INFLUENCE OF *AZOSPZRZLLUM* SPP. ON THE YIELD AND NITROGEN NUTRITION OF DIFFERENT GENOTYPES OF MAIZE

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INTRODUCTION

Azospirillum - plant associations has been studied because of their economic and scientific importance (1). These diazotrophic bacteria can increase the radical volume of the inoculated plants, (2) and in consequence their ability to absorb ions (3) under greenhouse and laboratory conditions. There are many reports which show grain yield increases in ***Azospirillum*** inoculated plants in field conditions (1,4).

Occurrence of biological nitrogen fixation (BNF) was reported in maize (5) and some authors suggested the possibility of breeding this crop in relationship with BNF (6).

Although the available data about ***Azospirillum* - *maize*** associations were made considering that ***Azospirillum*** inoculations could increase corn yield and supply of N. It would be necessary to study the response mechanisms of maize plants, growing at conditions as similar as possible to the field, to ***Asozpirillum*** inoculation. It was thought that those responses could be explained through two mechanisms: BNF and increase the uptake of available N from the soil.

This paper shows ***Azospirillum*** inoculation effects on several Argentinean and Brazilian maize genotypes grown in field plots and pots with semi-controlled conditions. Changes of biomass production, grain yield, N accumulation were observed between wild strains and nitrate-reductase negative (NR-) phenotypes of ***Azospirillum* ; *these*** were included to study the importance of bacterial nitrate-reductase activity on N accumulation of inoculated maize plants. In order to study the mechanism of BNF in ***Azospirillum*** - maize associations the ^{15}N isotope dilution technique was used.

Different strategies to transfer these ***Azospirillum*** - maize associations to the farmers as well as impacts on environmental resources are discussed.

MATERIALS AND METHODS

Site and General Experimental Characteristics.

Two field and one pot experiments were conducted during 1989- 1990 and 1990- 199 1 at the experimental station of the Department of Plant Production, University of Buenos Aires, Argentina. The characteristics of the soils and weather are in Table 1 and 2 respectively.

The experiments consisted of three and four randomized complete blocks with maize genotypes and inoculations treatments which are described below.

Seventeen months before planting, the soil of the pots was enriched with Amonium Sulfate with 2 1% atoms ^{15}N in excess and the initial soil ^{15}N enrichment was 0.15% atoms ^{15}N in excess with respect to % total N of the soil. (Boddey 1987).

Every pot was placed in a plastic bag to avoid the roots growing into the surrounding soil. This system was put inside the ground to minimize the temperature effects on roots. Each pot had two plants of maize growing during whole period of cropping and *Panicum maximun cv. KK 16* which was placed inside every pot and used as non-N2-fixing control as described by Miranda et *al.* (1990). Its growth was restricted to a circle of 10 cm diameter inside every pot.

TABLE 1: SOIL CHARACTERISTICS OF THE EXPERIMENTS

Classification		Previous crop	NO_3	P	C/N
			(ug/g)		
Field Experiments (1)					
1989-1990	Vertic Arguidol	sunflower	30	25	9.7
1990-1991	Vertic Arguidol	maize	50	23	9.6
Pot Experiments (2)					
1990-1991	Humic Hapludol	sunflower	60	17	9.6

(1): Analysis of *O-20* cm horizon.
(2): From upper 20 cm layer of a plot, sited at General Viamonte, 365 km from Buenos Aires city.

The soil was enriched with Amonium Sulfate with 2 1% atoms ^{15}N in excess, seventeen months before planting. The initial soil ^{15}N enrichment was 0.15% atoms ^{15}N in excess with respect to % total N of the soil (Boddey 1987).

TABLE 2: ORIGIN AND CHARACTERISTIC OF THE *AZOSPIRILLUM* STRAINS USED (1).

