

# Symbiotic Interactions Between Strains of *Rhizobium phaseoli* and Cultivars of *Phaseolus vulgaris* L.<sup>1</sup>

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## ABSTRACT

Several cultivar-strain combinations of the *Phaseolus vulgaris* L.-*Rhizobium phaseoli* association were evaluated for N<sub>2</sub> fixation, N assimilation, and primary (biomass) production. Plants were grown in a greenhouse. Nitrogen fixation rates were estimated from H<sub>2</sub> and C<sub>2</sub>H<sub>4</sub>-production data. The relative efficiency of N<sub>2</sub> fixation was less than 0.60 indicating a loss of reducing power to H<sub>2</sub> evolution. Copious production of H<sub>2</sub> by all effective strains used in this study suggests that energy losses through H<sub>2</sub> evolution may limit the productivity of the *Phaseolus-Rhizobium* symbiosis. Assimilation of N by the symbiotic association in response to N<sub>2</sub> fixation was influenced by both strain and cultivar effects. Analysis of variance showed significant differences due to both symbionts in most growth parameters measured. However, there was no significant correlation ( $P < 0.05$ ) between N assimilation and the estimated rates of N<sub>2</sub> fixation in the various effective host-endophyte combinations. Assimilated N is a better, more direct measure of symbiotic efficiency than the estimate of N<sub>2</sub>-fixation rate by C<sub>2</sub>H<sub>4</sub> and H<sub>2</sub> production, and the merits of different growth parameters as measures of symbiotic effectiveness are compared and discussed.

**Additional index words:** Bean, Hydrogen evolution, Nitrogen fixation, Nodulation, Nitrogenase, Nitrogen assimilation.

THE effectiveness (Nutman, 1954) of symbiotic N<sub>2</sub> fixation is expressed in terms of enhanced growth and nutrition of the host plant and has been linked to genetic differences among strains of *Rhizobium* and compatible legume hosts. Strain characteristics of *R. phaseoli* (Burton et al., 1954) influence the response of *Phaseolus vulgaris* L. to inoculation. Host genotypic factors affecting nodulation and nodule activity have recently been identified in pulse legumes (Holl, 1975; Graham and Rosas, 1977; Westermann and Kolar, 1978; Zary et al., 1978; Graham, 1981). Screening of rhizobia for efficiency in N<sub>2</sub> fixation has led to the isolation of superior strains of *R. japonicum* (Caldwell and Vest, 1970), *R. leguminosarum*, (Nelson and Child, 1981), the chickpea miscellany (Minguez and Ruiz-Argueso, 1980), and rhizobia colonizing peanuts (Wynne et al., 1980).

Host-endophyte interactions have been investigated in some major field crops but only recently have received attention in *P. vulgaris* (Hohenberg et al., 1982). Variability in field responses to nodulation in *P. vulgaris* has been ascribed to unsatisfactory host-microsymbiont interactions (Graham, 1981), competition between *R. phaseoli* strains (Das and Bhaduri, 1974), and in temperate climates, to the environment (Sprent, 1982). A screening program for efficient strains under controlled conditions is therefore desirable (Date, 1976). Considerable scope exists for

selecting and modifying the genotypes of both *P. vulgaris* and *R. phaseoli* (Sprent, 1982). However, efficient strains must be matched with compatible hosts and the resulting association selected for suitable environments (Rys and Bonish, 1981) to achieve superior N<sub>2</sub>-fixing associations. The purpose of this study was to evaluate several cultivar-strain combinations of the *P. vulgaris* × *R. phaseoli* symbiosis, and to measure the effect of N<sub>2</sub> fixation on N assimilation and primary production.

