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INHERITANCE OF N_2 FIXATION EFFICIENCY IN COWPEA[1]

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SUMMARY

Host plant genetics of N_2 fixation in the cowpea (*Vigna unguiculata* (L.) WALP.) – *Rhizobium* symbiosis was investigated in the field with a mixed strain inoculum and in the greenhouse with a single strain inoculum. Five cowpea genotypes, including 'H-Brown Crowder' and 'L-Bush Purple Hull', were used to generate populations for genetic studies. Diallel analysis [illegible]eral combining ability (GCA), specific combining ability (SCA) and reciprocal effects was carried o[illegible] he N_2 fixation variables nitrogenase activity, nodule number, nodule weight, and top dry weight, in the field study. Generation mean analysis and heritability estimates were performed on the greenhouse population which included P_1 ('H-Brown Crowder'), P_2 ('L-Bush Purple Hull'), F_1, F_1', F_2, BC_1, and BC_2. Positive and significant correlations were observed between nitrogenase activity and both nodule weight and nodule number. Correlation coefficients between top dry weight and the other 3 variables were not significant. Estimates of SCA were highly significant for all variables except top dry weight, while those of GCA were significant only for nodule weight. Generation mean analysis revealed that additive gene action was more prominent than dominance and interallelic gene action for nodule number and nitrogenase activity, while the opposite was true for nodule weight and top dry weight. Narrow sense heritability estimates were moderately high for nodule number (0.55) and nitrogenase activity (0.62), and low for nodule weight (0.29) and top dry weight (0.17).

INTRODUCTION

Cowpeas, because of their drought tolerance, are an important proteinacious crop throughout the semiarid regions of the world (FAO, 1981; PATERSON & BLACKHURST, 1958). Like many members of the Leguminosae, cowpeas possess the ability to fix N, due to a unique symbiotic relationship with certain soil-borne bacteria of the genus *Rhizobium*. Symbiotic N_2 fixation depends on the interaction among the host plant, microbial symbiont and environment. Both partners of the association are subject to genetic variation. While considerable effort has been expended on study of the bacterial aspects of the symbiosis, surprisingly little has been expended on the host plant component.

Infection of the root hairs is the first step in the host plant-*Rhizobium* interaction,

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which results in effective N_2 fixation. Failure of infection in the presence of normally effective *Rhizobium* strains is controlled by the host plant and causes non-nodulation. Non-nodulating plants have been observed in several legume species and are usually determined by recessive genes (GORBET & BURTON, 1979; HOLL, 1975; NUTMAN, 1949; WILLIAMS & LYNCH, 1953). In addition, following infection and nodulation, there can be failure leading to ineffectiveness at any of the subsequent developmental stages in this complex process. Ineffective N_2 fixation following nodulation has also been reported in several legumes. Genetic investigations have shown this trait to be controlled by dominant (CALDWELL, 1966; VEST, 1970; VEST & CALDWELL, 1972), recessive (HOLL, 1975; NUTMAN, 1954; NUTMAN, 1957; NUTMAN, 1968; PETERSON & BARNES, 1981), and modifying (GIBSON, 1969; NUTMAN, 1957) host genes.

Once effective nodulation has been achieved, quantitative differences in N_2 fixation efficiency can still exist. This variation is evidence for genetic control of the trait and has been reported in several grain legumes, including *Vicia faba* L. (broad bean) (EL SHERBEENY et al., 1977), *Pisum* sp. (field pea) (HOLL & LARUE, 1975), *Phaseolus vulgaris* L. (common bean) (GRAHAM & ROSAS, 1977; GRAHAM, 1979; WESTERMANN & KOLAR, 1978), *Glycine max* (L.) MERRILL (soybean) (WACEK & BRILL, 1976), and *Vigna unguiculata* (L.) WALP. (cowpea) (ZARY & MILLER, 1980; ZARY et al., 1978). Although non-nodulation and ineffectiveness are of interest in the study of N_2 fixation per se, it is this third area of quantitative differences in efficiency that offers the greatest opportunity for development of superior N_2 fixing cultivars. The genetics of these quantitative differences is not as well understood as that of non-nodulation and ineffectiveness. Therefore, the identification and understanding of the host plant genetics of these differences in imperative before effective breeding for enhanced N_2 fixation in cowpea can occur. Thus, the principal objective of this investigation was to study the inheritance and determine the heritability of quantitative differences in the ability of cowpea genotypes to fix atmospheric N, with the ultimate goal of increasing vegetable protein and yield in cowpea by developing superior N_2 fixing cultivars.

