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HERITABILITY OF $N_2(C_2H_2)$ FIXATION RATES AND RELATED CHARACTERS IN PEAS (*Pisum sativum* L.)

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To study the genetic variability of $N_2(C_2H_2)$ fixation rates in peas, 85 genotypes were screened in the field. Genotype means differed ($P<0.01$), and ranged from 1.5 to 12.5 μmoles $C_2H_2 \cdot h^{-1} \cdot plant^{-1}$ and from 10 to 49 μmoles C_2H_2 $h^{-1} \cdot$(g nodulated root)$^{-1}$. Under environmentally controlled conditions the rankings of six genotypes, selected to represent the full genotypic range of fixation, were significantly correlated to those in the field. A diallel mating design among the six genotypes showed that heterosis occurred in fixation per plant, probably partly due to nonallelic interaction. In fixation per gram nodulated root, however, nonadditive effects were small. Heterosis occurred in total shoot N and in shoot weight, probably due in the latter case to both nonallelic interaction and overdominance. Total dominance, associated with decreased expression, was evident for percent shoot N. Study of the genes controlling fixation may best be done by examining $N_2(C_2H_2)$ reduction on a per-gram nodulated root basis because results are less affected by the genetics of correlated whole plant characters. Fixation expressed in this way might be improved by simple selection.

Pour étudier la variabilité génétique du taux de fixation de $N_2(C_2H_2)$ chez le pois, on a examiné au champ 85 génotypes de cette espèce. Les moyennes génotypiques révélaient des différences significatives ($P<0,01$) variant de 1,5 à 12,5 μmoles C_2H_2 par plante par heure et de 10 à 49 μmoles par heure et par gramme de racine nodulée. En conditions d'ambiance contrôlée, le rang de six genotypes choisis pour représenter tout l'éventail génotypique de fixation était significativement corrélé au classement obtenu au champ. Un dispositif de croisement diallèle comportant les six génotypes a révélé un phénomène d'hétérosis dans le taux de fixation par plante, probablement dû en partie à une interaction non allélique. En revanche, le taux de fixation par gramme de racine nodulée n'a montré que de faibles effets non additifs. L'effet d'hétérosis s'est manifesté également sur la teneur totale en N et sur le poids des pousses (parties aériennes), ce qui, dans le dernier cas, celui du poids, s'expliquerait à la fois par une interaction non allélique et par un effet de superdominance. Des effets de dominance complète liés à l'expression limitée du caractère sont apparus pour la teneur totale en N des parties vertes. La meilleure façon d'étudier les gènes qui modulent la fixation de l'azote serait d'examiner la réduction de $N_2(C_2H_2)$ par gramme de racine nodulée, parce que les résultats risquent moins d'être faussés ou masqués par le mode de transmission des caractères corrélés de la plante entière. Ainsi exprimée, l'intensité de la fixation pourrait être améliorée par sélection simple.

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The increasing cost of N fertilizers in agriculture has prompted interest in the possibility of replacing some of their use by the manipulation of symbiotic N_2 fixation. Improving the ability of crop legumes to fix atmospheric N_2 is one method by which such a goal might be met (Barnes et al. 1978). Although this improvement may be achieved by genetic modification of either the plant or bacterial symbiont, investigations have concentrated mainly on the bacteria (Schwinghamer 1977). The information available concerning the potential for increasing the N_2-fixing ability of the host is, therefore, sparse; however, there are indications that such plant selection may be possible.

Quantitative host genetic variability for fixation is reported by Pinchbeck et al. (1980) in Spanish clover (*Desmodium sandwicense* E. Mey); by Zary et al. (1978) in southern pea (*Vigna unguiculata* (L.) Walp); and by Seetin and Barnes (1977), Duhigg et al. (1978) and Hoffman and Melton (1981) in alfalfa (*Medicago sativa* L.). In the latter crop there is some evidence of intracultivar variability exceeding intercultivar variability (Hoffman and Melton 1981). The genetic control of fixation has been investigated in a few instances; major genes in peas (*Pisum sativum* L.) affected both nodulation and fixation (Holl 1975) and quantitative genetic variation in overall fixation was due solely to general combining ability in Spanish clover (Pinchbeck et al. 1980). Furthermore, plant selection has actually resulted in some improvement of fixation in alfalfa (Seetin and Barnes 1977; Duhigg et al. 1978).

In legume species grown on N-free nutrient media, N_2 fixation, photosynthesis and plant size are closely associated (Hardy and Havelka 1976; Pate 1977). The genetic analysis of any single feature may be complicated, therefore, if observed genetic variability in the character under examination is affected, or caused by association with another trait which is itself genetically variable. The significant correlation between plant size and N_2 fixation per plant (Seetin and Barnes 1977; Duhigg et al. 1978; Pinchbeck et al. 1980) may make the genetic analysis of the control of either character imprecise, but N_2 fixation expressed on an appropriate weight basis may be dissociated from plant size and hence may be a better way of examining the fixation process.