## MATERIALS AND METHODS

### Biological Materials

Seeds of *P. vulgaris* L. cvs. Red Kidney, Topcrop, and Dwarf Horticultural (obtained from Burpee Co., Warminster, PA 18974)<sup>3</sup> were weighed and selected within a narrow range (0.6 to 0.7 g for Red Kidney, 0.4 to 0.5 g for Topcrop, and 0.5 to 0.6 g for Dwarf Horticultural cultivars). Seeds were surface sterilized successively in 70% (v/v) ethanol (1 min), 0.1% (w/v) HgCl<sub>2</sub> (10 min), and 0.01 N HCl (10 min) with a thorough wash between and after treatments, and were germinated for 3 days at 28°C. Three seedlings were aseptically transferred to pots containing 600 cm<sup>3</sup> of autoclaved perlite. Two plants per pot were removed after 1 week with the remaining plants selected to produce a uniform stand.

Cultures of *R. phaseoli* (strains 127K14, 127K46, 127K48, 127K59 originally supplied by J. C. Burton, Nitragin Co., Milwaukee, WI, 53209, and strains 2667, 2668, RCR3610, CIAT632, TAL182 obtained originally from H. H. Keyser, USDA, Beltsville, MD 20705) were maintained on yeast-mannitol agar slants at 4°C. Individual strains were cultured separately in Vincent's (1970) yeast-mannitol broth prior to inoculation, and dilution plate counts of viable cells were made. To eliminate differences in nodulation due to variable *Rhizobium* number, pots were inoculated with a saturating number of cells (approximately 10<sup>9</sup>) of the appropriate strain at planting and 7 days after planting to insure a high number of viable cells were present in the inert potting media during the initial period of infection. Controls were given 5 mL of sterile Vincent's medium to compensate for N input due to the nutrient broth.

### Growth Conditions

A total of 120 plants were grown in a greenhouse in Albany, Calif. between July and September 1981. Plants of each of the three cultivars were inoculated with one of the nine strains of *R. phaseoli*, or were left uninoculated as controls. The experiment was replicated four times, one replication per week, at 1-week intervals. Contamination between pots was minimized by placing them on a wide wire mesh screen above a corrugated fiberglass catchment system which was sterilized three times a week by spraying with 70% (v/v) ethanol.

Temperature and relative humidity varied from day to day within the day/night ranges of 20/32°C and 55/89%, respectively. Photosynthetic photon flux density (PPFD) varied between 450 and 900 μmol m<sup>-2</sup> s<sup>-1</sup> on cloudy or sunny days. Pots were rotated twice weekly to minimize

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<sup>3</sup> Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

Table 1. Tukey's Multiple Comparison Tests for plant and N<sub>2</sub>-fixation characteristics among *Rhizobium* strains combined over three *Phaseolus vulgaris* cultivars.

<i>Rhizobium</i> strain	Assimilated N†	Plant dry wt	Nodule dry wt	C <sub>2</sub> H <sub>4</sub> produced	H <sub>2</sub> evolved	SNA‡	RE§
	mg/plant	g/plant	g/plant	μmol C <sub>2</sub> H <sub>4</sub> /plant·h	μmol H <sub>2</sub> /plant·h	μmol N <sub>2</sub> /(g nod·h)	
TAL182	66.5 a*	2.45 a	0.235 b	21.00 b	9.8 b	14.9 cde	0.51 bc
127K46	55.9 b	1.94 bc	0.251 ab	23.41 a	10.4 ab	17.7 abc	0.53 ab
127K14	55.8 b	2.08 bc	0.217 c	18.76 c	9.0 c	12.8 e	0.51 bc
CIAT632	55.3 b	2.16 ab	0.240 ab	22.42 a	9.4 bc	20.2 a	0.58 a
2667	55.2 b	2.05 bc	0.174 d	17.43 c	9.2 bc	15.6 cde	0.45 c
127K48	53.4 b	2.09 bc	0.241 ab	23.18 a	11.5 a	16.4 bcd	0.59 a
127K59	53.2 b	1.89 c	0.255 a	22.75 a	11.5 a	14.3 de	0.48 bc
RCR3610	36.2 c	1.55 d	0.205 c	18.68 c	8.4 c	18.8 ab	0.54 ab
2668	2.2 d	1.42 d	0.038 e	0.18 d	0.0 d	2.06 f	—
Control	0.0 d	1.29 d	0.0 f	0.0 d	0.0 d	0.0 f	—

\* Means having common letters within a column are not significantly different at the 5% level of significance.

