

Use of Hill Plots for Genetic and Breeding Studies of Bean¹

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Abstract. The precision of a 9-plant hill-plot design in which plants were sown 15 cm apart in a 3 × 3 arrangement compared favorably to that of 3-m-row plots containing approximately 75 plants for the estimation of pod yield of snap beans *Phaseolus vulgaris* L. Quality traits and sieve size distribution based on pod diameter in both plot designs were similar. Using the square design, the entire 9-plant plot can be taken as the unit of selection, or single plant selection can be practiced when the test plant is grown in the center hill surrounded by 8 uniform guard plants. Single plant selection using this design has been used effectively to modify traits of beans having moderate to high heritability. Selection based on family means should be used for traits with low heritability. Efficiency of the hill-plot design is realized in terms of smaller plot size, fewer required seeds per plot and reduced harvest time.

Experimental techniques that are statistically precise and economically efficient contribute substantially to identification of superior individual plants or families within genetically variable populations. Ideally the type of field plot used should reflect current or anticipated cultural practices under which the crop is grown commercially. Since most snap and dry beans are grown in rows, row plots have been used to evaluate yield and quality traits. The number of rows per plot, distance between rows and the use of guarded or unguarded plots vary with the experimental requirements and resources available.

Mack and Hatch (8) have shown that plant spacing and planting design affect yield of snap beans. In an effort to raise pod yield and improve quality, commercial producers are now interested in the potential offered by growing plants at an optimum density in narrow rows or in a uniformly-spaced arrangement rather than in the conventional widely-spaced rows. Current row plot designs are inadequate for experiments involving this concept.

Row plots are of limited value in bean breeding programs that utilize either early generation single plant selection or selection between advanced families. In the first instance, the progeny of plants spaced far enough apart within each row to allow easy evaluation and selection often respond differently at higher plant densities. Higher plant densities in experimental plots also cause difficulties during selection and harvest. Selection between families requires that adequate seed is available from specified parent(s) to plant a given progeny plot. Single bean plants usually do not produce more than 100-150 seeds. Row plots 3-3.5 m long require 25-35 seeds per m of row and provide limited opportunity for adequate replication. Families must often be bulked and advanced to later generations for replicated trials. That procedure is expensive and time consuming. Plots that employ few plants without greatly sacrificing accuracy and precision would be useful.

Snap bean breeders must work within even greater restrictions. Instead of harvesting mature dry seed, succulent pods are evaluated. Meaningful evaluations of pod yield and quality traits must be made during a short period when the pods are at optimum quality. Furthermore, it is desirable to make multiple harvests of each experimental line to determine yield levels and the rate of development of pods, seeds and fiber. The necessity for harvesting and evaluating large amounts of pods in a short time increases the desirability of utilizing small plots that contain relatively few plants.

The concept of hill plots is not new. In 1947, Bonnett and Beaver (3) discussed the use of hill plots in selecting for disease resistance in cereals. The reports of Jellum et al. (6), Frey (5) and Baker and Leisle (1) suggest that they provide a useful technique for genetic and breeding studies and early generation selection in certain cereal grains.

The use of hill plots has also been reported in legume breeding programs. Torrie (11) found that relative performances of soybean cultivars in hill and row nurseries were similar. He concluded that more replicates would be needed using hill plots to estimate yield differences between cultivars, but for other traits the precision was similar. Schutz and Brim (9) employed a hill plot design in which soybean plots consisted of 9 hills (3 × 3 hills equally spaced with 2 plants growing in each hill) of which only the 5 guarded hills were harvested (i.e., corner hills were excluded). They concluded that the plot yield included only about 30% of the competition bias expressed by an unbordered hill. The coefficients of variation for hill and row plots were 19% and 14% respectively, and comparisons with row plots indicated that in both designs the genotypes ranked in the same order of performance. Each row plot, however, required approx. 4 times more space than a corresponding hill plot.

The objectives of the research reported herein were to develop an experimental plot technique suitable for studies involving equally-spaced arrangements of plants, to compare the precision of hill and row plots and to determine whether hill plots can be used effectively in selection programs aimed at improving quantitatively inherited traits of common bean, *Phaseolus vulgaris* L.

