
3-Way Interactions	8881504.032	4	.021	1024580.293	4	.275
V S T	1302767.014	1	.176	240047.506	1	.271
V S B	6192771.867	1	.004	438332.380	1	.139
V T B	554949.816	1	.374	9875.515	1	.822
S T B	1600428.877	1	.134	272640.876	1	.241
4-Way Interactions	6517.014	1	.923	39605.488	1	.653
V S T B	6517.014	1	.923	39605.488	1	.653
Explained	162840618.612	18	.001	23065347.254	18	.001
Error	31684450.250	46		8894374.500	46	
Total	194525068.862	64		31959721.754	64	

Table 11. Analysis of variance for cowpeas; percent and total nitrogen (low temperature study).

	% N			Total N		
	Sum of Squares	DF	Sig. of F	Sum of Squares	DF	Sig. of F
Source of Variation						
Main Effects	50.095	4	.001	234398.520	4	.001
V	4.928	1	.001	19.253	1	.883
S	4.738	1	.001	123484.941	1	.001
B	40.429	2	.001	110894.325	2	.001
Interactions						
2-Way	6.460	5	.001	66232.264	5	.001
V S	1.062	1	.037	112.853	1	.721
V B	1.280	2	.073	4507.533	2	.089
S B	4.118	2	.001	61611.878	2	.001
3-Way	.080	2	.839	6198.345	2	.039
V S B	.080	2	.839	6198.345	2	.039
Explained	56.635	11	.001	306829.129	11	.001
Error	8.175	36		31392.930	36	
Total	64.810	47		338222.059	47	

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Table 10. Cowpea percent nitrogen[1] and total nitrogen[2] as influenced by *Rhizobia* spp. strain and salt level for low temperature (27 C).[3]

		Speckled Purple Hull		Big Boy	
		% Nitrogen	Total Nitrogen (mg/plant)	% Nitrogen	Total Nitrogen (mg/plant)
	Rhizobia spp. strain				
Nutrient Solution	CB756	3.76	176.8	3.52	220.0
	591	4.04	192.6	3.33	133.4
	Control	1.14	4.0	1.06	6.9
Nutrient Solution + Salt	CB756	3.05	30.7	2.12	28.7
	591	3.37	26.5	1.94	32.6
	Control	1.52	2.7	1.07	3.9

[1,2]Significant interactions analyzed at the 5% level using LSD or SNK tests.

[3]See Table 11 for significant interactions.

Boy was superior in nitrogen fixation efficiency and shoot accumulation at the low salt level, but no efficient system was found under stress conditions. In general, nitrogen fixation efficiency decreased with increased temperature and salt level. Perhaps the high temperature, $33^{o}C$, was not high enough to separate stress results in these strains and varieties.

Mung Beans

The Mung Bean Leonard Jar Studies show a consistant but non-significant trend in which the combination of strain CB756 and cultivar P1246130 produced more nodule mass under stress conditions. Under conditions of no stress, the combination of strain 484 and P1246130 produced significantly more nodule mass. Introduction of a temperature stress factor reduced nodule mass to the point where no significant differences were found for any of the combinations. Addition of salt stress factor further reduced nodule mass. Reductions due to increased salt at either temperature was greater than when temperature was increased at high salt. P1246130 consistently produced greater nodule mass, however, on introduction of any stress condition, nodule mass was not significantly different from either cultivar. Note that reduction of nodule mass due to an increase of stress are greater with strain 484 than CB756, indicating that CB756 may be more stress tolerant (Table 12).

Cultivar P1246130 consistantly produced more nodules than P1376963, except when the two cultivars were combined with strain 484 at the high salt, high temperature stress level where nodule numbers were equal. However, the nodules produced by the combination of P1246130 and strain 484 had greater mass/nodule at this stress level. Strain CB756 consistently produced as many or more nodules than 484 with the exceptions of the non-stressed conditions with P1246130. Increasing salt or temperature alone caused significant decreases in nodule number for cultivar P1246130 with both strains, but not for cultivar P1376963. Lack of a significant response to stress for cultivar P1376963 was due to overall poor

TABLE 12. Mung bean nodule weight, nodule number and acetylene reduction as influenced by plant cultivar, strain of rhizobia, plant cultivar temperature and salt level.

Cultivar	Strain of Rhizobia	Nodule Mass[1] (mg) 35°C	Nodule Mass[1] (mg) 27°C	Nodule Number[2] (mg) 35°C	Nodule Number[2] (mg) 27°C	Acetylene Reduction[3] (um/plant/hour) 35°C	Acetylene Reduction[3] (um/plant/hour) 27°C
P.I. #376963							
Nutrient Solution	CB756	13.00c[4]	51.25bc	7.75c	13.25C	0.38a	0.45a
	484	8.50c	69.50b	5.75c	8.00c	0.47a	0.40a
Nutrient Solution + Salt	CB756	3.00c	5.75c	4.00c	7.25c	0.00a	0.00a
	484	2.00c	3.33c	4.00c	3.67c	0.00a	0.00a
P.I. #246130							
Nutrient Solution	CB756	19.75c	83.00b	20.50c	49.00b	1.02a	0.92a
	484	19.50c	167.75a	12.50c	82.00a	0.85a	0.55a
Nutrient Solution + Salt	CB756	8.25c	15.50c	9.75c	23.00c	0.02a	0.00a
	484	6.00c	11.75c	4.00c	10.25c	0.12a	0.00a

1 Significant interactions analyzed at the 5% level using LSD test: BxSxT; VxSxT; SxT; BxT; BxS; VxT; VxS.
2 Significant interactions analyzed at the 5% level using LSD test: BxSxT; VxSxT; SxT; BxT; BxS; VxT; VxS.
3 No significant interactions:
4 Values without letters in common are significantly different ($p<0.05$) according to the Neuman-Kuel Range Test.
B = strain of rhizobia V = plant cultivar T = temperature s = salt.

nodulation of this cultivar. Cultivar P1246130 appeared to be superior for nodule initiation (Table 12).