A full understanding of the genetic control of a character is often necessary before it can be successfully improved in a breeding program (Breese 1972). The objective of this study was to examine the quantitative variation of N_2 fixation and associated characters in peas so that selection procedures for their improvement might be identified. Dinitrogen fixation was estimated by C_2H_2 reduction, and the results were expressed as both rate per plant and per gram nodulated root. The size and nature of the genetic component of variability was determined.

MATERIALS AND METHODS

Eighty-five genotypes from the world collection of *P. sativum* L., including Trapper and Alaska as representatives of locally grown cultivars, were tested for $N_2(C_2H_2)$-reducing ability in 1979. Plants were grown in the field in a randomized split-plot design with two replicates. Four main plots within replicates were time of measurement; subplots were genotypes. Soil was of the Chernozemic, Orthic Dark Brown subgroup and was of the Bradwell association consisting of fine sandy glaciolacustrine deposits (Acton and Ellis 1978). Peas were sown on barley stubble; soil nitrogen content was less than 20 ppm.

Genotypes were planted within main plots in hills at 1-m spacing. At each hill five seeds, treated with commercial inoculum (Nitragin Inoculator Co., Milwaukee, Wis. 53209), were planted at a depth of 6–8 cm. Planting date was 24 May.

Acetylene reduction assays were made over the 4 wk following 26 June, one main plot from each replicate being analyzed per week. Plants were excavated, the soil was shaken from the roots, and the roots were excised and placed in 900-mL glass jars. In the laboratory,

an airtight lid (with septum incorporated) was screwed onto each jar and 50 mL of purified C_2H_2 were injected. After a 30-min incubation at 23°C, two 1-mL gas samples were taken from each jar and analyzed for C_2H_4 in a gas chromatograph. Roots and nodules were then washed, dried and weighed.

On the basis of these results, five genotypes that showed no genotype × time of season interaction for fixation (PI177054, PI212916, PI244180, PI244223, PI244253) were chosen to represent the full genotypic range of C_2H_2 reduction calculated as rate per plant and rate per gram nodulated root. These were grown, with Trapper, in a controlled environment room with a 16-h photoperiod ($200\mu E \cdot m^{-2} \cdot sec^{-1}$) and day/night temperatures of 20°C/15°C. Pots were inoculated 1 wk after planting with the same commercial inoculum as used in the field and irrigated daily with water and twice-weekly with N-free nutrient solution (Mahon 1977). For each genotype three seeds were planted per pot in 'Turface' (Wyandotte Chemicals, Wyandotte, Mich.), and plants were later thinned to two per pot. A randomized split-plot design was used with two replicates. Five main plots within replicates were time of measurement; subplots were genotypes. Each genotype was represented by one pot in each main plot. C_2H_2 reduction assays were performed on one main plot per replicate at each of five, half-weekly intervals beginning 3.5 wk after planting. These timings were selected to provide an average N_2 fixation rate over a period when this would be at its peak.

A complete diallel mating design was produced by cross-pollinating the six selected genotypes in all possible combinations. F_1 progenies from the crosses, including the selfed parents, were assayed in a controlled environment. Conditions of planting and growth were as above. A completely randomized design was used with four replicated pots for each entry. Acetylene reduction assays were performed on one randomly selected replicate of each of four half-weekly intervals beginning 3.5 wk after planting. Shoots were dried and weighed. N content was analyzed by the Kjeldahl method at the POS Pilot Plant Corp., Saskatoon, Sask.

Data were analyzed by factorial analysis of variance and Duncans's multiple range test. Using the diallel data, percent heterosis over mid-parental values was estimated as (F1-MP)/MP × 100 and percent heterosis over the higher parent as (F1-HP)/HP × 100. Heritability was calculated as the regression coefficient of F_1 means plotted against mid-parent means. Relationships between the variance (Vr) and parent-offspring covariance (Wr) of members of the same array in the diallel were analyzed by the graphical method given by Mather and Jinks (1971) for each character measured.

RESULTS

Differences existed among the 85 genotypes for $N_2(C_2H_2)$ fixation both on a per-plant and on a per-gram nodulated root basis ($P<0.01$). Genotypic mean fixation per plant ranged from 1.5 to 12.5 μmoles $C_2H_2 \cdot h^{-1}$, approximately normally distributed around a mean of 6.4 μmoles $C_2H_2 \cdot h^{-1}$ (Fig. 1A). Fixation per gram ranged from 10 to 49 μmoles $C_2H_2 \cdot h^{-1}$, approximately normally distributed around a mean of 32 μmoles $C_2H_2 \cdot h^{-1}$ (Fig. 1B).

When retested under controlled environmental conditions, six genotypes (including Trapper), selected to show the full range of genotypic variability for both methods of expressing fixation, again showed genotype differences. The means for genotypes grown in the field and in the growth room for this selected population are given in Table 1. Despite the obviously greater fixation by PI244180 under controlled conditions, the overall means of $N_2(C_2H_2)$ fixation were similar in the two environments; and there was a significant correlation between genotype rankings under the two sets of conditions ($N_2(C_2H_2)$ fixation per plant, $r_s=0.829$, $P<0.05$; $N_2(C_2H_2)$ fixation per gram, $r_s=0.886$, $P<0.05$). This indicated that the genotypic differences in fixation rates were consistent in different environments.