Materials and Methods

Commercial cultivars commonly grown in Wisconsin and promising advanced breeding lines of snap and dry beans were studied. Experiments were conducted primarily at the Hancock, Wisconsin Experiment Station where the soil is Plainfield sand. Supplemental overhead irrigation was used as needed to prevent drought stress. Eptam (3.4 kg/ha) and 5N-4.3K-24.9K fertilizer (170 kg/ha) were applied prior to planting. Some additional studies were conducted on commercial farms in central Wisconsin with similar cultural practices.

Row plot experiments were arranged in a randomized complete block design with 4 replicates. Each plot consisted of a single row 3.7 m long (trimmed to 3 m prior to harvest) planted 90 cm apart. A V-belt hand seeder was used for planting at rates of 30-35 seeds/m of row. Stands were thinned to approx 25 plants/m. At each location, 2 replications of row plots were harvested on each of 2 days to allow machine grading, counting, weighing and visual evaluation of pods on the same day as harvested. Yields of row plots were recorded as total pod wt from all plants in the 3 m row. Pods were separated

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Table 1. Coefficients of variation, $(s \cdot 100)/x$, for snap bean trials when cultivars were grown in row and hill plots in replicated nurseries in central Wisconsin (pod yield/plot).

Year	Plot design ^z	No. of replications ^y	Location		
			Hancock station	Knutson farm	Reid farm
1966	R	4	25	20	17
1967	R	4	17	16	21
1969	R	4	15	21	12
1970	R	4	10	20	
1970	H	4	14	23	
	H	4	17	14	
	H	4	17	17	
1971	H	4	18	19	
	H	4	20	12	
	H	4	14	16	
1972	H	4	14		
	H	4	18		
	H	4	12		
1973	H	2	18		
	H	2	17		
	H	2	18		

^zR = row plots, H = hill plots.

^yAll trials were planted in randomized complete block designs except in 1973, when a simple lattice design was used.

by a commercial grader into 3 sieve sizes according to diam, and the percent by weight of each size was recorded.

Each hill plot consisted of 9 plants (spaced 15 cm apart in both directions), grown in a 3 x 3 plant arrangement, with plots 45 cm apart. Planting was facilitated by the use of a board having pegs to make depressions in the soil. Seeds were dropped into these depressions, covered by hand and irrigated with an overhead sprinkler to settle the soil around the seeds. Two seeds were planted in each hill to insure a complete stand, and 2 weeks after emergence the plants were thinned to 1 per hill. When whole plots were used as the unit of selection all 9 plants were harvested to provide the data. Yields of hill plots were recorded as total pod wt per plot. Pods were separated manually with the use of a small pocket grader into 3 sieve sizes corresponding to those of the machine grader, and the percent by weight of each size was recorded.

When the hill plot design was used in single plant selection experiments, the middle hill was planted with experimental seeds from the segregating family. The 8 hills at the perimeter of the plot were planted with a standard cultivar to provide uniform competition to each test plant.

Table 2. Comparisons of pod yield (g/plot) and sieve size distributions (% by wt) for snap bean cultivars grown in row and hill plots at 2 locations, 1970.