STRAIN	CHARACTERISTICS	ORIGIN
7M	***Azospirillum brasilense*** resistant to160 $ug.ml^{-1}$ of streptomycin.	Chloramine-T treated (15 min) maize roots grown in a field in Pergamino, Buenos Aires Province.
10M	***Azospirillum brasilense*** resistant to 160 $ug.ml^{-1}$ of streptomycin.	ditto
40 M (*)	***Azospirillum brasilense*** resistant to 100 $ug.ml^{-1}$ of streptomycin.	Chloramine-T treated (15 min) maize roots grown in a field in Trenque Lauquen, Buenos Aires Province.
42 M (*)	***Azospirillum brasilense*** resistant to 100 $ug.ml^{-1}$ of streptomycin.	ditto
BSL 505 (*)	***Azospirillum lipoferum*** resistant to 100 $ug.ml^{-1}$ of streptomycin.	Washed maize roots grown in Km 49, Rio de Janeiro.
242 (*)	***Azospirillum lipoferum*** resistant to 100 $ug.ml^{-1}$ of streptomycin.	***Sterilized maize*** roots grown in Km 47, Rio de Janeiro
S 82	***Azospirillum lipoferum*** resistant to 100 ug.ml -1 of streptomycin.	Sterilized sorghum roots in Km 47, Rio de Janeiro

(1): More details of the strains in Garcia de Salamone (1993).

(*): Strains with respective negative nitrate-reductase mutants obtained as described Ferreira ***et al.*** (1987).
All the strains were included in the MIXTURE of strains used in the 1989-1990 field experiment. Only the strains with (*) and their nitrate reductase negative (NR-) mutants were included the MIXTURE and MIXTURE NR- of the 1990-1991 field and pot experiments.

Genotypes

Several Argentinean and Brazilian genotypes were included in these experiments. Fifteen and seven maize genotypes were included in the first and second field experiments, respectively. Two genotypes were included in the pot experiment in order to study the occurrence of BNF. They have different breeding histories which can have important effect on BNF occurrence.

Inoculation

Table 2 described ***Azospirillum*** strains included in this work. All of them had spontaneous resistence to 100 ug.ml-1 streptomycin and they were selected in a previous work (Garcia de Salamone 1993). The three experiments included a mixture treatment with all of the individual strains. In pot experiments negative nitrate reductase mutants (NR-1) were included for three inoculation treatments: *A. brasilense 42M* , *A.lipoferum 242* and the mixture. These mutants were obtained previously (Garcia de Salamone 1993). There are two kinds of controls: one with 100 kg $N.ha^{-1}$ added as urea in two equal doses, (at planting and 45 days after) and the other without N fertilizer. The last one was not included in the field experiments. The controls included heat killed bacteria. The inoculants were prepared using 48 h old cultures of every strain in liquid NFb medium containing 1 $g.l^{-1}$ NH_4Cl and 100 $ug.ml^{-1}$ streptomycin sulphate which were put together and mixed with neutralized sterilized peat in a 1: 1 ratio. One gram of the respective inoculant was applied to each seed hole resulting in over 10^8 $ufc.seed^{-1}$.

Determinations

Evaluation of the establishment of the inoculated bacteria was made 15 days after planting by Most Probable Number (MPN) according to Baldani et *al* (1986). The inoculated strains were identified by the transference of the three vials containing the highest dilution showing *Azospirillum growth* of each sample, into NFb medium containing 100 $ug.ml^{-1}$ streptomycin sulphate. Control vials were evaluated in the same way.

At maize ripening, plants of both crops were harvested. Top and grain maize plants were, separated for the three experiments. They were dried at 60ºC to constant weight and % N content of each sample was determined according to Bremmer and Mulvaney (1982).

The N isotope ratio of both parts of maize plants and *Panicum maximum cv. KK 16* for the pot experiment was determined in aliquots evaporated to dryness in glass vials using a VG 900 mass spectrometer (VG Isogas, Middlewich, Cheshire, Englandm); (Urquiaga et *al.* 1992). To calculate the percentage of plant N derived from BNF (%NF) the McAuliffe (1958) equation, cited by Chalk (1985); Urquiaga et *al.* 1992) was used.