There were no significant interactions for acetylene reduction assay. Significant main effects were found for plant cultivar and salt stress. Cultivar P1246130 reduced significantly more acetylene than P1376963 at the low salt level only. There was essentially no acetylene reduced at high salt levels. Increasing temperature caused no significant reduction in ethylene production, in fact for cultivar P1246130 ethylene production was a little higher at the higher temperature (Table 12).

The most pronounced effect shown by analysis of these interactions was the poor response of cultivar P1376963 when combined with strain 484. Even under conditions of no stress, this combination produced significantly less shoot weight than any other combination. Increasing salt stress caused reductions in shoot weights, regardless of strain, cultivar, or temperature, although these reductions were not significant at the high temperature. Rhizobia strain CB756 consistantly produced more shoot tissue than 484, except in conditions of no stress, the combination of CB756 and cultivar P1246130 produced more tissue. Although most of these differences were not significant, it appears that under stress conditions the combination of CB756 and P1246130 is a more reliable combination for shoot production than the other three. Introduction of salt or heat stress reduced shoot weight (Table 13).

Increasing temperature at the low salt level caused a significant reduction in root weight regardless of Rhizobia strain or plant cultivar. Analysis of interactions showed that a significant salt effect occurred only at the lower temperature which might indicate that root production

TABLE 13. Mung bean shoot and root weight as influenced by plant cultivar, strain of rhizobia, temperature and salt level.

Cultivar	Strain of Rhizobia	Shoot Weight[1] 35°C mg/plant	Shoot Weight[1] 27°C mg/plant	Root Weight[2] 35°C mg/plant	Root Weight[2] 27°C mg/plant
P.I. #376963					
Nutrient Solution	CB756	360.50d	1695.25a	75.50c	735.75a
	484	245.75d	826.75c	61.00c	395.25b
Nutrient Solution + Salt	CB756	101.50d	155.50d	45.50c	170.25c
	484	84.00d	149.33d	39.25c	98.33c
P.I. #246130					
Nutrient Solution	CB756	395.25d	1420.75ab	64.50c	468.00b
	484	345.50d	1344.50b	57.00c	504.25b
Nutrient Solution + Salt	CB756	172.00d	290.75d	55.25c	161.00c
	484	157.25d	240.25d	85.75c	155.25c

[1] Significant interactions analyzed at the 5% level using LSD test: VxBxS; BxS; SxT; BxT; VxB.

[2] Significant interactions analyzed at the 5% level using LSD test: VxBxS; BxS; SxT; BxT; VxB.

[3] Values without letters in common are significantly different ($p<0.05$) according to the Neuman-Keul Range Test.

B = strain of rhizobia V = plant cultivar T = temperature S = salt

is more sensitive to temperature than salt. Cultivar P1376963 produced significantly more root weight at the lower temperature than P1246130 when combined with strain CB756. At the high temperature, there were no significant differences in root production (Table 13).

In all cases but one, increasing salt decreased percent N. In treatments when this was not true, i.e., cultivar P1376963 at low temperature, the percent N value is probably a reflection of cotyledon reserves as many of the plants in the high salt treatment did not live past seedling stage. Increasing temperature did not cause a consistent response. Care must be taken in interpretation of percent N, since cotyledon reserves can confound the data when little plant growth occurs.

Although no one combination of cultivar and strain of Rhizobia produced significantly more total N per plant, under stress conditions strain CB756 and cultivar P1246130 were consistently higher in total N. Increasing salt stress on CB756 at low temperature caused a significant reduction in total N regardless of the cultivar. The reduction due to increasing salt at the low temperature was greater than the reduction due to a temperature increase at a low salt level. Thus, N accumulation in the shoot from this combination may be more sensitive to salt than to temperature (Table 14).

Note that the combination of 484 with P1376963 was particularly inefficient in N accumulation, especially in the non-stress situation. When combined with P1246130, 484 was much more efficient at BNF. It is also of interest that the higher nodule mass, associated with 484 in the non-stress situation with either cultivar, did not translate into higher N accumulation.

TABLE 14. Mung bean percent nitrogen and total nitrogen as influenced by plant cultivar, strain of rhizobia, temperature and salt level[3].

Cultivar	Strain of Rhizobia	Percent Nitrogen[1] 35°C mg/plant	Percent Nitrogen[1] 27°C mg/plant	Total Nitrogen[2] 35°C mg/plant	Total Nitrogen[2] 27°C mg/plant
P.I. #376963					
Nutrient Solution	CB756	3.65ab[3]	1.97de	12.82c	33.45b
	484	2.99abcd	1.35e	7.83c	11.07c
Nutrient Solution + Salt	CB756	2.54bcde	2.51bcde	2.47c	4.00c
	484	2.13cde	2.15bcde	1.77c	2.90c
P.I. #246130					
Nutrient Solution	CB756	3.95a	3.41abc	15.90c	47.15a
	484	3.64ab	3.78a	13.15c	46.60a
Nutrient Solution + Salt	CB756	2.45bcde	2.60bcde	4.12c	7.68c
	484	2.11cde	2.51bcde	3.30c	5.75c