MATERIALS AND METHODS

Field study using mixed strain inoculum. Five cowpea genotypes, 'H-Brown Crowder', 'H-Calico Crowder', 'H-Knuckle Purple Hull', 'L-Bush Purple Hull', and 'L-TX 460', representing a broad range of horticultural types and previously identified for their high (H) or low (L) N_2 fixing potential (ZARY et al., 1978) were crossed in a 5 × 5 reciprocal diallel mating design. Populations comprising 5 parents, 10 F_1 and 10 reciprocals were included in the genetic analysis.

Planting of the populations took place in May, 1979 on well worked, moist 5 m long plots. The soil type was a Lufkin fine sandy loam (Vertic Albaqualf, fine, Montmorillonitic, thermic), with a pH of 6.7. Fertilizer was applied in a preplant band 10 cm below the soil surface at a rate of 45 kg/ha of 0-46-0. Trifluralin (2,6-dinitro-N,N-dipropyl-4-[trifluoromethyl]benzenamine) was also applied at a rate of 1.2 l/ha preplant. All seeds were hand planted 45 cm apart within rows, 1 m apart, inoculated with a commerical mixed strain of rhizobia (32H1, 150B1, 41Z2, 176A22, & 32Z3) and then covered with sterile sand to insure prompt and uniform emergence. Irrigation was provided as needed by overhead sprinklers. The statistical design was a rando-

mized complete block with 5 replications.

At 44 days after planting, 4 plants from each plot were decapitated, the roots were carefully dug and the following N_2 fixation variables were measured: nitrogenase activity of the nodules by the $C_2H_2 - C_2H_4$ reduction assay as described by ZARY et al. (1978), nodule number/plant, nodule weight/plant, and top dry weight/plant. Plot means were computed from these 4 plants and used in the analysis. Diallel analysis for general combining ability (GCA), specific combining ability (SCA) and reciprocal effects was carried out using Griffing's method 1, fixed model (GRIFFING, 1956) on log transformed values.

Greenhouse study using single strain inoculum. 'H-Brown Crowder' and 'L-Bush Purple Hull' were used to generate F_1 and F_1' (reciprocal) hybrids, F_2, BC_1, and BC_2 populations. Seven populations, including the 2 parents, were grown in the greenhouse in the fall of 1980. The experimental design was a randomized block with 4 replicates where each replicate contained 5 plants of P_1, P_2, F_1, and F_1', 20 plants of BC_1 and BC_2 and 60 plants of F_2.

Plants were grown in 3.7 l black plastic pots in a greenhouse, at a temperature of 25 ± 5 C. Potting medium contained equal amounts of sand and vermiculite (v/v) and was steam sterilized at 82 C for 2 h. Seeds were surface sterilized with 6% sodium hypochlorite. At planting, 2g of peat-based cowpea single strain 32H1 inoculum were used and pots were watered with N free nutrient solution. At 6 weeks after planting, all plants were decapitated and those variables described in the field study were measured. Population mean, variance, and standard error were computed for all 4 N_2 fixation variables.

Genetic analysis. The generation mean analysis of MATHER & JINKS (1971) was used for each trait.

