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ANALYSIS OF HOST GENOTYPE X RHIZOBIAL STRAIN INTERACTION IN N_2 FIXATION

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KEY WORDS

Vigna radiata, mungbean, *Rhizobium*, average fixing ability, specific fixing ability, breeding for N_2 fixation, disease resistance, N_2 fixation index.

SUMMARY

A method of analyzing host genotype × rhizobial strain interaction for N_2 fixation potential, based on the principle of structural relationship analysis, is proposed. When this interaction is significant, selecting genotypes for high N_2 fixation potential is incorrect, because the N_2 fixation efficiency is dependent on the rhizobial strain used. Under such circumstances, grouping genotypes based on average fixing ability (AFA), a linear response to the effectiveness of rhizobial strain, and specific fixing ability (SFA), deviation from the linear response in terms of the magnitude of the error variance, is useful before initiating a breeding program for enhanced N_2 fixation. Host genotype × strain interaction effect is partitioned into two components, and two parameters are derived which estimate AFA and SFA. N_2 fixation data from 3 mungbean genotypes and 6 rhizobial strains are used to illustrate this method.

INTRODUCTION

Nitrogen fixation efficiency in the *Rhizobium*-legume symbiosis involves a sequence of interactions between the 2 symbiotic partners in which each partner influences the gene expression of the other. A significant amount of host genotype × rhizobial strain interaction has been found for the efficiency of N_2 fixation in broad bean (MYTTON et al., 1977), field pea (HOLL, 1975), soybean (CALDWELL & VEST, 1970), common bean (WESTERMANN & KOLAR, 1978), cowpea (MINCHIN et al., 1978), and mungbean (FERNANDEZ & MILLER, 1985). Ambiguous results were obtained in inheritance studies of differential N_2 fixation in cowpeas inoculated with a mixed strain inoculum (MILLER et al., 1986). Significantly different narrow sense heritability estimates for the same trait with different rhizobial strains have also been reported in mungbean (FERNANDEZ & MILLER, 1985). Thus, use of single strain inoculum as opposed to mixed strain has been emphasized in inheritance studies (MILLER et al., 1986; LIE & MULDER, 1979).

Superior rhizobial strains and the presence of significant host genotype × rhizobial strain interaction could be effectively identified from linear statistical models involving

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a host genotype × strain interaction term. However, in the presence of a significant genotype × strain interaction, grouping genotypes based on N_2 fixation potential is incorrect, because the N_2 fixation potential of a genotype is dependent on the rhizobial strain used. From a breeding point of view, evaluating host genotypes for average fixing ability (AFA), i.e. linear response of N_2 fixation to the effectiveness of the rhizobial strain, and specific fixing ability (SFA), i.e. deviation of N_2 fixation response from AFA, allows the identification of host genotypes with high N_2 fixation potential following inoculation with differrent rhizobial inoculum. This situation is analogous to characterization of yield stability of cultivars over varying environments (TAI, 1971). Therefore, the objective of this paper is to propose a method for evaluating host genotypes for AFA and SFA when significant host genotype × rhizobial strain interaction exists.

STATISTICAL ANALYSIS

Analysis of host genotype × rhizobial strain interaction. Assuming fixed genotypic effects and random rhizobial strain effects the general linear model for m genotypes inoculated with n rhizobial strains is:

$$Y_{ijk} = \mu + G_i + S_j + (GS)_{ij} + R_{jk} + \varepsilon_{ijk} \quad (1)$$

$$(GS)_{ij} = \alpha_i S_j + \delta_{ij} \quad (2)$$

where Y_{ijk} = the observation on the ith genotype grown in k^{th} replicate of strain j,
μ = mean across all genotypes and all strains,
G_i = the effect of ith genotype, i = 1...m,
S_j = the effect of jth strain, j = 1...n,
$(GS)_{ij}$ = the interaction between i^{th} host genotype and j^{th} strain,
α_i = the coefficient of linear response of ith genotype on the strain effects. (a measure of AFA).
δ_{ij} = the deviation in N_2 fixation from linear response of the ith genotype on the jth strain, (a measure of SFA).
R_{jk} = the effect of kth replicate of the jth strain, k = 1...p.
ε_{ijk} = random error.