1 Significant interactions analyzed at the 5% level using LSD test: VxSxT; SxT; VxT; VxS.

2 Significant interactions analyzed at the 5% level using LSD test: VxSxT; SxT; VxT; VxS; VxB.

3 Values without letters in common are significantly different ($p<0.05$) according to the Neuman-Keul Range Test.

B = strain of rhizobia V = plant cultivar T = temperature S = salt

CONCLUSIONS

Genetic variability was found for mung bean cultivars and Rhizobia strains under controlled conditions of heat and salt stress. When the cultivars and strains selected from two initial screenings were used in a Leonard jar study, significant variation was found under conditions of no stress. Symbiotic systems varied in nodule mass, nodule number, shoot weight, root weight and total N. When no stress was present, Rhizobia strain 484 produced more nodule mass than CB756 in most cases, however, these nodules were less efficient because total nitrogen and shoot accumulations were higher for strain CB756. This is especially evident when strain 484 is combined with cultivar PI376963 where even though nodule mass was greater for strain 484, total N was significantly lower. Under stress conditions, no significant differences were found between symbiotic combinations. However, the combination of CB756 and PI246130 consistantly produced more shoot weight, total nitrogen, nodule weight, nodule number, and acetylene reduction. It is interesting to note that salt stress consistantly reduced BNF (C_2H_2>C_2H_2) while temperature had very little effect. Total nitrogen was also more strongly decreased by salt stress than by temperature. It appears that in mung beans, nitrogen fixation and accumulation is more sensitive to salt than to temperature.

This research demonstrates the genetic variability of BNF by mung beans under stress conditions. The variable response indicates the necessity of repeated screenings using both symbiotes in order to maximize efficiency of fixation.

III. Evaluation of Heat and Salt Tolerant Cowpeas and Mung Beans and Interacting Rhibozian Strains Under Field Conditions

In order to evaluate field performance of selected cowpeas, mung beans, and Rhizobia strains, a field trial composed of four experiments was initiated. In addition to strains #484 and CB756, inocula prepared with promising strains was provided for evaluation by the Nitragin Co, Milwaukee, Wisconsin and North American Plant Breeders (NAPB).

Cowpea - Experiment I

Entry	Rhizobia strain	Entry	Rhibozia strain
P.I. 293518	"O"	P.I. 211641	"O"
P.I. 293518	R.P. 292-1 (NAPB)	P.I. 211641	R.P. 292-1
P.I. 293518	R.P. 259-2	P.I. 211641	R.P. 259-2
P.I. 293518	R.P. 361-2	P.I. 211641	R.P. 361-2
P.I. 211109	"O"	P.I. 211642	"O"
P.I. 211109	R.P. 292-1	P.I. 211642	R.P. 292-1
P.I. 211109	R.P. 259-2	P.I. 211642	R.P. 259-2
P.I. 211109	R.P. 361-2	P.I. 211642	R.P. 361-2

Cowpea - Experiment II - 1978

Entry	Rhizobia strain	Entry	Rhizobia strain
Big Boy	"O" inoculum	P.I. 353332	"O" inoculum
Big Boy	CB-756	P.I. 353332	CB756
Big Boy	#484	P.I. 353332	# 484
Speckled Purple Hull	"O" inoculum	P.I. 353380	"O" inoculum
"	CB-756	P.I. 353380	CB-756
"	# 484	P.I. 353380	# 484

Mung Beans - Experiment I - 1978

Entry	Rhizobia strain	Entry	Rhizobia strain
P.I. 246130	"O"	P.I. 376961	"O"
P.I. 246130	Lx726 (Nitragin)	P.I. 376961	Lx726
P.I. 246130	Lx724	P.I. 376961	Lx724
P.I. 246130	Lx721	P.I. 376961	Lx721
P.I. 376956	"O"	P.I. 376989	"O"
P.I. 376956	Lx726	P.I. 376989	Lx726
P.I. 376956	Lx724	P.I. 376989	Lx724
P.I. 376956	Lx721	P.I. 376989	Lx721

Mung Beans - Experiment II

Entry	Rhizobia strain	Entry	Rhizobia strain
P.I. 377005	"0"	P.I. 376963	"0"
P.I. 377005	CB756	P.I. 376963	CB756
P.I. 377005	# 484	P.I. 376963	# 484
Berken	"0"	P.I. 376963	"0"
Berken	CB756	P.I. 376963	CB756
Berken	# 484	P.I. 376963	# 484

Cowpea and mung bean seed were pelleted, placed into envelopes and refrigerated until planting. The field was pre-irrigated and fertilized with 3Kg N and 67Kg treble super phosphate/ha. Each entry, replicated 4 times, was planted on the sides of beds 5 meters long with 2 rows/ replication.

Due to severe salt stress aggravated by prolonged irrigation intervals, all mung beans were dead by June 1. Hundred percent mortality also occurred in the Mung Bean Observation Nursery (Table 15). Starting 24 days after planting, surviving cowpeas were examined biweekly until termination of the experiment for nodulation. Whenever possible, 10 plants/plot were observed. No nodules were evident on any inoculated or uninoculated plants. Since salt stress resulted in extremely poor stands, no yield data were taken.

Table 15. Mung Bean Observation Nursery.