GAMBLE'S (1962) notation was used in defining the parameters.

m = midparent value
a = pooled additive effects
d = pooled dominance effects
aa = pooled additve × additive effects
ad = pooled additve × dominance effects — digenic interaction terms
dd = pooled dominance × dominance effects

Expectations of the generation means using GAMBLE'S (1962) notations are as follows:

$\bar{P}_1$ (high parent) = m + a + aa
$\bar{P}_2$ (low parent) = m − a + aa
$\bar{F}_1$ = m + d + dd
$\bar{F}_2$ = m 1/2d + 1/4dd
$\bar{B}_1$ = m + 1/2a + 1/2d + 1/4aa + 1/4ad + 1/4dd
$\bar{B}_2$ = m − 1/2a + 1/2d + 1/4aa − 1/4ad + 1/4dd

First, a 3 parameter model, consisting of m, a, and d was tested for all 4 traits, and these parameters were estimated by the weighted least square method described by ROWE & ALEXANDER (1980). The weighting factor, reciprocal of the variance of the means, not only accounted for the unequal sample size, but also standardized the

differences in magnitude in the residual SS caused by differences in units. Expected generation means were estimated by three-parameter models in which epistasis was disregarded. To test the adequacy of the model, the residual SS was tested for goodness of fit using χ^2 (4).

Secondly, a six-parameter model, which included aa, dd, and ad, digenic interactions terms, in addition to m, a, and d, was also tested using the weighted least square method. Based on these 2 models, the magnitude and the sign of each genetic component was estimated.

Estimation of the variance components. The components of genetic variance of these 4 N_2 fixation traits were estimated by the inclusive method of MATHER & JINKS (1977). This method consists of setting the equations of expectation equal to the corresponding observed variances and solving by least squares for additive variance (D), dominance variance (H) and environmental variance (E). The equations of expectation were:

$\sigma^2P_1 + \sigma^2P_2 + \sigma^2F_1 + \sigma^2F_1' = 4E$

$\sigma^2F_2 = 1/2D + 1/4H + E$

$\sigma^2BC_1 + \sigma^2BC_2 = 1/2D + 1/2H + 2E$

where:

σ^2A – additive genetic variance = 1/2D

σ^2D – dominance genetic variance = 1/4H

σ^2E – environmental variance = E

Because this model assumes no significant digenic interaction, the analysis was carried out on log transformed values to eliminate the possibility of these interactions.

RESULTS AND DISCUSSION

Field study

The genotypes selected for this investigation had been previously subjected to vigorous study (ZARY et al., 1978; ZARY & MILLER, 1980). Upon collection of field data, parental populations were subjected to analysis of variance to test similarities to previously established patterns (ZARY & MILLER, 1980). Distinct separation between H and L fixing genotypes was observed for nitrogenase activity (μ moles C_2H_4/plant/h) and nodule weight (g/root) (Table 1). Dry weight of plant tops at 44 days after planting; however, bore little relation to fixation ability, indicating that another factor such as photosynthetic efficiency was affecting N_2 fixation rates. Nodule number was not a consistent indicator of nitrogenase activity suggesting that nodule weight was more critical in affecting rate of fixation than absolute number of nodules. These results can be interpreted as confirmation that differences do exist between these host plant genotypes for nitrogenase activity.

Correlation coefficients for various N_2 fixation variables at flowering. Among the parents used in this study, positive and significant correlations were observed between nitrogenase activity and both nodule weight (0.56) and nodule number (0.39) (Table 2). A significant correlation was also observed between nodule weight and number (0.54). These results confirmed that nitrogenase activity, among these parental cowpea

Table 1. Nitrogenase activity, nodule number, nodule weight and plant dry weight, for the 5 parental genotypes used in the field study.

Genotype	Nitrogenase activity (μmoles C_2H_4/plant/h)	Nodule number (number/plant)	Nodule weight (g/root)	Plant dry weight (g/plant top)
H-Brown Crowder	43.7a[1]	80.0a	1.35a	63.5ab
H-Calico Crowder	41.4a	66.4b	1.06b	58.8b
H-Knuckle Purple Hull	40.9a	57.8b	1.30a	74.2a
L-TX 460	14.3b	63.9b	0.62c	74.6a
L-Bush Purple Hull	3.4c	34.7c	0.30d	57.5b

[1] Mean separation in columns by Duncan's Multiple Range Test, 5% level.

genotypes, is controlled in large part by the plant's ability to form large, efficient nodules and not by its ability to form vegetative matter. However, the correlation coefficients between top dry weight and the other 3 N_2 fixation variables were not significant, suggesting that top dry weight/plant at the time of flowering is not a good indicator of N_2 fixation potential.