All data were analyzed statistically by factorial analysis with MSTAT program.

RESULTS

Tables 4 and 5 describe the results obtained from both field experiments. The other tables and figures describe the results obtained from pot experiment which had soil enriched with ^{15}N.

TABLE 3: Most probable number (mpn) of ***azospirillum*** spp. and recovery of inoculated strains, from surface-sterilized roots of four maize genotypes inoculated with streptomycin-resistant ***azospirillum strains*** and nitrate reductase negative (nr-) mutants

	GENOTYPES			
	Dekalb 4D-70		CMS 22	
	(1)	(2)		
	MPN 10^3	**REC**	**MPN** 10^3	**REC**
CONTROL	1.56	0	1.68	0
A.brasilense				
42 M	1.91	3 ab	1.54	3 c
42 M NR-	7.36	0	6.64	3 b
A.lipoferum				
242	1.12	3 b	8.04	3 ab
242 NR-	5.10	3 a	-------N.D.------	
MIXTURE	3.23	3 ab	21.2	3a
MIXTURE NR-	----------N.D.------------		1.88	3 c
A.brasilense				
40 M	1.94	2 ab	21.4	0
A.lipoferum				
BSL 505	4.85	2 a	12.9	2 ab
+N Control 100 kg $N.ha^{-1}$	0.12	0	--------N.D.--------	

MIXTURE: of strains 40M, 42M, 242 y BSL 505. (See Table 2). MIXTURE NR-: NR- Mutants of MIXTURE

(1): MPN x103: Bacteria per grame of fresh weight of sterilized roots after 20'min in 1% cloramine-T

(2): REC: Recovery : The three vials of the highest ***Azospirillum*** - positive dilutions in MPN counts were replicated into NFb medium containing 100 **ug.ml**$^{-1}$ streptomycin. The controls were evaluated with same method.

N.D.: not determinated.

TABLE 4. Nitrate reductase activity (ma) in ear leaves at silking stage, total N accumulation and grain yield of maize genotypes in two growing seasons in the control plots.

	NRA (1) (ug.NO2.h-1.g-1 DM)		TOTAL N (2) ACCUMULATION (kg.ha- 1)		GRAINYIELD (2) (kg.ha-1)	
	Year I	Year II	Year I	Year II	Year I	Year II
ARGENTINEAN GENOTYPES						
CARGILL R-157	359	-	119	-	5495	-
TRIHYBRID ACA	379	-	101	-	5182	-
DEKALB 4F-37	253	235	134	96	7344	4647
DEKALB 2F-11	178	234	86	106	5643	3154
DEKALB 3F-24	209	-	104	-	6453	-
DEKALB XL 670	187	-	81	-	2843	-
DEKALB 4D-70	317	305	53	132	4664	3775
MORGAN 251	284	248	100	59	7135	3484
MORGAN 318	328	314	68	98	3745	6496
MORGAN 315	384	342	124	79	6921	4421
MORGAN 400	317	-	110	-	5547	-
MORGAN 505	206	-	70	-	4276	-
BRAZILIAN GENOTYPES						
C.M.S. 22	336	326	92	102	2934	3307
C.M.S. 29	331		92	-	1722	-
BR 201	311		123	-	2199	-
M.S.D. (p:0.05)	107	110	16	26	580	1015
c.v (%)	30	35	30	21	21	23

(1): means of 7 plants per plot. (2): mean of 3 plot. C.V.: Coefficient of variation.

TABLE 5. Effect of maize genotypes on percent increases or decreases of nitrate reductase activity (NRA) in ear leaves at silking stage, total n accumulation and grain yields, due to inoculation with a mixture of *azospirillum* spp. strains (1).