V-2106*	V-1336	V-1773	V-1331	V-1947
V-1360	V-3488	V-2273	V-1364	V-1388
V-1134	V-1682	V-1847	V-2040	V-1774
V-1548	V-1590	V-1830	V-2065	V-2278
V-1533	V-1813	V-1329	V-1322	V-2272
V-2283	V-1180	V-1353	V-1660	V-1785
V-1388	V-1958	V-1959	V-1953	V-1837
V-1067	V-1476	V-1009	V-1016	V-2105
V-1851	V-1946	V-2075	V-1042	V-1390
V-1066	V-1930	V-1954	V-1339	V-1337

V = lines obtained from AVRDC-Taiwan.

Conclusions

Although pelleting *Rhizobia* strains has been proposed as a means of insuring rhizobial survival in stress environments, we found this method to be unsatisfactory.

IV. Evaluation of Inoculant Placement on Rhizobial Survival in Saline-Alkali Soils.

Results from the previous field studies enabled us to conclude that 1 - indigenous infective strains were not present in the soil and 2 - pelleting *Rhizobia* strains with peat-based inocula did not assure survival of sufficient numbers of organisms in hot, dry, saline soils. Based on data from the growth pouch and Leonard Jar studies where cowpeas nodulated under stress we decided to evaluate the potential of granular inoculant placed both with the seed and at 10-15 cm depth. Soil temperatures at planting in excess of 30°C (Figure 1) and high salt levels (Figure 2) were thought to contribute to poor nodulation.

In this study, eight cowpea lines, P.I. 293518 (1), P.I. 353332 (2), Speckled Purple Hull (3), P.I. 211642 (4), California #5(5), P.I. 353011 (11), P.I. 353380 (12), and P.I. 180494 (19) were used. Although not completely satisfactory agronomically, each had demonstrated a fair degree of salt tolerance. Inoculant was prepared with strain CB-756. The Nitragin Company, Milwaukee, Wisconsin, USA, formulated and provided the granular peat inocula.

Since a moist seed-bed was reasoned to be conducive to rhizobial survival, cowpeas were planted on pre-irrigated beds with 1.2 m centers.

FIGURE 1. BI-WEEKLY AVERAGES OF DAILY SOIL TEMPERATURES AT 5, 10 AND 20 CM DEPTH. GROWING SEASON - 1980.

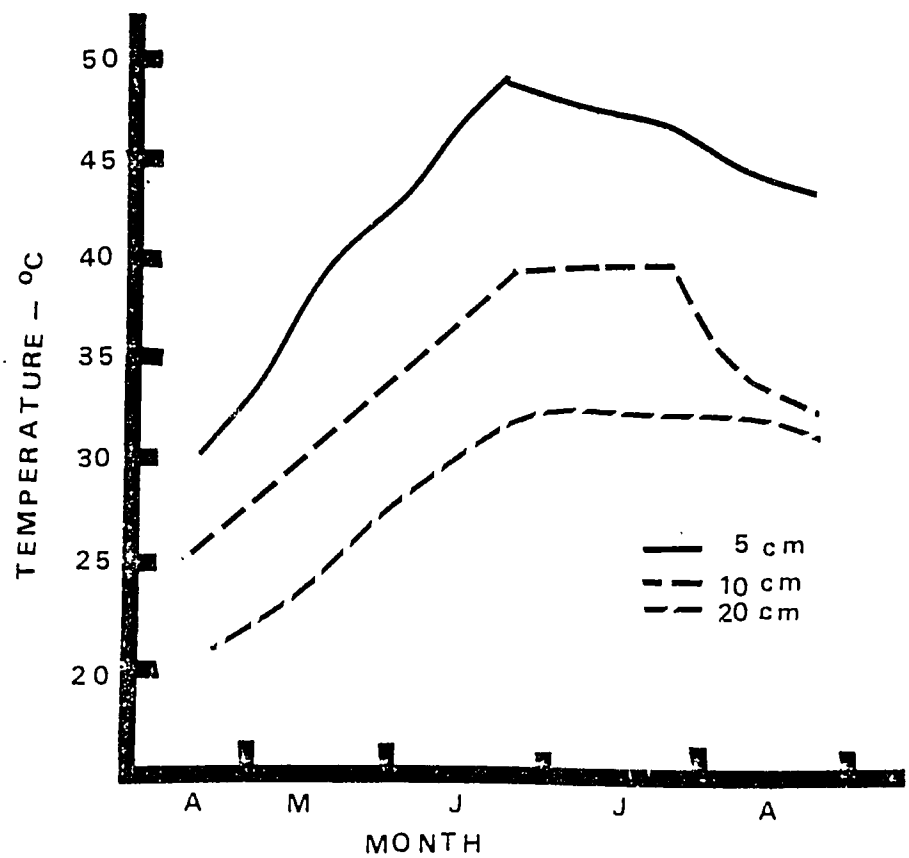


FIGURE 2. AVERAGE DRY WEIGHTS OF NODULES/PLANT AT 23, 39, 53 AND 93 DAYS.