The effectiveness of strain j is measured as the strain effect S_j, which is the mean of all genotypes with strain j minus the grand mean. TAI (1971) used the same approach to estimate stability parameters of potato genotypes when genotype (G) × environment (E) interaction was significant. He partitioned the interaction component (GE) into the linear response to environmental effects and the deviation from the linear response. Based on this approach, using the principles of structural relationship analysis (KENDALL & STUART, 1961), when $(GS)_{ij}$ is significant, it can be partitioned into two components which describe the nature of a cultivar's symbiotic fixing ability. Two parameters, α_i and γ_i are thus derived. The parameter α_i measures the linear response to the strain effect S_j, and γ_i the deviation from the linear response in terms of the magnitude of the error variance. The analysis of variance for the above model is shown in Table 1.

The parameters α and γ for N_2 fixing ability can be estimated as follows.

$$\hat{\alpha}_i = \frac{MSS}{MSS-MSR}(b_i - 1)$$

$$\lambda_i = \left(\frac{m}{m-1}\right)\left(\frac{n-2}{n-1}\right)\frac{s_d^2}{MSE/p} - \hat{\alpha}\left(\frac{b_i - 1}{m-1}\right)\frac{MSR}{MSE}$$

where MSS = mean square for strains, MSR = mean square for replicates within strains, MSE = mean square error (Table 1), b_i is the regression coefficient and s_d^2 the deviation from the regression mean square in the following linear regression model.

$Y_{ij} = \mu_i + b_i S_j + \varepsilon_{ij}$

where

Y_{ij} = the mean phenotypic value of the ith genotype inoculated with j^{th} strain

μ_i = mean of ith genotype over all strains

b_i = the regression coefficient

S_j = strain effect

ε_{ij} = the deviation from regression of the mean phenotypic value i^{th} genotype on j^{th} strain effect

The mean squares, MSS and MSR, are equivalent to MSL and MSB, respectively, in TAI's (1971) stability analysis.

Thus, the magnitudes of AFA and SFA are estimated by $\hat{\alpha}_i$ and λ_i, respectively. A host genotype with a value of $(\alpha,\lambda) = (0,1)$ represents a perfect average fixing genotype, where the N_2 fixation potential is linear to the effectiveness of the strain because $b = 1$. A host genotype of $(\alpha,\lambda) = (-1,1)$ may be referred to as a rhizobial strain independent genotype and probably does not exist. However, non-nodulating genotypes might be classified as one extreme of strain independent genotypes.

When $\hat{\alpha}_i$ is significantly greater than 0, then i^{th} genotype responds greatly to the change in the effectiveness of rhizobial strain. Such a genotype could be identified as a high N_2 fixing genotype. When $\hat{\alpha}_i$ is significantly lower than 0, then i^{th} genotype responds less to the change in the effectiveness of the strain, and could be identified as low N_2 fixing. The value λ_i measures the deviation from the linear response in terms of the magnitude of the error variance; therefore, when λ_i is greater than 1 the fixing ability is unpredictable and the genotype's fixing ability is probably strain specific.

Critical areas when testing $\alpha = 0$ for different λ_i values can be computed by TAI's

Table 1. Analysis of variance with fixed genotypic effects and random replicate and rhizobial strain effects.

Source of variation		Degrees of freedom	Mean squares	Expected mean squares
Strain index	S_j	n – 1	MSS	$\sigma_\varepsilon^2 + m\sigma_{R_k}^2 + mp\sigma_s^2$
Replicates within strains	$R_k(S)$	n(p – 1)	MSR	$\sigma_\varepsilon^2 + m\sigma_R^2$
Genotypes	G_i	m – 1	MSG	$\sigma_\varepsilon^2 + p\sigma_{sg}^2 + np\sum_{i=1}^{m} g_i^2/(m-1)$
Strain × Genotype	$(GS)_{ij}$	(n – 1)(m – 1)	MSSG	$\sigma_\varepsilon^2 + p\sigma_{sg}^2$
Linear response	α_i	(m – 1)		
Deviation from linear response	δ_{ij}	(m – 1)(n – 2)		
Error	ε_{ijk}	n(m–1)(p-1)	MSE	σ_ε^2