Means of reciprocals were used as raw data in calculations using data from the diallel of the six genotypes. These means are presented in Table 2 for all characters. The parental mean rankings for $N_2(C_2H_2)$ fixation per plant differed slightly from those in the preliminary indoor experiment (Table 1), and were generally lower in this second indoor trial as the plants were not

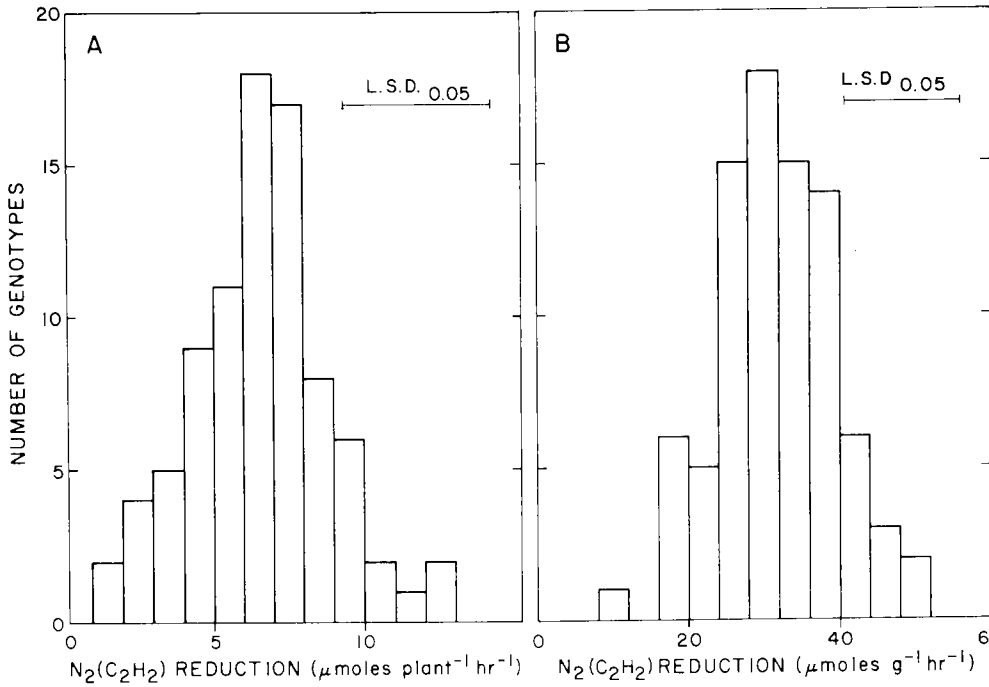

Fig. 1 Distribution of genotype means for $N_2(C_2H_2)$ reduction per plant (A) and per gram nodulated root (B).

measured over the same period of growth (four harvests and not five were measured). However, parental rankings of $N_2(C_2H_2)$ fixation per gram nodulated root were the same in both indoor experiments.

Generation means, estimates of heterosis and heritability values calculated from the diallel cross are presented in Table 3. Heterosis was evident for $N_2(C_2H_2)$ fixation on a per-plant basis, but not on a per-gram basis. Heterosis of fixation per plant was not only expressed as an increase of F_1 over mid-parental values, which might have indicated that dominant alleles in-

Table 1. Means of N_2 (C_2H_2) fixation for six selected genotypes grown in the field and under controlled environmental conditions

	Field (μmoles $C_2H_2 \cdot h^{-1}$)		Controlled environment (μmoles C_2H_2 h^{-1})	
Genotype	plant^{-1}	g·nodulated root^{-1}	plant^{-1}	g·nodulated root^{-1}
PI177054	5.0 *bc*	42 *ab*	7.4 *c*	32 *b*
PI212916	12.5 *a*	48 *a*	9.4 *a*	38 *a*
PI244180	2.0 *c*	10 *c*	8.3 *bc*	26 *c*
PI244223	4.3 *bc*	25 *bc*	8.4 *bc*	24 *c*
PI244253	9.2 *ab*	47 *a*	9.3 *a*	34 *ab*
Trapper	6.4 *bc*	38 *ab*	8.5 *abc*	33 *b*
Mean	6.6	35	8.6	31

a-c Any two means within a column not connected by a common letter differ at the 5% level of Duncan's multiple range test.