	NRA (1) (ug.NO2.h- 1 .g- 1 DM)		TOTAL N (2) ACCUMULATION (kg.ha- 1)		GRAIN YIELD (2) (kg.ha- 1)	
	Year1	Year II	Year I	Year II	Year I	Year II
ARGENTINEAN GENOTYPES						
CARGILL R- 157	-20	-	+31*		-7	
TRIHYBRID ACA	-18		+36*		+20	
DEKALB 4F-37	+19		-23”	-42*	-32*	-39*
DEKALB 2F- 11	+176	+38	+11	+5	+5	+12
DEKALB 3F-24	+11		+47*	-	+17*	
DEKALB XL 670	+46	-	+15		-*	
DEKALB 4D-70	+86*	+52*	+82*	+32	+45*	+24
MORGAN 25 1	+31	+23	-7	+28	-9	+42*
MORGAN 3 18	-9	-6	+39*	+28*	+40*	+27*
MORGAN 3 15	-55*	-32	+4	-21	-7	-21
MORGAN 400	-10	-	+13		+7	
MORGAN 505	+61*		+4		+10	
BRAZILIAN GENOTYPES						
C.M.S. 22	-37*	-36*	+38*	+2	+37*	+11
C.M.S. 29	-26	-	-4		+94*	
BR 201	-46*	-	+13*		+21*	

(1): See Table 2 for more details of the mixture of *Azospirillum strains.*
(*): Difference between inoculated and control significant at p:0.05. Test Tukey.

TABLE 6. **Grain** yield and total n accumulation of two maize genotypes inoculated with different streptomycin-resistant ***azospirillum strains*** and nitrate reductase negative (nr-) mutants.

	GRAINYIELD		N TOTAL ACCUMULATION	
GENOTYPES	**D4D-70**	CMS22	**D4D-70**	CMS22
	(g/plant-1) (*)			
INOCULANTS (1)				
1. N CONTROL	31.9 c(3)	62.7 abc	0.98 b	2.52 ab
A.brasilense				
2.42 M	87.9 ab	66.7 abc	2.45 a	3.15 ab
3.42 M **NR-**	53.7 abc	99.9 ab	1.51 ab	2.41 ab
A.lipoferum				
4.242	31.6 c	40.3 cd	1.01 b	2.06 bc
5.242 **NR-**	43.3 bc	9.3 e	1.04 b	0.61 c
6. MIXTURE	90.0 ab	69.0 abc	1.75 ab	3.20 ab
7. **MIXTURE NR-**	98.8 a	53.1 **bcd**	1.61 ab	3.19 ab
8. +N CONTROL				
100kgN.ha^{-1}	95.9 a	108.2 a	2.29 a	3.90 a
C.V. (%) (p: 0.05) **-** (3)	64	65	61	**64**

(*): Means of four replications, D4-70: Dekalb 4D-70. (1): See Table 2 for more details of the ***Azospirillum strains.***
(2): Means in the same column followed by the same letter are not significantly different at p:0.05 (Duncan's multiple range test). Data transformed as log10(x) for analysis of variance and comparisons of means.
(3): Coefficient of variation of untransformed data.

TABLE 7. 15N enrichment of two maize genotypes grown in 1 5N-labelled soil and inoculated with different streptomycin-resistant *azospirillum* strains and the non-fixing control plant *panicum maximum cv. kk* 16.

	DEKALB 4D-70		C.M.S. 22	
	whole plant (*)	Panicum maximum	whole plant (*)	Panicum maximum
	weighted mean	cv. kk 16 (@)	weighted mean	cv. kk 16
	---------Atoms % ^{15}N EXCESS ----------			
INOCULANTS (1)				
1. - N CONTROL	0.13679 a (2)	0.108	0.05901 b	0.096
A.brasilense				
2. 42 M	0.05122 c	0.121	0.05111 b	0.095
3. 42 M NR-	0.11554 ab	0.125	0.09154 b	0.101
A.lipoferum				
4. 242	0.11689 ab	0.125	0.05349 b	0.078
5. 242 NR-	0.07515 bc	0.114	0.17013 a	0.122
6. MIXTURE	0.07926 bc	0.109	0.07457 b	0.100
7. MIXTURE NR-	0.08732 abc	0.120	0.06488 b	0.091
p: (%)	5	ns	1	ns
C.V.(%) (3)	34	19.5	49	27