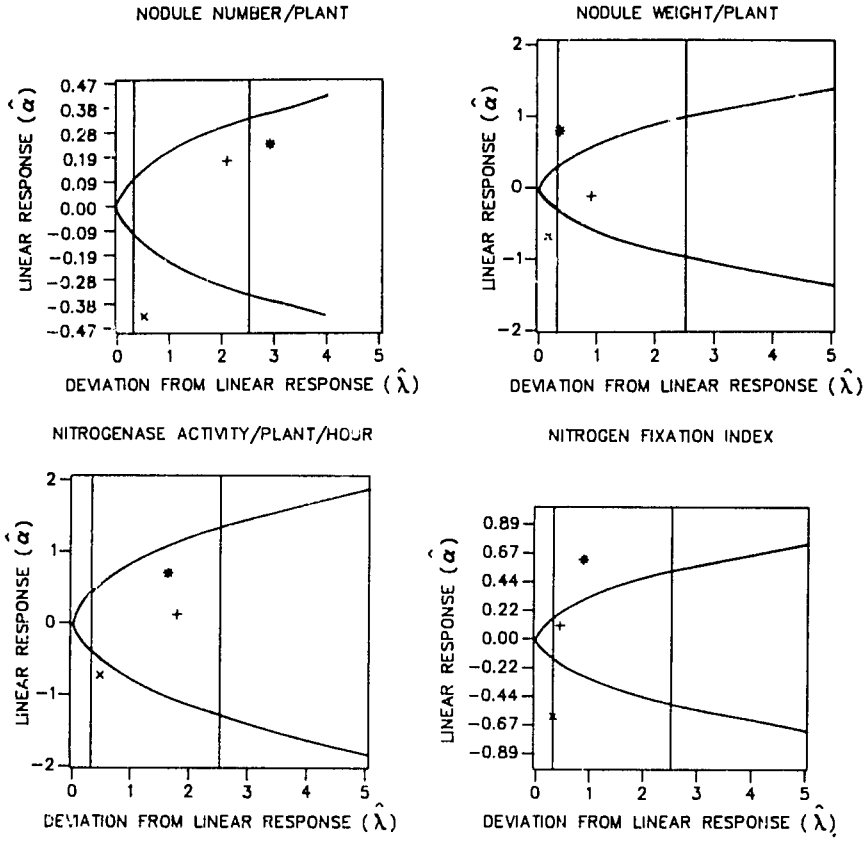

Fig. 1. Estimates of linear response ($\hat{\alpha}$) and deviation from linear response ($\hat{\lambda}$) parameters for 4 N_2 fixation related variables in 3 mungbean genotypes. Genotypes legend: x = TX39, + = TX33, * = TX72.

(1971) method. The regions corresponding to non rejectable values (α,λ) are identified in a plot with axes representing linear response (α) and deviation from linear response (λ) (Figure 1). The hyperbola in Figure 1 delineates non-rejectable $\hat{\alpha}_i$'s (P = 0.95) if the true α is zero. The two vertical lines are the critical values when testing $\lambda = 1$.

Genotypes which fall into the region enclosed by the parabola and the vertical lines do not differ significantly from average fixing ability ($\alpha = 0$) and are predictable ($\lambda = 1$), while other regions encompass genotypes whose fixing ability is significantly above or below average fixing ability and are unpredictable.

MATERIALS AND METHODS

A field study was conducted to measure N_2 fixation potential of mungbean genotypes at College Station, Texas on a vertic albaqualf fine, montmorillonitic, thermal soil at pH 6.7. Analysis of soil samples by the Texas A&M Soil Testing Laboratory indicated that extractable levels of N, P and K were 6, 10, and 100 ppm, respectively, in the 15 to 30 cm depth.

The N_2 fixing potential of 3 mungbean genotypes, TX33, TX39, and TX72, was

measured under 7 inoculation treatments: control (no inoculation), 5 single strains (176A22, 150B1, 32H1, 31Z3 and 41Z2), and a commercial mixed strain inoculum (ELG63). Each inoculation trial was comprised of a randomized complete block design with 3 replicates and separated to prevent contamination.

Three seeds per hill were planted at 15 cm spacing on a 1 m row, 1 m apart. Seeds were inoculated at planting with peat-based inoculum at the rate of 1 g/hole. Two weeks after germination, plants were thinned to 1 plant/hole. The plants were irrigated as needed.

At the time of flowering (7 weeks after planting), two adjacent plants from each plot were harvested and the following N_2 fixation variables measured on each plant: nitrogenase activity by the C_2H_2 C_2H_4 assay in µmoles C_2H_4/hour as described by ZARY et al. (1978), number of nodules, nodule weight, top dry weight, root weight, and percentage N of the plant tops based on Kjeldahl analysis. The N_2 fixation index was estimated by a linear combination of the 5 fixation variables using principal component analysis (JOHNSON & WICHERN, 1982). Principal component analysis consists of transformation of the original (correlated) variables into a new set of uncorrelated variables or principal components.