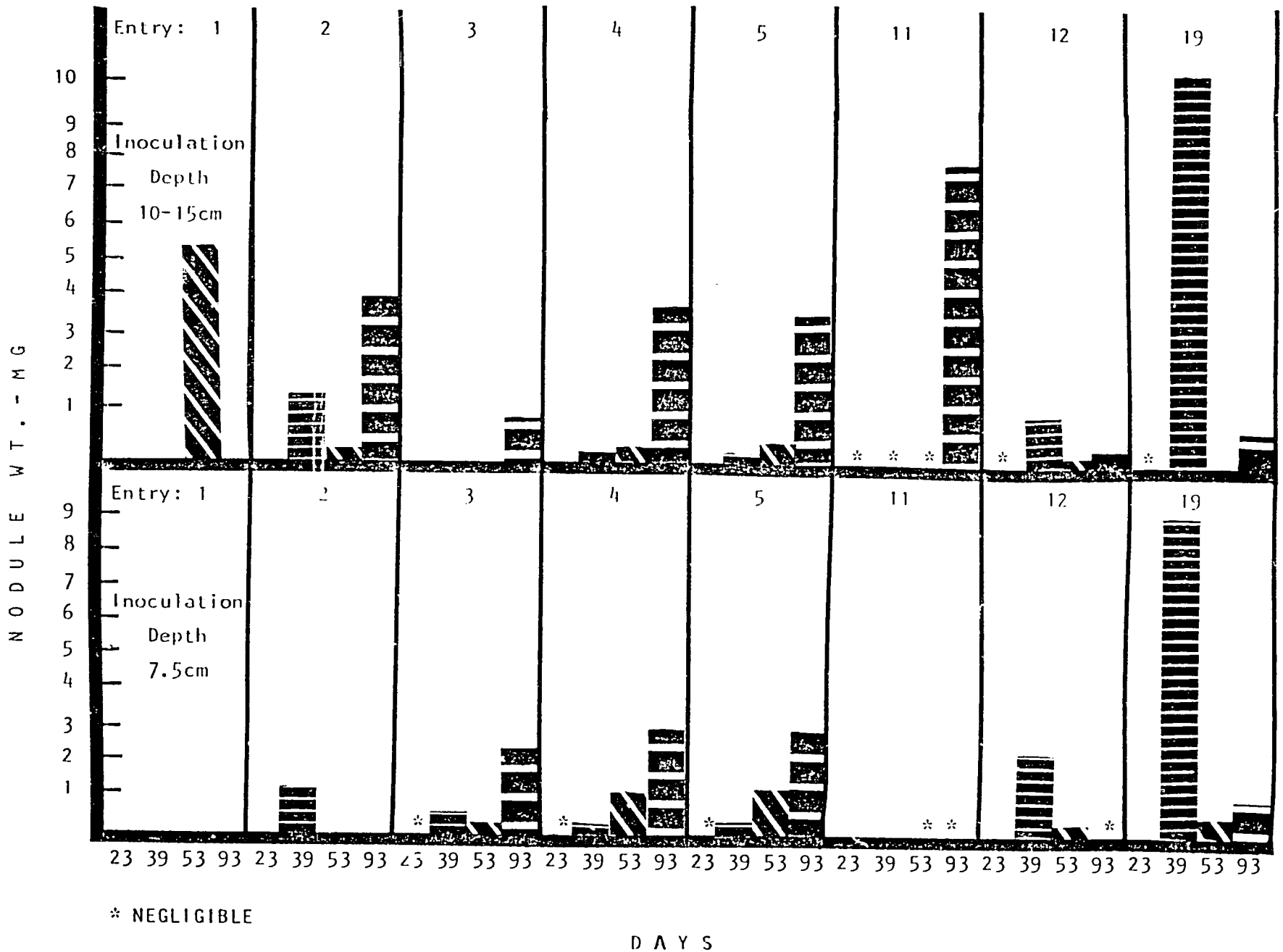


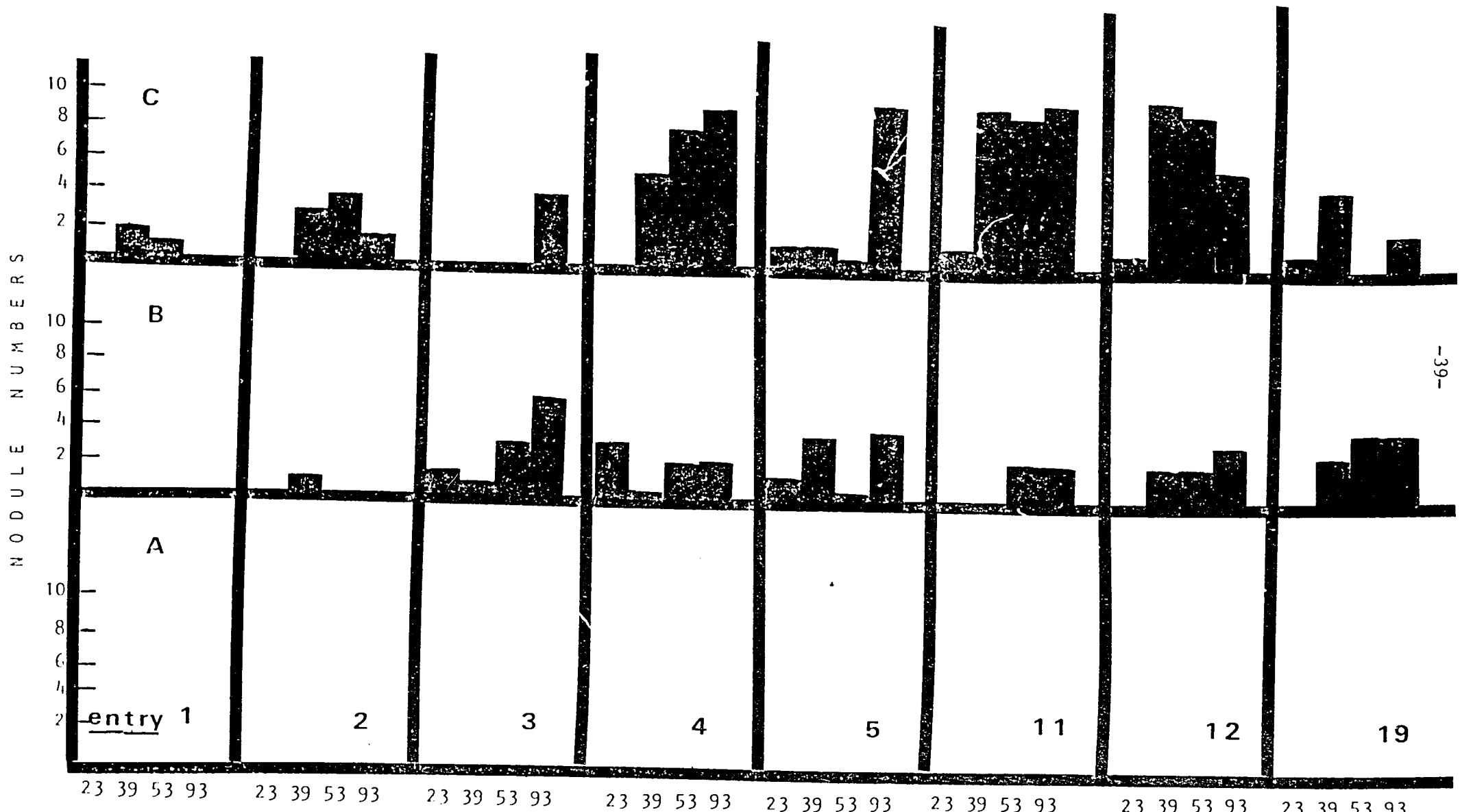
Table 16. Soil Data - 1980 Salt Tolerance Screening Trials
University of Arizona Experimental Farm, Safford, Arizona