Table 2. Means for crosses from a 6×6 diallel mating design

Cross		$N_2(C_2H_2)$ fixation·plant^{-1} (μmoles $C_2H_2 \cdot h^{-1}$)	$N_2(C_2H_2)$ fixation·g^{-1} (μmoles $C_2H_2 \cdot h^{-1}$)	Shoot weight (g)	Percent shoot N (%)	Total shoot N (mg)
PI177054	× PI177054	4.76	37.9	0.64	31.6	21.0
	× PI212916	7.42	39.6	1.31	2.85	37.7
	× PI244180	8.05	35.0	1.23	2.94	36.8
	× PI244223	7.73	36.4	1.21	2.85	34.8
	× PI244253	7.27	41.5	1.04	3.07	32.1
	× Trapper	7.71	40.5	0.99	2.90	29.0
PI212916	× PI212916	7.89	44.4	1.17	2.56	31.1
	× PI244180	8.88	39.3	1.16	2.80	32.2
	× PI244223	9.08	37.6	1.65	2.67	45.3
	× PI244253	7.83	36.8	1.21	2.58	31.7
	× Trapper	8.54	44.3	1.46	2.77	41.6
PI244180	× PI244180	5.99	35.4	0.90	2.92	27.4
	× PI244223	9.47	33.8	1.64	2.75	46.0
	× PI244253	6.38	38.1	0.80	3.21	25.7
	× Trapper	7.14	37.9	1.01	3.02	31.0
PI244223	× PI244223	7.20	34.5	1.08	3.87	42.6
	× PI244253	7.94	33.9	1.24	2.98	36.8
	× Trapper	7.38	35.7	1.35	2.97	40.5
PI244253	× PI244253	6.12	43.1	0.79	3.23	26.5
	× Trapper	6.51	42.3	0.97	3.04	29.7
Trapper	× Trapper	5.74	40.4	0.76	2.91	23.6

creased the expression of the character, but also as an increase of F_1 over the higher parent in each cross. In contrast, the F_1 values of $N_2(C_2H_2)$ fixation per gram were significantly lower than the higher parent in each cross and not significantly different from the mid-parental values. This indicated that there was little, if any, dominance associated with $N_2(C_2H_2)$ fixation expressed per gram.

The entries in the diallel also differed with respect to the other characters measured, i.e. shoot weight, percent shoot N, and total shoot N. Shoot weight, not shoot weight + root weight, was used to estimate plant size because not all the root system could be harvested. The F_1 values indicated heterosis over both mid-parent and highest parent values in shoot weight and total shoot N (Table 3). Percent shoot

Table 3. Overall, parental and F_1 means, heterosis and narrow-sense heritability estimates from a 6 × 6 diallel mating design

	Overall mean	Parental mean	F_1 mean	% heterosis over highest parent	% heterosis over mid-parent	Heritability
$N_2(C_2H_2)$ fixation·plant^{-1} (μmoles·h^{-1})	7.4	6.3	7.8	13.1*	23.8**	0.76±0.30
$N_2(C_2H_2)$ fixation·g nodulated root^{-1}) (μmoles·h^{-1})	38.4	39.3	38.1	−8.6**	−3.0	0.85±0.25
Shoot weight (g)	1.12	0.89	1.22	20.5**	37.3**	1.43±0.39
Percent shoot N (%)	2.95	3.10	2.89	−13.0**	−7.03**	0.11±0.17
Total shoot N (mg)	33.5	28.7	35.3	9.0*	23.1**	0.93±0.28

*,**significant at the 5% and 1% probability levels, respectively.

N F_1 values, however, were significantly lower than mid-parent values but not lower than the lowest parents in each cross. Therefore, only heterosis below the mid-parent values was present and dominant alleles appeared to be associated with a decrease in the expression of this character.

Narrow-sense heritability estimates for the selected population were significant in all characters except percent shoot N (Table 3).

The Wr/Vr graph for $N_2(C_2H_2)$ reduction per plant (Fig. 2) has a regression coefficient that is significantly different from both 1 and 0. This indicates that epistasis is present and that the inheritance of the character cannot be explained in terms of a simple additive-dominance model. The removal of each array in turn and the recalculation of the diallel, as suggested by Jinks (1954) to test whether any single array is the cause of the non-allelic interaction, did not produce any Wr/Vr graph with a regression coefficient approaching 1.

The regression coefficient of the Wr/Vr graph for $N_2(C_2H_2)$ reduction per gram nodulated root was significantly different from 1 but not from 0 due to a large scatter of array coordinates (Fig. 3). However, reclaculation omitting array PI244253 produced a regression coefficent significantly different from 0 but not from 1 (Fig. 4), indicating that much of the non-allelic interaction was due to crosses involving PI244253 and that an additive-dominance model fitted the data in the reduced diallel. The positive Wr/Vr intercept indicated partial dominance. The two genotypes with the highest mean rates were positioned on the graph close to the upper intercept of the regression line and the limiting parabola and the genotype with the lowest mean rate approximated to the lower intercept. These relative positions indicated that dominant alleles were associated with low $N_2(C_2H_2)$ fixation per