A combined analysis of variance was carried out using SAS (1982), with fixed genotypic effects and random strains and replicates. Average fixing ability (α) and specific fixing ability (λ) parameters were estimated for each genotype separately.

RESULTS AND DISCUSSION

Analysis of variance results for the N_2 fixation variables are shown in Table 2. Effects of genotype were significant for all variables, while the strain effects and strain × genotype interaction were significant for nodule number, nodule weight, nitrogenase activity and the N_2 fixation index. The % N in plant tops did not vary significantly among genotypes; therefore, it was not included in the analysis.

Sums of squares due to host genotype × rhizobial strain interaction were partitioned into linear response to strain effect and the deviation from the linear response to the variables nodule number, nodule weight, nitrogenase activity and the N_2 fixation index when the interaction was significant. The results indicated that the linear response to strain effect was highly significant for the above 4 variables (Table 2).

Mungbean genotypes TX33 and TX72 produced significantly larger shoot and root sytems than did genotype TX39 (Table 3). Three single rhizobial strains, 32H1, 31Z3 and 41Z2, appeared to be more effective in fixing N_2 than the other two single strains and the mixed inoculum (Table 4). Generally, TX33 and TX72 showed higher N_2 fixation potential than TX39. More and larger nodules expressing higher nitrogenase activity were produced in the genotype TX33 when inoculated with strain 32H1 or 31Z3 than when inoculated with the other strains. In TX72, strains 31Z3 and 41Z2 were equally effective in producing more and larger nodules; however, nitrogenase activity was higher with 41Z2 than with 31Z3. This suggested that to fully exploit biological N_2 fixation, mungbean genotype TX33 should be inoculated with either 32H1 or 31Z3, while TX72 should be inoculated with 41Z2.

The N_2 fixation index, the linear combination of 5 N_2 fixation variables, is a good indicator of N_2 fixation potential, because ANOVA results were in close agreement

Table 2. Analysis of variance of N_2 fixation variables.

Source of variation	df	Sums of squares					
		nodule no./plant	nodule weight plant	nitrogenase activity/ plant/h	top dry weight/ plant	root weight/ plant	N_2 fixation index[1]
Strain	6	3151.27***	0.595***	201.07***	87.09	11.12	60.12***
Replicates (strain)	14	521.28**	0.205**	124.70**	262.63	32.93**	15.01*
Genotypes	2	552.72***	0.267***	144.76***	11079.84***	179.99***	77.28***
Strain × Genotypes	12	569.90**	0.372*	176.86**	273.94	17.39	19.20**
Linear response	2	295.10***	0.275***	94.58***	[2]		15.62***
Deviation from response	10	274.80*	0.097	82.27*			3.64
Error	28	319.92	0.386	143.23	398.53	28.86	12.40

[1] N_2 fixation index computed from the linear combination of the 5 N_2 fixation variables by principal component analysis.

***, **, * Significant at 0.1%, 1% and 5% levels, respectively.

[2] Interactions between Strain × Genotype were not statistically significant and thus were not partitioned as linear effects and deviation from linear effects.

with the other N_2 fixation variables (Table 2). Also, more than 60% of the information in all N_2 fixation variables was condensed into a single variable the N_2 fixation index (Table 5). Thus, this index could be used to screen genotypes for N_2 fixation potential. Nitrogenase activity, nodule number and nodule weight contributed more to the N_2 fixation index than did root and shoot weights (Table 5).

Correlation analysis indicated that N_2 fixation variables nodule number, nodule weight and nitrogenase activity, were significantly correlated among themselves (Table 5). Likewise, correlation between root weight and shoot weight (plant growth variables) was also very high. However, correlations between the two groups were not high. Similar results were reported in cowpea (MILLER et al., 1986) and other legumes (KREMER & PETERSON, 1983; HEICHEL et al., 1981).

Estimates of AFA and SFA. The $\hat{\alpha}$ values for genotype TX39 were negative. The were significantly different from 0 for the N_2 fixation index (Table 6). This indicated that the linear response to the strain effect was below average for TX39. However, λ values were not statistically different from 1, indicating that the deviation from the linear

Table 3. Genotypic values for top dry weight and root weight per plant harvested at flowering.

Genotype	Top dry weight (g/plant)	Root weight (g/plant)
TX33	12.9a	5.56a
TX39	3.8b	2.07b
TX72	12.3a	5.74a

Means followed by the same letter within a column do not differ significantly according to the DUNCAN-WALLER test (KRATIO = 100).