† Represents total N less seed N.

‡ Specific nodule activity (SNA) was calculated as  $[(C_2H_4 \text{ produced} - H_2 \text{ evolved})/3]/(\text{g nodule dry wt} \cdot \text{h})$ .

§ Relative efficiency (RE) was calculated as  $[1 - (H_2 \text{ evolved}/C_2H_4 \text{ produced})]$ .

positional effects. An additional 20 uninoculated control plants served to indicate if chance contamination occurred among treatments.

Pots were watered to field capacity 5 days a week with a nutrient solution (pH 6.4) consisting of 1.5 mM CaCl<sub>2</sub>, 0.5 mM KH<sub>2</sub>PO<sub>4</sub>, 0.25 mM K<sub>2</sub>SO<sub>4</sub>, and 0.25 mM MgSO<sub>4</sub>. Micronutrients were according to Johnson et al. (1957) at one-quarter strength and included 0.6 μM CoCl<sub>2</sub> and 20 μM Fe supplied as FeEDDA (ferric ethylenediamine di-(o-hydroxyphenyl)acetic acid). One day a week the pots were flushed with deionized water. Harvest was planned to coincide with high rates of nodule activity (Bethlenfalvai and Phillips, 1977b). Plants were harvested over a 3-day period during the 5th week after planting.

### Assays

Nodule activity (Pate, 1977) of excised roots (ATP-dependent H<sub>2</sub> production and C<sub>2</sub>H<sub>2</sub> reduction) was determined as described previously (Bethlenfalvai and Phillips, 1977a), with the following modifications. Ethylene analyses were made with a Varian Model 1400 gas chromatograph equipped with a flame ionization detector using a 0.32 × 183 cm stainless steel column filled with 80 to 100 mesh Poropak N. Hydrogen was measured with a Hewlett-Packard model 5880 gas chromatograph equipped with a thermal conductivity detector using a 0.32 × 183 cm column filled with 60 to 80-mesh molecular sieve 5A. Helium served as the carrier gas for C<sub>2</sub>H<sub>4</sub>, and N<sub>2</sub> for H<sub>2</sub>, both at a flow rate of 30 mL/min. Oven temperatures were 85°C for C<sub>2</sub>H<sub>4</sub> and 50°C for H<sub>2</sub>. Nitrogen fixation was estimated from C<sub>2</sub>H<sub>2</sub>-dependent C<sub>2</sub>H<sub>4</sub> production and H<sub>2</sub>-evolution data as: N<sub>2</sub> fixed = (C<sub>2</sub>H<sub>2</sub> produced - H<sub>2</sub> evolved)/3. The relative efficiency (RE) of electron transfer to N<sub>2</sub> via nitrogenase was calculated as  $RE = 1 - (H_2 \text{ evolved}/C_2H_4 \text{ produced})$  according to Schubert and Evans (1976).

Plant N content was determined by Kjeldahl digestion (Assoc. Official Analytical Chem., 1980). Assimilated N was calculated as the difference between total N and seed N. Dry weights of plant parts were measured after drying at 80°C for 2 days.

The experiment was of completely random design with 30 cultivar × strain combinations (including uninoculated controls) arranged over an area of homogeneous growth conditions. The experiment was repeated four times. Additional controls, used to check contamination, were interspersed among the experimental plants. An analysis of variance was carried out to determine the effect of strain, cultivar, or strain × cultivar interactions on symbiotic

growth (Dunn and Clarke, 1974). Linear regression analysis was performed according to Draper and Smith (1966) to determine the correlation between N assimilation and the estimated rate of N<sub>2</sub> fixation. Comparisons of significant differences among cultivars or strains for various parameters measured were made by Tukey's multiple comparison test (1953).