Location and cultivar	Row plots ^z			Hill plots ^y				
	Yield (g/plot)	Distri- bution (%)			Yield (g/plot)	Distri- bution (%)		
		Sieve size	5+	4		3	Sieve size	5+
<i>Hancock Expt. Station</i>								
Tempo	3895	64	22	14	421	36	45	19
Sprite	2401	8	67	25	367	19	50	31
Prossessor	3078	53	30	16	356	21	46	33
Slenderwhite	2361	22	52	26	306	22	58	21
Cascade	2518	25	36	39	367	30	54	16
Tenderette	2771	36	42	22	314	34	52	14
Astro	2432	40	35	26	371	27	53	20
W.S. Tendercrop	2276	22	43	35	313	32	47	21
BBL 274	2939	26	41	33	429	32	53	15
Tenderwhite	3220	34	49	17	350	30	49	21
Earligrreen	3392	27	54	18	313	24	56	19
Slimgreen	2838	16	52	31	327	27	59	14
Mean	2843	31	44	26	353	28	52	20
<i>s_x</i>	83				17			
<i>Knutson Farm</i>								
Tempo	2303	47	34	19	252	43	39	18
Sprite	3531	7	72	22	292	6	67	27
Prossessor	3681	43	41	16	274	17	62	21
Slenderwhite	2798	18	58	24	245	17	57	26
Cascade	4039	41	41	18	289	35	40	25
Tenderette	3591	51	36	12	288	42	39	19
Astro	2998	46	33	22	310	24	55	21
W.S. Tendercrop	3165	18	54	29	314	31	45	24
BBL 274	4045	64	25	11	415	48	33	19
Tenderwhite	2879	37	47	16	275	35	46	18
Earligrreen	3058	24	59	17	216	24	55	21
Slimgreen	2992	11	57	32	262	12	61	27
Mean	3257	34	46	20	286	28	50	22
<i>s_x</i>	189				17			

^zMeans of 4 replications.

^yMeans of 12 replications.

^xSize 3, approx 64 mm diam; 4, 79 mm diam; 5, 97 mm diam. (Per- cents are by wt.)

A randomized complete block design was used when comparisons involved a relatively few homozygous strains and the whole plot was the unit of selection. For comparisons among 64 advanced families and standard cultivars of snap beans, a simple lattice design was employed for each of 3 harvest dates. Suitable statistics were used to compare the

Table 3. Mean yields and estimates of sources of variation among 12 snap bean cultivars grown in row and hill plots in replicated nurseries at the Hancock Experiment Station (H) and Knutson Farm (K).^z

Year	Plot yield (pod wt/plot)		CV(%)		VG		VE		VG/ (VG + VE)	
	H	K	H	K	H	K	H	K	H	K
	1970 - Rows	2843	3257	10	20	219418	170124	83168	427388	.73
1970 - Hills										
Harvest 1	301	290	14	22	1502	3537	1865	4338	.45	.45
2	368	284	16	14	1629	636	3775	1662	.30	.28
3	390	283	16	17	1685	3623	4103	2371	.29	.60
1971 - Hills										
Harvest 1	477	308	18	19	7039	985	7569	3554	.48	.22
2	599	432	20	12	8899	757	12075	2609	.42	.22
3	608	482	15	16	8991	3423	8022	6236	.53	.35

^zAll figures based on 4 reps.

CV = coefficient of variation; VG = variation among cultivars; VE = environmental variation.

precision and efficiency of hill and row plots and to assess their utility for evaluation trials and selection experiments (7, 10).

Results and Discussion

Whole plots as the unit of selection. Coefficients of variation (CV) for pod yield per plot were calculated for commercial snap bean cultivars grown in hill and row plots in replicated nurseries in central Wisconsin (Table 1). Although few data are available in the literature for comparison, the values for row plot experiments appeared to be representative, and they were consistent over several years and at different locations. The CV's for row plots appeared to be smaller, suggesting that additional replication of hill plots would be desirable for equal precision. More hill plots can easily be added because of the modest amount of seed, land, and labor required for each additional replication. The relative efficiency of hill plots vs. row plots for evaluating seed yield of dry beans was similar to that of snap bean yield. In 1973, a CV of 22% was determined for a hill plot nursery of 20 cultivars at the Hancock Experiment Station. CV's for seed yield of dry beans grown in row plots in Wisconsin have varied from 7% to 17%, depending on year and location of the trial (4).

In 1970, 12 snap bean cultivars were grown in hill and row plots in nurseries at 2 locations. The values of Kendall's coefficient of rank correlation were computed to be .41 at the Hancock Experiment Station and .10 at the Knutson farm. These values suggest little relationship between the ranks based on pod yield of cultivars grown in hill and row plots at Hancock, but a strong relationship at the Knutson farm. The sieve size distributions for each cultivar were similar regardless of the type of plot (Table 2). Visual estimates of pod color, curvature, seed size and interocular cavitation provided no indication of differences due to type of plot.