(*) Weighted mean of 15N enrichment in leaves-stems and grain. Means of four replications.
(@) As described Miranda et *al.* (1990).
(1): See Table 2 for more details of the *Azospirillum* strains.
(2): Means in the same column followed by the same letter are not significantly different at p:0.05 (Duncan's multiple range test).
(3): Coefficient of variation.

CONCLUSIONS

- Inoculation response of maize to inoculation with *Azospirillum* is dependent on both the plant genotype and bacterial strain.

- The responses to *Azospirillum* inoculation were largely consistent in field and pot experiments.

- The increase in grain yield of the genotype DEKALB 4D-70 promoted by a mixture of *Azospirillum* strains was aproximately equivalent to the application of 100 kg N / ha. This suggests that if suitable maize genotypes are selected N fertilizer can be at least partially replaced by *Azospirillum* inoculation.

- The Figure 1 illustrates the fact those maize plants which accumulated most N showed the lowest 15N enrichment. This is exactly what would be expected for plants which obtained significant inputs from BNF as have suggested by Boddey (1987) and Chalk (1991).

• The quantities of N derived from BNF by the maize genotypes were estimated using the 15N enrichment of the uninoculated DEKALB 4D-70 maize as non-N- fixing control. The data of this study indicate that the large reponses of total N accumulation were in this case due to BNF contributions **(Figures 2 & 3)**

LEGENDS FOR FIGURES

FIGURE 1: REGRESION OF TOTAL N ACCUMULATION (TNA) WITH WEIGHTED MEAN 15N ENRICHMENT (EN) OF TWO GENOTYPES OF MAIZE INOCULATED WITH VARIOUS STREPTOMYCIN-RESISTANT STRAINS OF *AZOSPIRILLUM* SPP. REGRESSION FOR ALL TREATMENTS WITH TWO MAIZE GENOTYPES: TNA= (-19.7 x EN) + 3.74 r = - 0.653*** , n=56.

FIGURE 2: TOTAL N ACCUMULATION (TNA) AND ESTIMATES OF N DERVED FROM BNF (Ndfa) BY MAIZE GENOTYPE Dekalb 4D-70 INOCULATED WITH VARIOUS STREPTOMYCIN-RESISTANT STRAINS OF *AZOSPIRILLUM* SPP. ESTIMATES CALCULATED USING 15N ENRICHMENTS OF INOCULATED PLANTS (ENI) AND OF UNINOCULATED CONTROL OF Dekalb 4D-70 GENOTYPE (ENUD) IN THE EQUATION: Ndfa = TNA x (1 - EN1 / ENUD).

FIGURE 3: TOTAL N ACCUMULATION (TNA) AND ESTIMATES OF N DERVED FROM BNF (Ndfa)BY MAIZE GENOTYPE C.M.S. 22 INOCULATED WITH VARIOUS STREPTOMYCIN-RESISTANT STRAINS OF *AZOSPIRILLUM* SPP. ESTIMATES CALCULATED USING 15N ENRICHMENTS OF INOCULATED PLANTS (ENI) AND OF UNINOCULATED CONTROL OF Dekalb 4D-70 GENOTYPE (ENUD) IN THE EQUATION: Ndfa = TNA x (1 - EN1 / ENUD).