## RESULTS

### Effect of *Rhizobium* Strain on Host Plant

Mean fresh weight for the seeds of the Red Kidney, Dwarf Horticultural, and Topcrop cultivars were 0.65, 0.55, and 0.45 g, respectively. Mean N content for the seeds of the Red Kidney, Dwarf Horticultural, and Topcrop cultivars were 18.8, 17.1, and 14.4 mg N, respectively.

Nitrogen fixation by the nine strains of *R. phaseoli* was the only source of N for N assimilation by the host (Table 1), as the N content of control plants was essentially unchanged from that found in the seeds. All strains were capable of nodulation, even though one of the strains (2668) was ineffective (Table 1). Relatively large amounts of H<sub>2</sub> were produced by the other eight effective strains, and approximately half of the electron flow to nitrogenase was apparently used to reduce H<sup>+</sup> to H<sub>2</sub> (Table 1). None of the control plants were nodulated.

An analysis of variance (Table 2) showed that the main effects due to strain were significant ( $P < 0.001$ ) for all plant and N<sub>2</sub>-fixation parameters. Estimated N<sub>2</sub> fixation (as defined in Methods) was significantly correlated with assimilated N (0.91\*\*, significant at the 0.01 level of probability), N content (0.89\*\*), nodule dry weight (0.93\*\*), plant dry weight (0.75\*\*), and C<sub>2</sub>H<sub>2</sub> reduction (0.99\*\*) at the 0.05 (\*, significant at the 0.05 level of probability) and 0.01 (\*\*) levels. Correlations between the plant parameters and C<sub>2</sub>H<sub>2</sub> reduction or estimated N<sub>2</sub>-fixation calculated from H<sub>2</sub> and C<sub>2</sub>H<sub>4</sub> data showed no significant differences ( $P > 0.05$ ) between these two measures of nodule activity (0.89\*\* and 0.90\*\*, respectively).

Comparison of significant differences within the plant parameters due to strain effects by Tukey's multiple comparison test (Table 1) revealed no significant ( $P > 0.05$ ) differences between the uninoculated con-

**Table 2. Analysis of variance for assimilated N, plant and nodule dry wt, and the nodule activity parameters.**

Source of variation	df	Mean squares					
		Assimilated N	Plant dry wt	Nodule dry wt	N <sub>2</sub> fixation/plant	SNA	RE
Strain (S)	9	6219***	1.59***	0.110***	29.42***	560.3***	0.518***
Cultivar (C)	2	400*	0.74*	0.018*	0.56	70.5*	0.017*
S × C	18	128	0.13	0.003	1.27	22.7	0.007
Error	90	116	0.13	0.003	1.30	15.9	0.005

\*,\*\*\* Differences were significant at the 0.05 and 0.001 levels, respectively.

**Table 3. Tukey's Multiple Comparison Tests for plant and N<sub>2</sub>-fixation characteristics among *Phaseolus vulgaris* cultivars combined over nine *Rhizobium phaseoli* strains and the control.**

Cultivar	Assimilated N†	Plant dry wt	Nodule dry wt	N <sub>2</sub> fixation‡	SNA§	RE#
	mg/plant	g/plant	g/plant	μmol N <sub>2</sub> / (plant·h)	μmol N <sub>2</sub> / (g nod·h)	
Topcrop	45.6 a*	1.86 b	0.204 a	2.75 a	13.5 b	0.60 ab
Red Kidney	43.6 a	2.04 a	0.203 a	2.99 a	14.7 b	0.63 a
Dwarf Horticultural	39.4 b	1.77 b	0.167 b	2.84 a	17.0 a	0.59 b

\* Means having common letters within a column are not significantly different at the 5% level.

† Represents total N less seed N in cultivars inoculated with effective strains of *R. phaseoli*.

‡ Dinitrogen fixation was calculated as [C<sub>2</sub>H<sub>4</sub> produced - H<sub>2</sub> evolved]/3/(h·plant).