Plot No.	Soil Depth cm	Soil Type	pH	ECe_3x10^3	Soluble Salts ppm	Na meq/l	ESP	N^+ ppm
3	0-10	Clay loam	8.00	5.44	5,327	0.53	0.90	22.80
3	10-20	Clay loam	8.30	2.32	1,624	13.79	7.58	5.05
19	0-10	Clay loam	7.65	42.01	29,407	154.00	15.57	16.00
19	10-20	Clay loam	8.40	1.58	1,106	12.66	12.34	8.80

Table 17. Influence of Inoculation Placement and Cultivar on Percentage of Plants Nodulated Over the Growing Season.

Cultivar	Days After Planting											
	23			39			53			93		
	Uninoculated %	Inocula Depth 7.5cm %	Inocula Depth 10-15cm %	Uninoculated %	Inocula Depth 7.5cm %	Inocula Depth 10-15cm %	Uninoculated %	Inocula Depth 7.5cm %	Inocula Depth 10-15cm %	Uninoculated %	Inocula Depth 7.5cm %	Inocula Depth 10-15cm %
1	0	0	0	0	0	40	0	0	11	0	0	0
2	0	0	0	0	20	30	0	0	100	0	0	83
3	0	10	0	0	40	0	0	100	0	0	100	75
4	0	30	0	0	10	100	0	100	100	0	100	100
5	0	10	8	0	30	10	0	0	0	0	100	100
11	0	0	100	0	0	100	0	100	100	0	100	100
12	0	0	40	0	30	70	0	100	100	0	100	90
19	0	0	17	0	40	60	0	100	0	0	100	75

FIGURE 3. AVERAGE NUMBERS OF NODULES/PLANT ON COWPEA ROOTS INOCULATED AT TWO DEPTHS WITH GRANULAR INOCULANT.



A = UNINOCULATED
B = INOCULANT DEPTH - 7.5 cm
C = INOCULANT DEPTH - 10 - 15 cm

In order to reduce salt stress, seed were placed to the side rather than in the middle of the irrigated rows. For the deep placement of inoculant, an Ortho fertilizer applicator which delivered 16 kg/ha inoculant 10-15 cm in the soil at planting was used. Uninoculated controls and cowpeas inoculated with 32 gm granular inoculant/33 m of row were included in the trials. The experiment was planted in a randomized complete block with four replications. Seeds were placed approximately 7.5 cm deep and the plots "capped" prior to seedling emergence. Ten plants/plot were examined for per cent nodulation, acetylene reduction assay, biomass production, nodule mass, and nodule numbers at 23, 39, 53 and 93 days. Final harvest and yield determination occurred at 124 days.

Both deep inoculation and the use of granular inoculant at seed level improved the percentage of cowpea plants nodulated over the growing season (Table 16). It is important to select host-plant-Rhizobia combinations that exhibit a high percentage of nodulation over the growing season. In general, total dry weights of nodules/plants (Figure 2) and nodule numbers (Fig. 3) were enhanced by deep placement of the inoculant. As would be expected, deep inoculant placement also affected nodule distribution, nodules occurring significantly lower in the soil profile with this treatment than when inoculant was placed with the seed (Fig. 4).

Variability among genotypes for nodule location on roots was not as great as with other characters studied. No significance was found between ethylene production (Table 18), total plant biomass, and yields (Figure 5).

Interestingly, a band of granular inoculant was still evident in the soil up to 93 days after planting. Lower soil temperatures, reduced

FIGURE 4. EFFECT OF INOCULANT PLACEMENT ON DISTRIBUTION OF NODULES ON COWPEA ROOTS AFTER 93 DAYS.