Analysis for combining ability. Results from analysis for GCA and SCA using F_1 data, including parents, is presented in Table 3. Specific combining ability estimates were highly significant for all characters analyzed except dry weight of plant tops, while GCA estimates were not significant except for nodule weight. No reciprocal effects were indicated by this analysis. These results suggest that SCA is a very important factor contributing to enhanced N_2 fixation in these populations. However, it must be remembered that interaction of specific cowpea genotypes with 1 or more separate rhizobial strains was occuring in this field study. Such apparent dominance (the significance of SCA) may be the reflection of a large interaction component. The only exception to this pattern was nodule weight where GCA and SCA were both significant. These results indicated that genetic studies using mixed strain inoculum are very complex and probably give ambiguous information on gene action involved in N_2 fixation.

Greenhouse study

The high N_2 fixing, indeterminate parent, 'Brown Crowder', produced larger and more

Table 2. Correlations among N_2 fixation variables at flowering for the parents used in the field study.

Index	Nirogenase activity (μmoles C_2H_4/plant/h)	Dry weight (g/plant top)	Nodule weight (g/root)	Nodule number (number/plant)
Nitrogenase activity (μmoles C_2H_4/plant/h)		0.14	0.56**	0.39**
Dry weight (g/plant top)			0.23	0.11
Nodule weight (g/root)				0.54**
Nodule number (number/plant)				

** Significant at the 1% level.

Table 3. Estimated mean squares for general (GCA) and specific (SCA) combining ability and reciprocal differences from a 5 – parent reciprocal diallel cross, including parents.

Source	df	Nitrogenase activity (μmoles C_2H_4/plant/h)	Dry weight (g/plant top)	Nodule weight (g/root)	Nodule number (number/plant)
Blocks	4	0.00019	0.00721	0.00631	0.00194
GCA	4	0.00033	0.02197	0.21085**	0.02309
SCA	5	0.00655**	0.01372	0.26224**	0.22954**
Reciprocal	10	0.00002	0.00516	0.05306	0.01178
Error	102	0.00025	0.05144	0.07121	0.05191

** Significant at the 1% level.

active nodules than the low fixing, determinate parent, 'Bush Purple Hull' (Table 4); however, 'Bush Purple Hull' produced more nodules than did 'Brown Crowder'. This indicated that nodule size reflects N_2 fixation potential more than does number of nodules, and supports our earlier correlation among variables. At the time of flowering, 'Brown Crowder' produced more dry matter than 'Bush Purple Hull'. The observed differences between the F_1 hybrid and the reciprocal F_1 were not found to be statistically significant for any of the 4 N_2 fixation variables. Heterosis, over the mid-parent value, was evident for all 4 traits; however, in the case of nodule weight and top dry weight, F_1 means were higher than the high parent. This suggests the involvement of a strong dominance gene action for these traits. Except for nodule number, the means of both BC populations were lower than those of the F_1. The F_2 means were intermediate between both parental and BC populations; but lower than those of the F_1. These results suggest that, for nodule number and nitrogenase activity, additive genes contribute more than dominance genes, whereas the opposite is true for the other two traits.

Additive-dominance (three-parameter model). The three-parameter model (MATHER & JINKS, 1977) includes m, the mid-parent value, [a] additive component and [d] dominance component, where: m = the mean of all possible homozygotes, irrespective of all loci at which there are allelic differences; [a] = net directional effect of additive

Table 4. Population size (n) means (x) and variances (σ^2) for N_2 fixation variables measured.