§ Specific Nodule Activity (SNA) was calculated as [C<sub>2</sub>H<sub>4</sub> produced - H<sub>2</sub> evolved]/3/(g nodule dry wt·h).

# Relative Efficiency (RE) was calculated as [1 - (H<sub>2</sub> evolved/C<sub>2</sub>H<sub>4</sub> produced)].

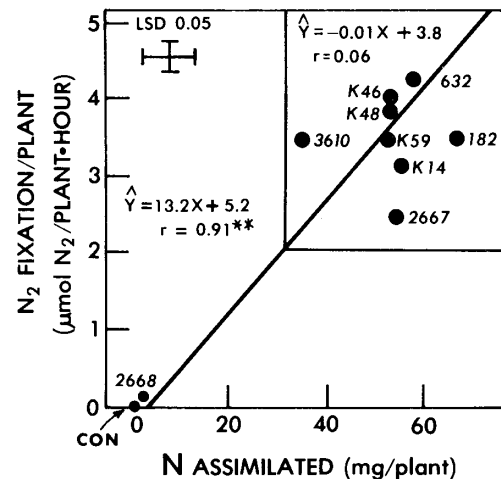
control and plants inoculated with strain 2668, with the exception of nodule weight. The correlation between assimilated N and estimated N<sub>2</sub> fixation was highly significant with the ineffective strain and control included but were not correlated among the effective strains (Fig. 1). Among the effective strains, TAL182 was superior and strain RCR3610 was least effective in providing N to the plant, even though both strains had similar rates of estimated N<sub>2</sub> fixation (Fig. 1). Host plants grown in association with the remaining strains assimilated amounts of N which were not significantly different, even though there were significant differences ( $P < 0.05$ ) for the estimated rates of N<sub>2</sub> fixation (Fig. 1) between strains.

#### Effect of Cultivar on Symbiotic Characteristics

Host genotype markedly influenced most of the symbiotic characteristics (Table 2), but no significant difference ( $P > 0.05$ ) in estimated N<sub>2</sub> fixation due to cultivar was found (Table 3). Dwarf Horticultural had lower dry weights, nodule weights, and assimilated N, while having significantly higher SNA than the other two cultivars. There were no significant ( $P > 0.05$ ) host × strain interactions (Table 2) indicating that no specific combination was superior over the broad range of all treatments.

#### DISCUSSION

The efficiency of N<sub>2</sub> fixation is often evaluated by relating the rate of C<sub>2</sub>H<sub>2</sub> reduction to other processes in a symbiotic legume association, such as ATP-dependent H<sub>2</sub> evolution (Schubert and Evans, 1976) or CO<sub>2</sub> fixation (Bethlenfalvay and Phillips, 1977c). These measures of N<sub>2</sub> fixation efficiency express transient cost-benefit relationships and do not consider other important aspects of the interaction between the symbionts, such as the transfer and utilization of fixed N by the host plant. The most meaningful measures of symbiotic effectiveness, N to C balance and association biomass (Phillips, 1980), are influenced by



**Fig. 1. Relationship between assimilated N and N<sub>2</sub> fixation for nine strains of *R. phaseoli* and an uninoculated control. Means and LSD were combined over three cultivars. Nitrogen fixation was estimated as defined in Table 3.**

the host's capability to assimilate N fixed in the root nodule. Assimilation, in turn, depends on total N input, integrated over the entire time span of effective nodule activity. However, the C<sub>2</sub>H<sub>2</sub> reduction and H<sub>2</sub> evolution assays provide only estimates of nitrogenase activity and are thus ill adapted to estimate total N input, especially if restricted to a single measurement.