Table 4. Means for N_2 fixation variables for plants harvested at flowering.

Genotype	Strain						
	Control	ELG63	176A22	150B1	32H1	41Z2	31Z3
	Nodule number/plant						
TX33	0c	7bc	3bc	5bc	10b	11b	29a
TX39	0c	0c	2c	2c	1c	6b	13a
TX72	0c	5bc	2c	6bc	12b	23a	26a
	Nodule weight (mg)/plant						
TX33	0d	63c	86c	121bc	283a	173b	274a
TX39	0a	4a	49a	24a	29a	83a	69a
TX72	0c	112bc	50bc	175b	543a	408a	543a
	Nitrogenase activity (μmoles C_2H_4 plant/hour)						
TX33	0.02c	2.08b	2.37b	3.64b	7.42a	3.42b	6.37a
TX39	0.05a	0.07a	0.37a	0.28a	0.14a	0.39a	1.23a
TX72	0.03c	0.03c	0.30c	1.57c	5.04b	10.41a	5.89b
	N_2 fixation index[1]						
TX33	0.88d	0.07cd	0.01cd	0.44bc	1.30ab	1.56ab	2.43a
TX39	2.11b	1.79ab	1.54ab	1.69ab	1.73ab	1.21ab	0.83a
TX72	0.89c	0.98c	0.05c	0.15c	1.81b	3.17a	2.89ab

[1] N_2 fixation index computed from the linear combination of the 5 N_2 fixation variables by principal component analysis.
Means followed by the same letter in a row do not differ significantly according to the Duncan-Waller test (KRATIO = 100).

response to the strain effect was not significant. Thus, genotype TX39 was in the low fixing ability region for N_2 fixation variables and N_2 fixation index (Figure 1).

In TX33, $\hat{\alpha}$ and $\hat{\lambda}$ values were not statistically different from 0 and 1, respectively, for all the variables. Thus, this genotype occupied the average fixing ability region for all N_2 fixation variables. This indicated that TX33's fixing ability is linearly related to the effectiveness of the rhizobial strain and less influenced by the specific host genotype × strain combination. When the effectiveness of the rhizobial strain is known, the fixing ability of TX33 can be predicted.

Table 5. Correlation coefficients among N_2 fixation variables (average of 2 plants) and normalized eigenvector for the principal component (N_2 fixation index) (n = 63).

N_2 fixation variables	NN	NW	NA	RW	TW	Prin 1[1]
Nodule number/plant (NN)		0.73**	0.76**	0.32	0.20	0.48
Nodule weight/plant (NW)			0.82**	0.36**	0.24	0.50
Nitrogenase activity/plant/h (NA)			–	0.37*	0.24	0.51
Root weight/plant (RW)					0.80**	0.40
Top dry weight/plant (TW)					–	0.33

[1] First principal component, which accounted for 60% of the variation, and normalized eigenvector for the 1st component.
**, * Significant at 1% and 5% levels, respectively.

Table 6. Means of N_2 fixation variables (x) and estimates α and λ parameters of mungbean genotypes inoculated with 6 rhizobial strains.

Genotype	nodule number (no/plant)			nodule weight (mg/plant)			nitrogenase activity (μmoles C_2H_4/plant/h)			N_2 fixation index[1]		
	x	$\hat{\alpha}$	λ	x	$\hat{\alpha}$	λ	x	$\hat{\alpha}$	λ	x	$\hat{\alpha}$	λ
TX33	10	0.17	2.11	144	0.12	0.90	3.6	0.07	1.80	0.69	0.06	0.48
TX39	4	-0.42*	0.52	37	0.65*	0.15	0.4	0.75*	0.47	1.56	-0.62*	0.33
TX72	9	0.24	2.92**	193	0.76*	0.36	3.5	0.67	1.64	0.87	0.58*	0.92

[1] Linear combination of N_2 fixation variables derived from principal component analysis.
* $\hat{\alpha}$ values significantly different from $\alpha = 0$ at 5% level.
** λ value greater than $F_{0.05}(n-2; n(m-1)(p-1))$, i.e. greater than $F_{0.05}(5,28) \cong 2.61$.