Generation	N	Nodule number (number plant)		Nodule weight (mg plant)		Nitrogenase activity (μmoles C_2H_4 plant h)		Top dry weight (g plant)	
		X	σ^2	X	σ^2	X	σ^2	X	σ^2
P_1[1]	20	20	35	310	5057	5.74	2.20	9.83	3.05
P_2[2]	20	40	73	252	4628	2.03	0.54	5.97	1.24
F_1	20	34	81	380	2853	4.68	2.74	11.18	3.64
F_1'[3]	20	38	99	355	3667	4.00	2.66	10.79	2.08
BC_1	80	32	97	331	7889	4.63	3.43	10.75	6.26
BC_2	80	41	188	283	6644	3.85	2.72	9.39	4.15
F_2	240	34	219	311	9247	4.34	4.57	9.31	3.96

[1] Brown Crowder; [2] Bush Purple Hull; [3] Reciprocal F_1 hybrids

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gene action; [d] = net directional effect of dominance gene action. The three-parameter model assumes that genes involved are independent of each other, or the magnitude of the epistatic gene effects are negligible, when compared to additive and dominance effects. In addition, the relative magnitude of different kinds of gene action and interaction cannot be inferred from estimates of the genetic components (MATHER & JINKS, 1977). Therefore, the net directional effects are all that can be reliably inferred from estimates based on generation mean analysis. However, by using the joint-scaling test on several populations, the adequacy of the additive dominance models can be tested.

Results of the joint-scaling tests, based on the additive dominance model for the 4 N_2 fixation variables, are given in Table 5. The additive dominance model is adequate for nodule number and nitrogenase activity, but not for nodule weight or top dry weight. With nodule number, both the net additive and dominance effects were positive and of the same order of magnitude. This indicated that alleles which increase nodule number are more important than those which decrease nodule number. For nitrogenase activity, the net effect of the additive component was positive and the frequency of both positive and negative alleles was approximately equal.

Based on the joint-scaling test and the resulting χ^2 stastistic a significant interallelic interaction was found to influence nodule weight and top dry weight. In addition, the net directional effect of dominant genes was positive and relatively larger in magnitude than net additive gene action.

Table 5. Genetic parameter estimates for N_2 fixation traits using the joint-scaling test based on the three parameter model.

Genetic parameter	Nodule number (number plant)	Nodule weight (mg plant)	Nitrogenase activity (μmoles C_2H_4 plant·h)	Top dry weight (g plant)
$\hat{m}$	30.88 ± 1.17[1]	270.16 ± 14.57	3.91 ± 0.18	7.78 ± 0.44
$(\hat{a})$	9.79 ± 1.24	17.51 ± 16.97	1.79 ± 0.18	1.64 ± 0.49
$(\hat{d})$	6.18 ± 2.08	92.13 ± 23.35	0.86 ± 0.33	3.65 ± 0.78
$\chi^2_{(4)}$	5.18	11.12*	4.91	20.75**

[1] Standard error of the estimate.
*, ** Significant at 5% and 1%, respectively.

Table 6. Genetic parameter estimates for N_2 fixation traits using the joint-scaling test based on the six-parameter model.

Genetic parameter	Nodule number (number plant)	Nodule weight (mg plant)	Nitrogenase activity (μmoles C_2H_4 plant·h)	Top dry weight (g plant)
$\hat{m}$	21.10* ± 9.90[1]	291.5* ± 83.7	4.75* ± 0.40	9.31* ± 0.1
$(\hat{a})$	9.70* ± 1.34	28.6* ± 15.3	1.85* ± 0.10	1.35* ± 0.53
$(\hat{d})$	38.50 ± 28.05	17.3 ± 239.5	1.02 ± 1.21	6.06* ± 1.14
$(\hat{aa})$	9.00 ± 9.98	16.6 ± 82.4	0.86* ± 0.40	3.02* ± 1.11
$(\hat{ad})$	0.50 ± 9.15	152.1* ± 80.8	2.14* ± 0.40	-3.28* ± 0.55
$(\hat{dd})$	23.90 ± 18.56	88.9 ± 158.4	0.86 ± 0.80	-5.65* ± 2.19
$\chi^2_{(1)}$	1.34	1.92	0.12	0.52

[1] Standard error of the estimate.
* Significant at the 5% level.