The present data illustrate the points made above. Comparison of the estimated N<sub>2</sub>-fixation rate and the assimilated N of the association shows that the two expressions do not always correspond (Fig. 1). Among the cultivars associated with effective strains, those with the highest (TAL182) or lowest (RCR3610) levels of assimilated N did not have the highest (CIAT632) or lowest (2667) rates of estimated N<sub>2</sub> fixation (Fig. 1). Although assimilated-N levels in the TAL182 and RCR3610 associations were signifi-

cantly different, their  $C_2H_4$ - and  $H_2$ -production rates were not (Table 1). Conversely, assimilated-N levels of the CIAT632 and 2667 associations were alike, while their estimated  $N_2$ -fixation rates were significantly different. This observation can be attributed to differences in the intensity of  $N_2$  fixation at the time of measurement and to different time spans of effective  $N_2$  reduction in different associations. This lack of correlation between assimilated N and estimated  $N_2$ -fixation rate (Fig. 1) does not diminish the value of the  $C_2H_2$ -reduction assay in measuring nodule activity per se, but points to its inadequacy as a measure of symbiotic effectiveness, unless continuous or repeated readings, permitting an integration of the results, can be made. It is concluded that an evaluation of *R. phaseoli* strains for symbiotic effectiveness based on  $C_2H_2$  reduction is meaningful only in conjunction with an N-assimilation determination.

Specific nodule activity (SNA) is even more misleading than the estimated  $N_2$ -fixation rate as an indicator of the effect of nodule activity on the host. Nodule weight is a host-plant parameter. There was, however, little relationship between nodule weight and other measures of plant development (Table 1). A ranking of the values for SNA showed no relationship to the ranking of values for assimilated N (Table 1). Although frequently used in the literature, we consider SNA to be a parameter which should be used with caution in describing either the symbiotic effectiveness of the association or the rate of  $N_2$  fixation.

While the range of cultivars used in this study was limited, main effects due to the host plant were still significant (Tables 2 and 3). Values for assimilated N, averaged over the eight associations infected with effective *R. phaseoli* strains, were significantly different for the cultivars Topcrop and Dwarf Horticultural, even though estimated  $N_2$ -fixation rates were not significantly different (Table 3). This finding can be best ascribed to a property of the host which affects the transfer of N from endophyte to host, the subsequent incorporation of N by the plant and the loss of N through root exudation or photorespiration, even though variations in estimated  $N_2$  fixation rates not detected by our sampling method cannot be excluded as a cause. Low nodule weights in Dwarf Horticultural did not affect the estimated  $N_2$ -fixation rates per plant, but significantly increased the SNA value (Table 3).

The copious production of  $H_2$  by all of the effective strains suggested that energy losses through  $H_2$  evolution may limit the productivity of the *Phaseolus-Rhizobium* symbiosis. Relative efficiencies (RE) for the eight effective strains (Table 1) indicated that more than 40% of the available electron flow to nitrogenase was used to reduce  $H^+$  to  $H_2$ , thus significantly curtailing the production of  $NH_4^+$ . By this criterion (Schubert and Evans, 1976), these strains could be considered inefficient at  $N_2$  fixation, as their RE values were all approximately 0.50 (Table 1). Under these conditions the  $H_2$ -evolution assay appears to be of little importance in making comparisons of nodule activity between strains with similar relative efficiencies.

The capability for  $H_2$  uptake, currently unreported

in *R. phaseoli*, would increase the RE and may enhance symbiotic effectiveness (Zablutowicz et al., 1980). In the absence of native strains capable of  $H_2$  uptake, efficient strains might be engineered by introducing the uptake hydrogenase gene on recombinant plasmids through genetic transformation (Selvaraj and Iyer, 1981). Thus inoculation of beans with a *R. phaseoli* strain capable of recycling  $H_2$  might increase the productivity of the association.