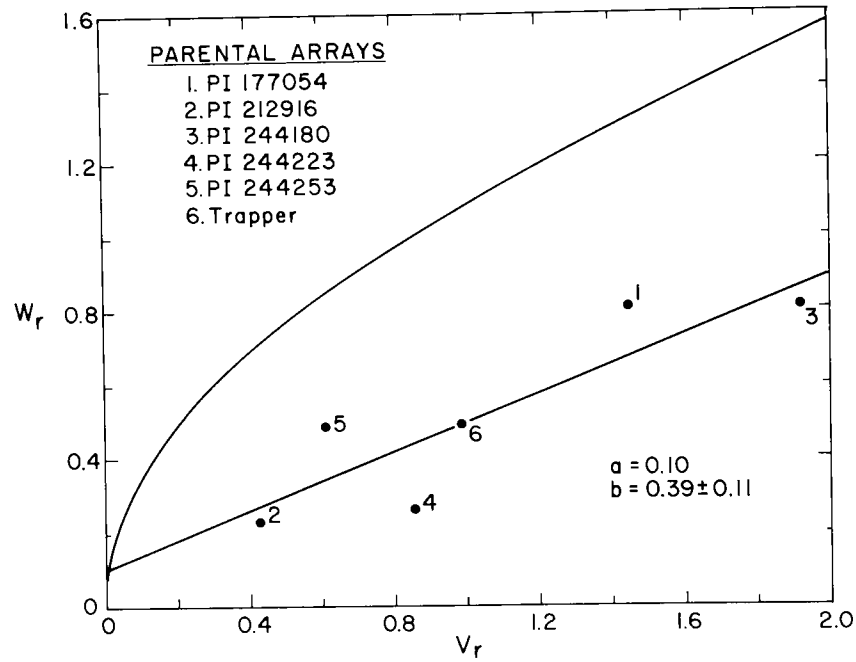

Fig. 2. Wr/Vr graph (parent-offspring covariance/variance, for members of the same array in a diallel) for $N_2(C_2H_2)$ reduction per plant.

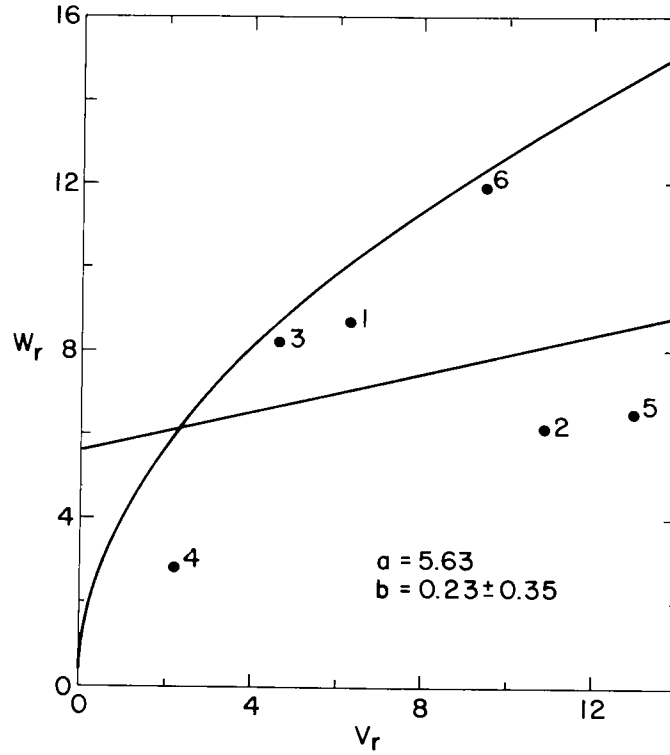

Fig. 3. Wr/Vr graph for $N_2(C_2H_2)$ reduction per gram nodulated root.

gram, although the correlation coefficient between Wr+Vr and the parental means was not significant due to the small number of arrays in the reduced diallel ($r_{\bar{p}:Wr+Vr} = 0.859$; $P>0.05$).

The Wr/Vr graph for shoot weight had a regression coefficient not significantly different from 1 or 0 (Fig. 5). The removal of array PI244223, however, produced a regression coefficient significantly different from 0 but not from 1 (Fig. 6). The negative Wr intercept indicated that the additive-dominance model of this reduced diallel tended towards overdominance. Dominant alleles were associated with increased shoot weight ($r_{\bar{p}:Wr+Vr} = -0.903$; $P<0.05$).

No epistasis was evident for total shoot N as the regression coefficient of the Wr/Vr graph was not significantly different from 1 and differed from 0 ($b=0.61\pm0.18$; $a=0.05$). The dominant alleles probably had an ambi-directional effect ($r_{\bar{p}:Wr+Vr} = -0.575$; $P>0.05$).

Table 4. Phenotypic correlation coefficient between N_2 (C_2H_2) fixation and associated characters

		1	2	3	4	5
$N_2(C_2H_2)$ fixation·plant^{-1}	1	-	0.062	0.872**	−0.428*	0.807**
$N_2(C_2H_2)$ fixation·g nodulated root^{-1}	2		-	−0.162	−0.019	−0.106
Shoot weight	3			-	−0.446*	0.948**
Percent shoot N	4				-	−0.181
Total shoot N	5					-

*,**significant at the 5% and 1% probability levels, respectively.