Table 7. Joint-scaling tests on log transformed data based on the three-parameter model and genetic parameter estimation for N_2 fixation traits.

Genetic parameter	Nodule number (number/plant)	Nodule weight (mg/plant)	Nitrogenase activity (μmoles C_2H_4/plant/h)	Top dry weight (g/plant)
$\hat{m}$	1.43* ± 0.02[1]	2.43* ± 0.02	0.52** ± 0.02	0.89* ± 0.03
$(\hat{a})$	0.14* ± 0.02	0.03 ± 0.02	0.22* ± 0.02	0.09* ± 0.03
$(\hat{d})$	0.10* ± 0.03	0.13* ± 0.03	0.12* ± 0.03	0.15* ± 0.04
$\chi^2_{(4)}$	0.34	0.72	0.32	1.67

[1] Standard error of the estimate.
* Significant at the 5% level.

Six-parameter model. Results of the joint-scaling test, based on the six-parameter model, are presented in Table 6. In addition to m, [a], and [d], 3 digenic interaction components, [aa], [ad], and [dd], are also included. The $\chi^2_{(1)}$ values indicated that the six-parameter model gave a very good fit and that significant digenic interactions exist for nodule weight and nitrogenase activity. The magnitude of epistatic components does not express the relative importance of each type of gene action; however, only [d] and [dd] reflect the net direction of the gene action and interaction. Thus, only these 2 components are used to classifiy the predominant type of epistasis (MATHER & JINKS, 1977). For nodule number and top dry weight, the [d] component was positive, but the [dd] component was negative. This indicates that the positive alleles were dominant and the epistatic gene action was duplicate (MATHER & JINKS, 1977). For nodule weight and nitrogenase activity, the magnitude of the [d] and [dd] components was not statistically significant. The [ad] component was the major digenic epistatic component.

Variance components and heritability estimates. Calculation of variance components and heritability estimates as proposed by MATHER & JINKS (1977) assumes no epistatic interaction. In the presence of significant epistasis, h^2 estimates are biased. MATHER & JINKS (1977) suggested that a log transformation might reduce the epistatic gene action, and that h^2 estimates on log transformed data would be unbiased.

Results of the joint scaling tests on log transformed data for the additive dominance model are given in Table 7. Non-significant $\chi^2_{(4)}$ values indicated that log transformation significantly reduced the epistatic contribution, especially for nodule weight and top dry weight. Therefore, h^2 estimation on log transformed data did provide unbiased

Table 8. Variance components and heritability estimation on log transformed data for the N_2 fixation traits.

Variance component	Nodule number	Nodule weight	Nitrogenase activity	Top dry weight
Additive variance (D)	0.033	0.018	0.074	0.004
Dominance variance (H)	0.002	0.126	0.016	0.017
Environmental variance (E)	0.013	0.008	0.180	0.005
Degree of dominance ($\sqrt{H/D}$)	0.270	1.200	0.470	2.100
Narrow sense heritability (h^2)	0.550	0.390	0.620	0.170

h^2 estimates.

The results of the variance components and h^2 estimates are given in Table 8. For nodule number and nitrogenase activity, dominance variance was smaller than the additive variance. Therefore, the average degree of dominance was small, while h^2 was moderately high. For nodule weight and top dry weight, the dominance variance was larger than the additive variance. Therefore, the average degree of dominance was very high and h^2 was moderately low. This explains why significant heterosis was expressed for nodule weight and top dry weight.

CONCLUSION

Inheritance studies of N_2 fixation traits using mixed strain inoculum produced ambiguous results. However, the use of a single strain overcame this ambiguity and provided useful estimates. Higher heritability estimates for nodule number and nitrogenase activity suggest that breeding for enhanced N_2 fixation in cowpea could be more effectively carried out using a single, efficient rhizobial strain.

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