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## Effects of Brown Midrib-3 on Yields and Yield Components of Maize<sup>1</sup>

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### ABSTRACT

The brown midrib-3 (*bm3*) gene associated with low lignin in corn (*Zea mays* L.) was evaluated for its effects on yield and yield components in Hawaii. Fifteen *bm3* hybrids representing a six-parent diallel and their 15 isogenic normal counterpart hybrids were planted as paired rows in two trials. Yields of the *bm3* hybrids were significantly lower than normals in both trials, with average reductions of 20% (range 7 to 29%) for grain and 17% (range 8 to 25%) for stover. Traits also affected negatively by *bm3* included kernel number per row (-12%), filled ear length (-10%), ear height (-7%), plant height (-5%), and five other measured traits. Correlation matrices of yields and all measured traits revealed few consistent differences among normal and *bm3* hybrids. Grain yields correlated highly with leaf area index for normal hybrids, but not for the isogenic *bm3* hybrids. Mean square values for general and specific combining ability (GCA, SCA) were significant for grain and stover yields and plant height, and GCA effects were significant for kernel number per row and ear length. The GCA/SCA ratios were higher for *bm3* hybrids than for normals for grain and stover yield. The gains in stover digestibility of *bm3* through lowered lignin content did not offset yield losses enough to justify separate breeding programs with *bm3*. Linkage of the *bm3* gene with yield-reducing genes was virtually excluded in this study as a cause for the yield reduction, and a role of colored lignins in reducing photosynthates is postulated.

**Additional index words:** Diallel analysis, General combining ability, Grain yield, Lignin, Silage yield, Specific combining ability, *Zea mays* L.

THE mutant gene brown midrib-3 (*bm3*) reduces dramatically the lignin contents of vegetative parts of corn (*Zea mays* L.) (11,15). Stover lignin reductions of up to 40% (16) were associated with increases of 10% or more in IVDMD (in vitro dry matter disappearance) and with significant increases in the feed value of *bm3* corn silage (1,6). Other brown midrib mutants also lowered lignin, but *bm3* was the most effective (11). Increased body weight gains and milk production were reported on *bm3* vs. normal

corn silage (5,7). Animals were found to have greater dry matter intakes and feed efficiencies on the brown midrib stover or silage.

The brown midrib trait has not attracted commercial interest despite its nutritional advantages. Decreased yields and increased lodging have been the evident reasons. Frenchick et al. (7) reported a 16.6% reduction in silage yield of a *bm3* hybrid vs. a normal that was nearly isogenetic (16.1 Mg/ha or t/ha vs. 19.3 Mg/ha). Lowered grain yields of three *bm3* hybrids vs. their normal counterparts at an intermediate stage in inbred conversion were observed by Tu and Bauman (18). The three hybrids ranged from 17.6 to 23.5% yield reduction due to the mutant gene, with greater reduction at 54 000 plants/ha than at 32 000 plants/ha. The data suggested that the *bm3* effect might be reduced by further backcrossing, since more advanced conversions showed less reduction (24%) than earlier conversions (32%). Recent studies of Miller et al. (13, 14) showed a 23% reduction in grain yield and 10% reduction in stover yield of hybrids based on first-generation inbreds, and 13% reduction in dry matter accumulation of the inbreds.

The present study was conducted to determine the effect of *bm3* on grain and silage yields and on yield components, using isogenic hybrids drawn from diallel crosses. The study was designed to assess the possibility of identifying superior hybrids for the *bm3* mutant, and for estimating combining ability for higher yield in the presence of this low-lignin gene.

### MATERIALS AND METHODS

Twelve inbreds and composites were converted in Hawaii to the *bm3* gene with five or more backcrosses. The gene was derived from H55*bm3* × H49*bm3* from the Maize Genetics Coop. Six of the most vigorous inbreds were chosen as parents for isogenic *bm3* and normal diallels, including three tropical inbreds—Hi27, Hi28, and Hi34—and three temperate inbreds—B37, Mo17, and Oh545. All six inbreds resulted from earlier conversions in Hawaii to genes for resistance to MMV virus, common rust, and Northern leaf blight (3,4,12).

Yield trials of the 15 hybrids were planted on the Waimanalo Research Station of the Univ. of Hawaii on 12 Aug.

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