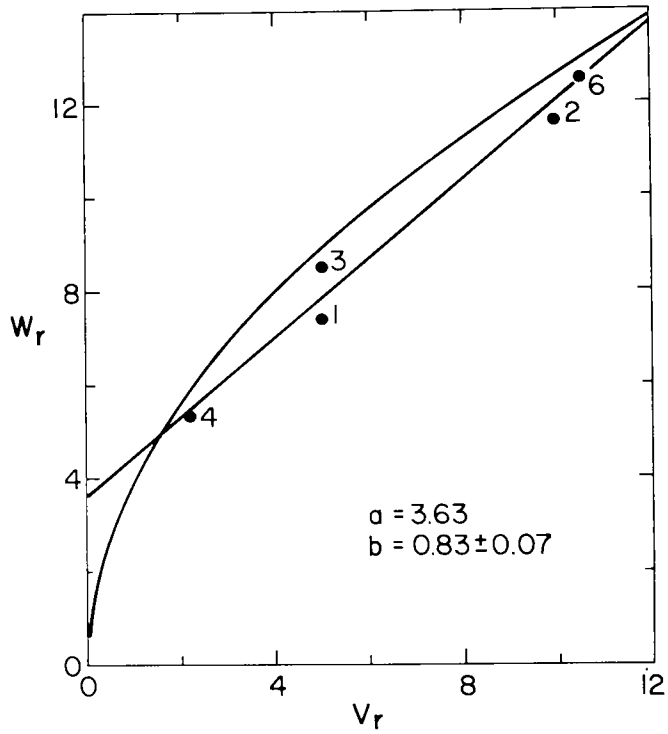

Fig. 4. Wr/Vr graph for $N_2(C_2H_2)$ reduction per gram nodulated root omitting array PI244253.

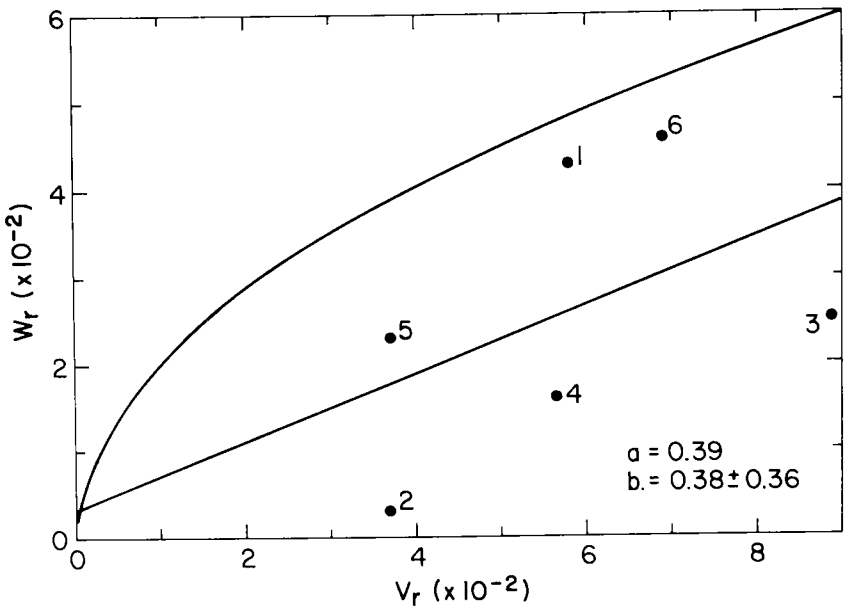

Fig. 5. Wr/Vr graph for shoot weight.

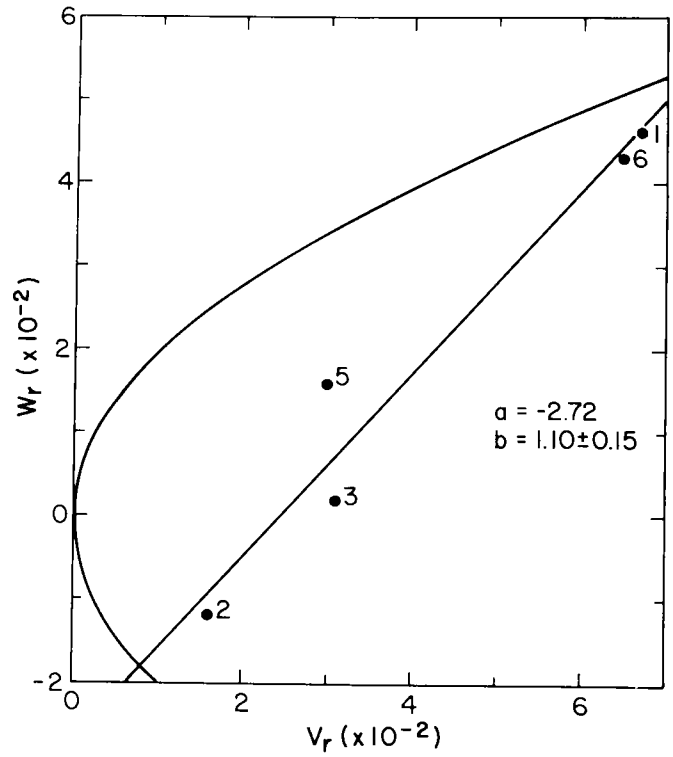

Fig. 6. Wr/Vr graph for shoot weight omitting array PI244223.

Epistasis was also not evident from the analysis of the Wr/Vr graph for percent shoot N ($b=0.97\pm0.11$; $a=-0.01$). Dominant alleles were associated with reduced N percent ($r_{p:Wr+Vr}=0.895$; $P<0.05$).

Phenotypic correlations between characters, calculated using the familly means within the diallel, are given in Table 4. Significant positive correlations were found between $N_2(C_2H_2)$ fixation per plant and total shoot N and between both characters and shoot weight. Significant negative correlations were found between percent shoot N and both $N_2(C_2H_2)$ fixation per plant and shoot weight.