{--- Figures at end of paper ---}

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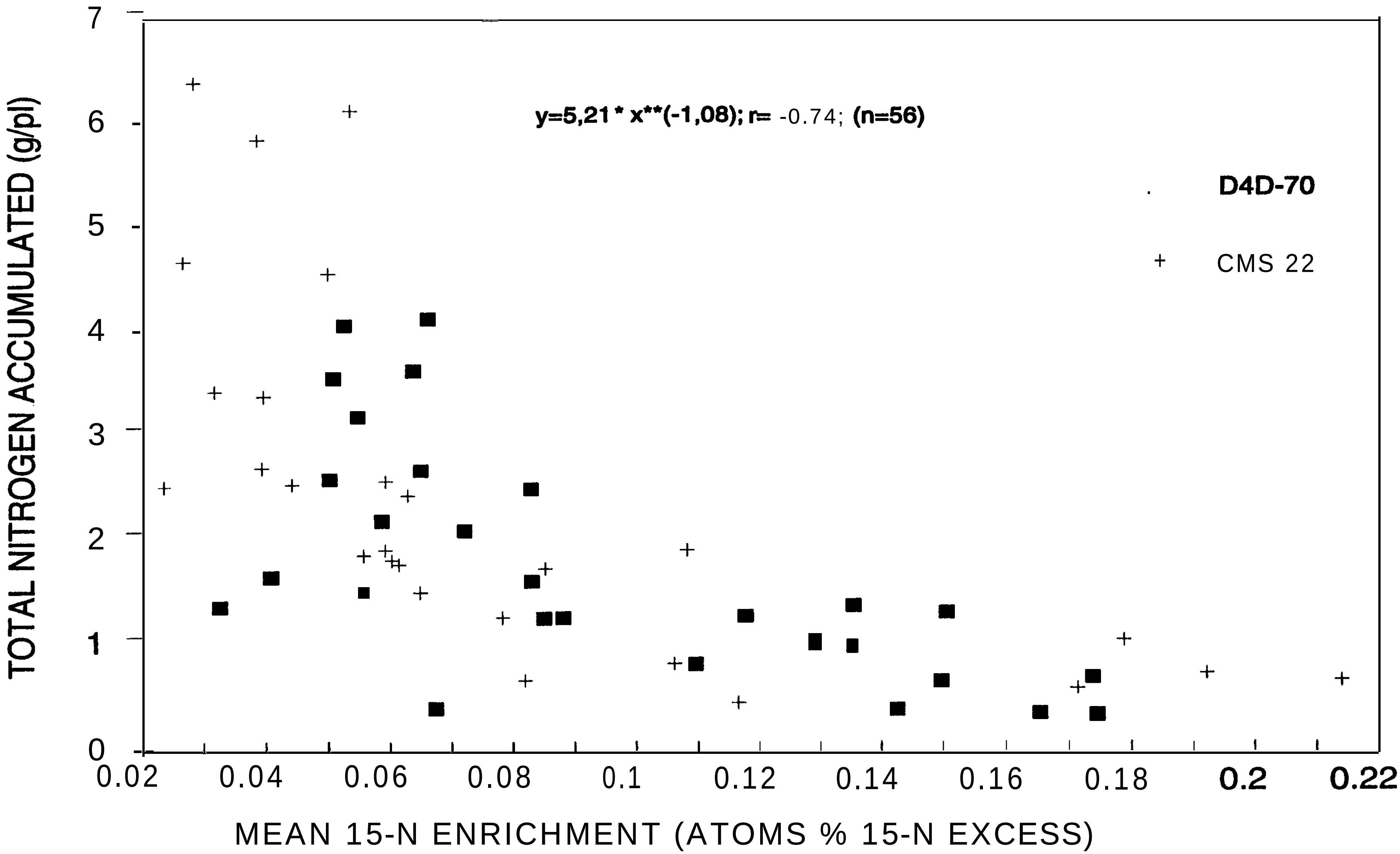
FIGURE 1



y=5,21 * x**(-1,08); r= -0.74; (n=56)
D4D-70
CMS 22
TOTAL NITROGEN ACCUMULATED (g/pl)
MEAN 15-N ENRICHMENT (ATOMS % 15-N EXCESS)
0
1
2
3
4
5
6
7
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0.18
0.2
0.22