In genotype TX72, $\hat{\alpha}$ values were positive and signficantly greater than 0 for nodule weight and the N_2 fixation index (Table 6). The λ value was significantly different from 1 for nodule number. This indicated that, based on the N_2 fixation index, TX72 had a greater response than the average fixing genotype TX33 to the effectiveness of the rhizobial strain. The deviation from linear response λ was significantly greater than 1 for nodule number only. Thus, TX72 can be identified as a high N_2 fixing cultivar and moderately predictable.

Because only three genotypes were included in this study, the results cannot be directly used in a breeding program. However, the data and results help to illustrate the methodology and usefulness of this analysis.

In conclusion, when the host genotype × rhizobial strain interaction is significant, this method is useful for identifying high, average and low fixing and predictable and unpredictable (strain specific) genotypes, when rhizobial strains with differential effectiveness are used. Furthermore, this method can be also be used in disease resistance studies to estimate the magnitude of horizontal and vertical resistance, when a disease is caused by several races of a pathogen.

REFERENCES

CALDWELL, B. E. & G. VEST, 1970. Effects of *Rhizobium japonicum* strains on soybean yields. Crop Sci. 10: 19–21.

FERNANDEZ, G. C. J. & J. C. MILLER, Jr. 1985. Estimatinon of heritability by parent-offspring regression. Theor. Appl. Gen. 70: 650–654.

HEICHEL, G. H., D. K. BARNES & C. P. VANCE, 1981. Nitrogen fixation of alfalfa in the seedling year. Crop Sci. 21: 330–335.

HOLL, F. B. 1975. Host plant control of the inheritance of dinitrogen fixation in the *Pisum-Rhizobium* symbiosis. Euphytica 24: 767–770.

LIE, T. A., & E. G. MULDER, 1979. Breeding for symbiotic nitrogen fixation. p. 341–348. *In*: J. Sneep and A. J. T. Hendriksen (eds.). Plant Breeding Perspectives. PUDOC, Wageningen.

JOHNSON, R. A. & D. W. WICHERN. 1982. Applied multivariate statistical analysis. Prentice-Hall, Englewood Cliffs, New Jersey.

KENDALL, M. G. & A. STUART. 1961. Advanced theory of statistics. Vol. 2. 3rd ed. Griffin & Co., London.

KREMER, R. J. & H. L. PETERSON. 1983. Field evaluation of selected *Rhizobium* in an improved legume inoculant. Agron. J. 75: 139–143.

MILLER, J. C., JR., K. W. ZARY & G. C. J. FERNANDEZ. 1986. Inheritance of N_2 fixation efficiency in cowpea. Euphytica 35: 551–560.

MINCHIN, F. R., R. J. SUMMERFIELD & A. R. J. EAGLESHAM. 1978. Plant genotype × rhizobial strain interaction in cowpea [*Vigna unguiculata* (L.) Walp]. Trop. Agric. (Trinidad) 55: 107–115.

MYTTON, L. R., M. H. EL-SHERBEENY & D. A. LAWES. 1977. Symbiotic variability in *Vicia faba*. III. Genetic effects of host plants, *Rhizobium* strain and host × strain interaction. Euphytica 26: 785–791.

SAS INSTITUTE. 1982. SAS user's guide: Statistics. SAS Institute, Cary, NC.

TAI, G. C. C. 1971. Genotypic stability analysis and its application to potato regional trials. Crop Sci. 11: 184–190.

WESTERMANN, D. T. & J. J. KOLAR. 1978. Symbiotic N_2 (C_2H_2) fixation by bean. Crop Sci. 18: 986–990.

ZARY, K. W., J. C. MILLER, JR., R. W. WEAVER & L. W. BARNES. 1978. Intraspecific variability for N_2 fixation in southern pea [*Vigna unguiculata* (L.) Walp]. J. Amer. Soc. Hort. Sci. 103: 806–808.

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MEMORANDUM

Date 2 Sep 88

TO: PPC/CDIE/DI, Tina Wilson/Acquisitions - 209 SA-18

FROM: S&T/AGR/RNR, Lloyd R. Frederick

SUBJECT: Reprints of Articles with Partial Support of A.I.D. Contracts/Grants

Attached is one copy of the following article with the supporting contract/grant number:

Fernandez, G.C.J. and J.C. Miller, Jr. 1987
Analysis of host genotype x rhizobial strain interaction in N_2 fixation. Euphytica 36: 903-911

PASA AG/TAB 610-9-76
~~Contract/Grant~~ Number: ~~BST-0610-P-AG-2170-00~~

Project: 931-0610

Attachments: a/s

cc: T.Gill
D. Bathrick

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