DISCUSSION

The destructive and time-consuming nature of the C_2H_2 reduction assay limited the number of possible replications. Reduced replication of the diallel cross, combined with the large error associated with individual $N_2(C_2H_2)$ reduction measurements, permitted only a graphical analysis of the overall diallel data. The data fit the general assumptions associated with diallel analysis as stated by Mather and Jinks (1971) as peas undergo diploid segregation, homozygous lines were used and epistasis was generally lacking. However, a fully partitioned analysis of variance for the diallel was not possible, because the number of plants within each cross necessary to provide sufficient precision of measurement would have been very large and was beyond the limits of the experiment.

These $N_2(C_2H_2)$ fixation measurements are estimates of the average rates of fixation during several physiological stages of plant development — from vegetative growth to flowering and early pod fill. LaRue and Kurz (1973) found that

$N_2(C_2H_2)$ fixation decreased in peas at the onset of pod setting and it is conceivable that the genotypic differences in fixation in this work (Fig. 1 and Table 1) are reflected in, or caused by, genotypic differences in the duration of certain physiological stages of development. However, no significant genotype × time (through season) interaction was found in the analysis of data from either environment, indicating that all genotypes had a similar seasonal pattern of fixation. Although the variation in the genotypic means about the overall mean was less under controlled conditions than in the field for both methods of expressing fixation, and although some genotypes (notably PI244180) reduced far greater amounts of $N_2(C_2H_2)$ in the growth cabinet (Table 1), the overall mean fixation values and the rankings of the genotypes were similar in both environments. Those differences that did exist between environments may have been due to the inability to match precisely the physiological stage at which the assay was done in the two environments, or to the fact that field plants were not growing on totally N-free soil.

Despite this apparent stability of means, it must be emphasized that results of the genetic analyses apply only to the six selected genotypes examined in the controlled environment. Evidence of phenotypic stability is not proof of genetic stability and the control of $N_2(C_2H_2)$ fixation under the semi-droughted, high irradiance, low N conditions in the field may be very different to its control in the freely watered, low irradiance, N-free conditions in the growth room.

The high narrow-sense heritability estimates for $N_2(C_2H_2)$ fixation found under our conditions (Table 3) indicated that selection for increased fixation in peas should be effective in a plant-breeding program. Such selection has already proved possible in alfalfa (Seetin and Barnes 1977; Duhigg et al. 1978). However, fixation per plant showed both heterosis (Table 2) and epistasis not associated with any single array (Fig. 2); heterosis has also been reported for this character by Seetin and Barnes (1977) and Pinchbeck et al. (1980). As this heterosis is expressed as a percentage, the higher the parental values the greater the heterosis in the F_1. Hence the narrow-sense heritability estimate of parent-offspring regression (Table 3) may be biased upwards and simple selection may not produce a rapid improvement of fixation expressed on this basis.

The nonallelic interaction present in the control of $N_2(C_2H_2)$ fixation expressed on a nodulated root weight basis (Fig. 3) was associated with a single array (PI244253), and the significant Wr/Vr regression coefficient produced when this array was removed (Fig. 4) indicated that some dominance was present in the control of this character. However, no significant difference was detected between F_1 and mid-parent means over all entries (Table 3) or when the entries involving PI244253 had been removed (% heterosis over mid-parent = 1.0 ± 1.0). The positive Wr intercept and the arrangement of most array points close to the limiting parabola (Fig. 4) also indicated that the degree of dominance was not large. This low proportion of nonadditivity in the control of this character, at least in selected populations, indicated that simple selection should be effective in increasing its expression.

Part of the reason for the differences in genetic control of $N_2(C_2H_2)$ fixation when expressed in different units, may be explained by examination of the close relationship between photosynthesis, shoot weight and $N_2(C_2H_2)$ fixation. Each of these characters has been shown to be correlated with the others (Hardy and Havelka 1976; Pate 1977) but the cause and effect in such correlations is unknown; plant size may be a direct function of available N produced through fixation, but total N_2 fixed may also be a direct function of plant size. (In the latter case the larger the plant, the greater the leaf area and,

therefore, the greater the amount of photosynthate available to the N_2 fixation process; alternatively, the larger the plant the larger the root area available for nodulation.) This interconnection of characters measured on a per-plant basis may mean that the examination of one particular feature, e.g. $N_2(C_2H_2)$ reduction, is not an investigation of that character alone, but is complicated by interactions with other traits.

Gritton (1975) found that heterosis in pea shoot weight also occurred in plants growing under conditions where nitrate was available, which indicates that its occurrence here (Table 3) was not dependant on heterosis for $N_2(C_2H_2)$ fixation. The heterosis of $N_2(C_2H_2)$ fixation per plant reported here may have been caused only by genes controlling fixation. However, fixation on a per-gram basis did not display heterosis, suggesting that heterosis in shoot size produced a correlated expression of heterosis in fixation per plant. The nature of the heterosis may also have been similar in these two characters. Shoot size heterosis may have been due to both non-allelic interaction and overdominance (Figs. 5 and 6); heterosis in fixation per plant was probably due in part to non-allelic interaction (Fig. 2).