FIGURE 2

DEKALB 4D-70

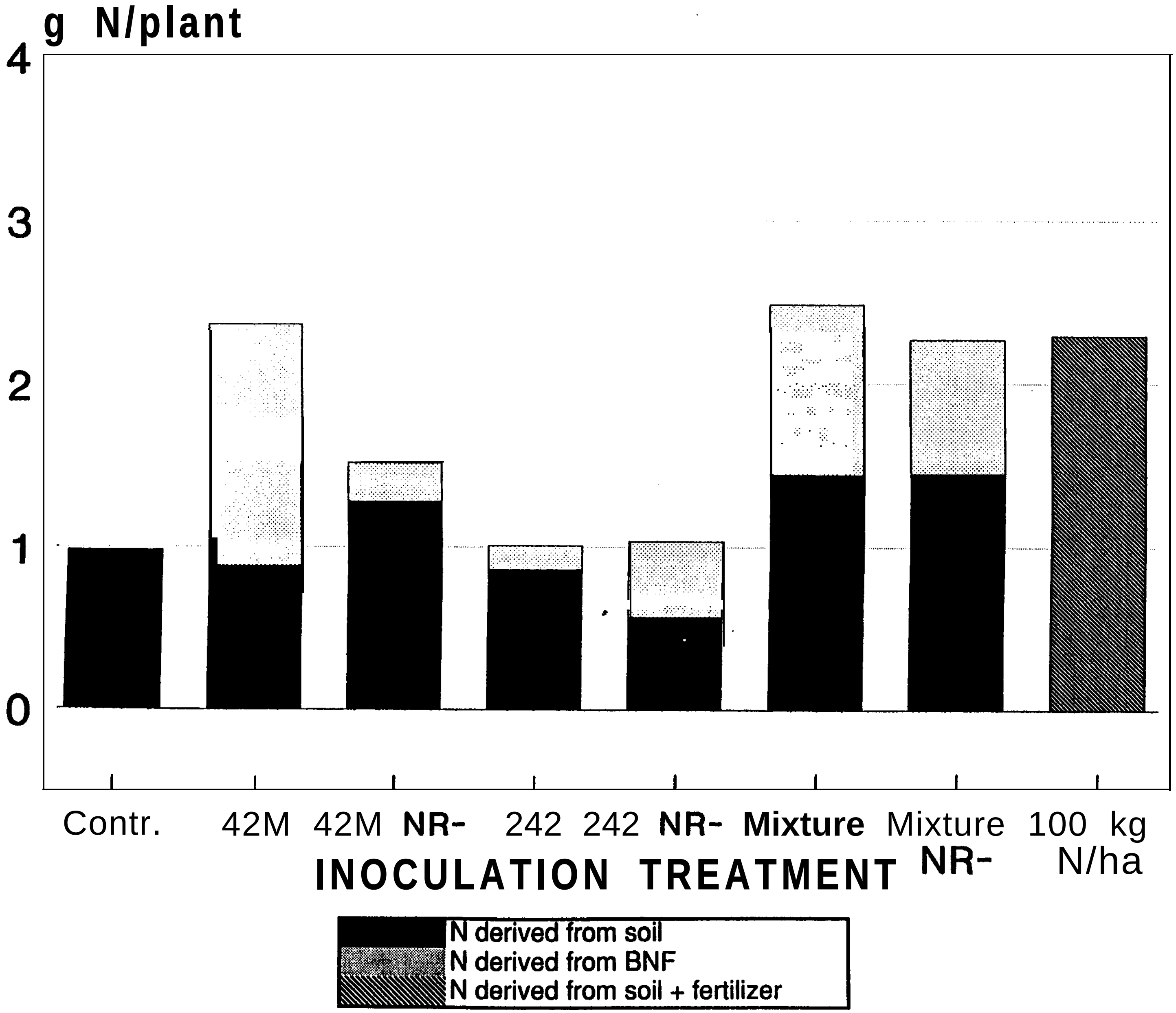


FIGURE 3

CMS 22