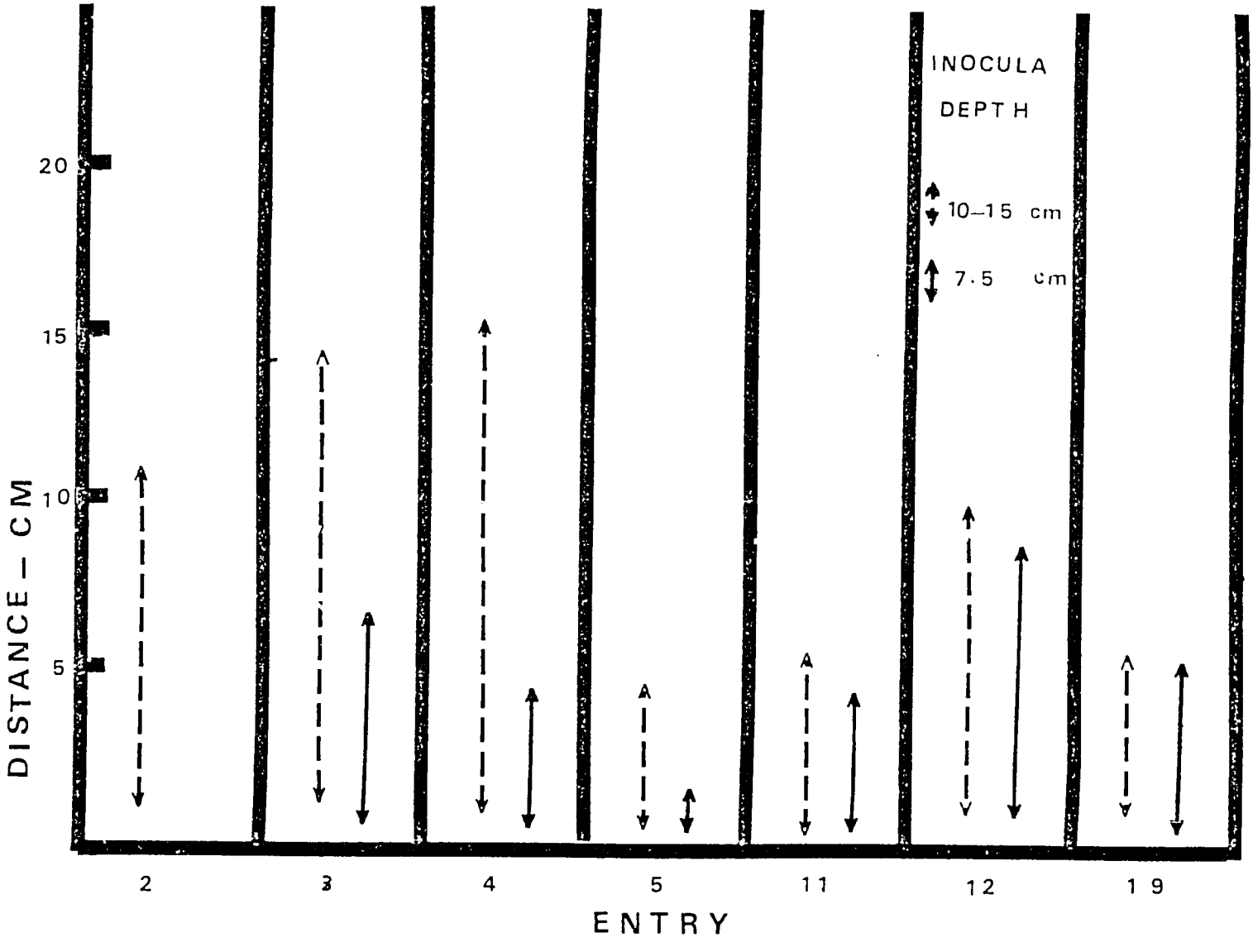
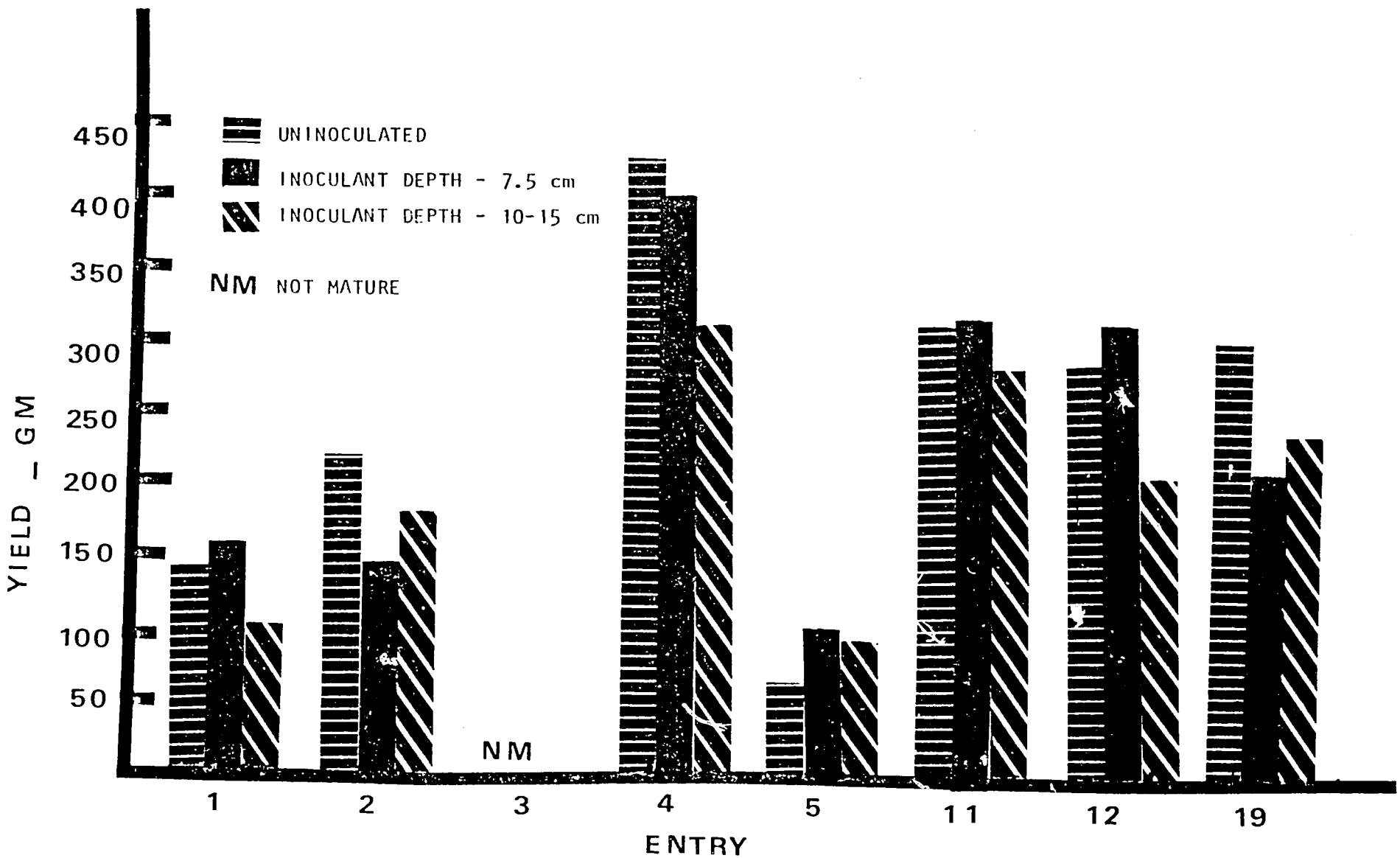