Shoot N also showed differences in genetic control depending on which unit of measurement it was based. Total shoot N showed heterosis (Table 3) and ambi-directionality of dominance. Percent shoot N showed no heterosis above the highest parent (Table 3) and dominance was associated with decreased percent N, as was also found by Pandey and Gritton (1975).

The correlations shown in Table 4 also indicated that the relationships between characters depend on the units of measurement used. There is a very close link between all traits measured on a per-plant basis, but this is biased by the fact that all such measurements are made in the same units (plant size). The expression of shoot N on a percent basis or fixation on a per-gram basis produces very different correlations between characters (Table 4).

The speed of improvement of a character by plant breeding is increased by knowledge of the genetic architecture of that character and Breese (1972) describes the breeding methods that best exploit the differing types of genetic control. Breeding for an increase in $N_2(C_2H_2)$ fixation per plant may best be done in this population by producing hybrid varieties to exploit the heterotic and epistatic nature of the genetic control of the character. Breeding for an increase in $N_2(C_2H_2)$ fixation per gram nodulated root might best be done by the production of inbred varieties because additivity is the predominant method of genetic control. It appears, therefore, that the best breeding method for increasing fixation will depend on the units in which measurements are expressed. Because pea varieties are invariably inbred lines, selection on a per-gram nodulated root basis may be preferable.

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ACTON, D. F. and ELLIS, J. G. 1978. The soils of the Saskatoon map area 73-B Saskatchewan, University of Saskatchewan, Extension Publ. 306, Saskatoon, Sask. 145 pp.

BARNES, D. K., DEVINE, T. E. and SCHRADER, L. E. 1978. Recommendations for research on selecting and breeding legumes for enhanced nitrogen fixation. *In* T. A. LaRue, ed. Selecting and breeding legumes for enhanced nitrogen fixation. Proceedings of a workshop at Boyce Thompson Institute, Cornell.

BREESE, E. L. 1972. Biometrical genetics and its application. Pages 135–146 *in* F. G. H. Lupton, G. Jenkins and R. Johnson, eds. The way ahead in plant breeding. Proc. Sixth Cong. Eucarpia.

DUHIGG, P., MELTON, B. and BALTENSPERGER, A. 1978. Selection for acetylene reduction rates in 'Mesilla' alfalfa. Crop Sci. **18**: 813–816.

GRITTON, E. T. 1975. Heterosis and com-

bining ability in a diallel cross of peas. Crop Sci. **15**: 453-457.

HARDY, R. W. F. and HAVELKA, U. D. 1976. Photosynthate as a major factor limiting nitrogen fixation by field grown legumes with emphasis on soybeans. Pages 421-439 *in* P. S. Nutman, ed. Symbiotic nitrogen fixation in plants. Cambridge University Press, Cambridge. 584 pp.

HOFFMAN, D. and MELTON, B. 1981. Variation among alfalfa cultivars for indices of nitrogen fixation. Crop Sci. **21**: 8-10.

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

JINKS, J. L. 1954. The analysis of continuous variation in a diallel cross of *Nicotiana rustica* varieties. I. The analysis of F_1 data. Genetics **39**: 767-788.

LaRUE, T. A. G. and KURZ, W. G. W. 1973. Estimation of nitrogenase in intact legumes. Can. J. Microbiol. **19**: 304-305.

MAHON, J. D. 1977. Root and nodule respiration in relation to acetylene reduction in intact nodulated peas. Plant Physiol. **60**: 812-816.

MATHER, K. and JINKS, J. L. 1971. Biometrical genetics. Chapman and Hall Ltd., London. 382 pp.

PANDEY, S. and GRITTON, E. T. 1975. Inheritance of protein and other agronomic traits in a diallel cross of pea. J. Am. Soc. Hortic. Sci. **100**: 87-90.

PATE, J. S. 1977. Functional biology of dinitrogen fixation in legumes. Pages 473-517 *in* R. W. F. Hardy and W. S. Silver, eds. A treatise on dinitrogen fixation. Section III, Biology. John Wiley and Sons, New York. 675 pp.

PINCHBECK, B. R., HARDIN, R. T., COOK, F. D. and KENNEDY, I. R. 1980. Genetic studies of symbiotic nitrogen fixation in spanish clover. Can. J. Plant Sci. **60**: 509-518.

SCHWINGHAMER, E. A. 1977. Genetic aspects of nodulation and dinitrogen fixation by legumes: The microsymbiont. Pages 577-622 *in* R. W. F. Hardy and W. S. Silver, eds. A treatise on dinitrogen fixation. Section III, Biology. John Wiley and Sons, New York. 675 pp.

SEETIN, M. W. and BARNES, D. K. 1977. Variation among alfalfa genotypes for rate of acetylene reduction. Crop Sci. **17**: 783-787.

ZARY, K. W., MILLER, J. C., WEAVER, R. W. and BARNES, L. W. 1978. Intraspecific variability for nitrogen fixation in southern pea (*Vigna unguiculata* (L.) Walp). J. Am. Soc. Hortic. Sci. **103**: 806-808.