Table 18. Ethylene Production of Cowpeas Inoculated at Two Depths with Granular Inocula (μmoles plant^{-1} hr^{-1}).

Entry	39 Days			53 Days			93 Days		
	Uninoculated	Inocula Depth		Uninoculated	Inocula Depth		Uninoculated	Inocula Depth	
		7.5cm	10-15cm		7.5cm	10-15cm		7.5cm	10-15cm
	μmoles	μmoles	μmoles	μmoles	μmoles	μmoles	μmoles	μmoles	μmoles
1	---	---	28.43	---	---	113.02	---	---	---
2	---	53.71	33.35	---	---	12.25	---	---	26.04
3	---	26.33	---	---	56.69	---	---	66.13	51.64
4	---	*	9.13	---	26.15	14.13	---	72.31	*
5	---	12.55	91.99	---	---	---	---	*	21.37
11	---	---	10.21	---	31.50	11.62	---	9.50	*
12	---	40.13	17.95	---	64.40	13.84	---	6.77	*
19	---	25.80	13.05	---	11.67	---	---	*	*

* Negligible

FIGURE 5. AVERAGE YIELDS OF COWPEAS INOCULATED AT TWO DEPTHS WITH GRANULAR INOCULANT.



salinity and more available moisture in conjunction with protection offered by granules at 10-15 cm depth may contribute to rhizobial survival under extreme conditions.

While nodulation was influenced by inoculation method and host genotype, there were no significant yield differences attributable to nodulation.

Conclusions:

Alternative inoculation technology in conjunction with modified management practices appears to provide a satisfactory solution to improving BNF in the arid and semi-arid tropics.

Use of a granular-type inoculant may enable rhizobial survival in hot, dry, saline soils. In addition, deep placement of granular-type inoculant may provide for a more favorable microclimate for infection.

Continued selection for cowpea genotypes more adapted to salinity identified host plants more amenable to infection, even though considerable variability existed among them for agronomic performance.

Implications to Developing Countries

The primary constraints to agricultural productivity in most developing countries are disease and insect outbreaks. Once these have been contained and low in-put agriculture is a priority, BNF assumes a major role in the arid and semi-arid tropics. Even though yield response to nodulation may not be evident with the legume crop, recent studies with various farming systems indicate significant yield responses to cereal intercrops, and relay crops following legumes. Therefore, among other

relevant considerations, our studies indicate that the following must not be over-looked.

1. Superior, stress adapted host-plant germplasms which give consistent, stable yields over seasons must be used in evaluating BNF.

2. Variability among both Rhizobia strains and host plants and their interactions to BNF must be identified in stress environments.

3. Results of controlled environment studies may not apply in field situations, thus necessitating verification of results in the field.

4. Constraints to successful BNF in stress environments must be identified, i.e., host plant, Rhizobia strain, inoculant technology, field management.

Training:

Before our project was initiated, there was no formal vehicle for initiation of BNF studies with crops adapted to SAT'S. Since that time, we have trained senior faculty as well as graduate students from various Developing Countries (D.C.). Except for one, these individuals were not directly associated with the project, but took advantage of the technology that was available.

Dr. G. Noor - Mohammadi - Ahwaz University, Iran.

Dr. F. Itulya - Kenya

Mr. Ibrahim Mohamad - Sudan

Mr. Haille Tewolde - Ethiopia

International Involvement:

Currently, we are involved in an on-going Cowpea Title XII CRSP

project in Senegal which was initiated as a direct consequence of being involved in this grant. Although not a primary objective, BNF studies are included in the priorities. Field studies will be initiated at the Louga and Bambay stations to assess symbiotic efficiency of the five major cultivars and identify constraints.

Indirect involvement in Developing Countries has included the sending to Morocco, Egypt, and Yemen salt-tolerant cowpeas for evaluation in their arid regions.

Publications:

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