Estimates of population parameters useful to the breeder are often computed from plot experiments. Variation in pod yields among hill plots (1970, 1971) and row plots (1970) was partitioned into components due to 1) differences among cultivars (VG), 2) environmental variation among plots (VE) and 3) differences among replications. The ratio, $VG/(VG + VE)$ provided an estimate of broad sense heritability (Table 3). The values estimated using either hill or row plots were similar. There was also consistency of estimates between years and locations when hill plots were used. Since hill plots were smaller, cheaper and easier to maintain, additional resources could be used for other determinations such as multiple harvests which facilitate estimation of yield increase relative to rate of pod development.

In 1973, 4 standard cultivars and 60 F₅ breeding lines derived from crosses of 'Cascade' and 'Tenderette' with OSC410-1816-1 were evaluated in an 8 x 8 simple lattice design. Using only 2 replications per harvest date, this experiment

provided good estimates of differences in pod yield, pod number and rate of development (Table 4). Inspection of the data for the 4 standard cultivars showed that the differences in yields were largely associated with the number of pods and the stage of pod maturity. Such information could be used to adjust yield to reflect differences in maturity and pod number, and provide more meaningful comparisons between families.

The importance of competition between adjacent plots in both hill plot and row plot experiments appears to vary with the species and strains being tested, the planting configuration and the distance between plots. The use of common borders between hill plots (6) and the inclusion of only certain hills from each plot for data collection (9) have been suggested to reduce unequal competition between hill plots. In the experiments reported herein, there was no evidence of competition between rows of snap beans grown 90 cm apart. Based on a limited investigation of border effects, no precision was gained by separating hill plots by common border plants instead of separating them by an unplanted space of 45 cm.

Single plants as the unit of selection. Single plant selection based on phenotypic expression in early segregating generations is often ineffective for improving quantitative traits having low heritabilities. The inability to grow experimental plants at population densities similar to those encountered commercially and non-uniform plant competition contribute substantially to a large non-genetic variance component which lowers heritability. Since beans cannot be readily propagated asexually, single plants cannot be replicated. Thus, effective selection for quantitative traits has usually been based on replicated trials of advanced generations (F₃ and beyond).

The hill-plot design was useful for studying genetic expression of percentage seed protein, for estimating variance components and for measuring gain from directional selection for this trait (2). Individual plants of segregating and non-segregating generations of experimental populations were grown in the middle hill of the 9-plant hill plot and surrounded by plants of a standard cultivar. Care was taken to use a standard cultivar that is not extremely large or competitive. The CV based on phenotypic expression of % protein among individual plants of non-segregating generations (parental and F₁) were low, while greater variability was present in the F₂ populations, suggesting the presence of an additional component due to genetic segregation in the latter (Table 5). Selection of phenotypically superior plants in the F₂ and F₃ generations was effective in raising the mean of the selected population above that of the unselected population and in identifying individuals that exceeded the better parent (2).

The effects of non-genetic factors on yield and yield components of both snap and dry beans are known to be substantial. Even with the precision provided through the use of hill plots, the non-genetic variability among plants remained

Table 4. Pod yield and pod number per plot from multiple harvests of standard snap bean cultivars and breeding lines grown in hill plots (8 x 8 simple lattice) at the Hancock Experiment Station, 1973.

Strain	1st harvest ^z			2nd harvest ^z			3rd harvest ^z		
	Plot wt (g)	% sieve (by wt)		Plot wt. (g)	% sieve (by wt)		Plot wt (g)	% sieve (by wt)	
		5+	4		3 pods	5+		4	3 pods
60 breeding lines (range)	273-746		53-141	442-972		86-151	779-1637		125-201
BBL 274	181	2	22 76 44	501	10	28 62 100	1034	25	34 40 170
Tenderette	445	12	35 54 494	755	25	37 37 133	1043	24	54 22 157
Cascade	570	4	48 48 100	801	15	34 51 140	1211	21	43 36 186
Provider	849	28	49 23 124	885	43	30 27 130	1417	47	31 23 178
CV	17.8%			16.5%			17.7%		

^zMeans of 2 replications, each having 9 plants per plot.

Table 5. Mean percentage of seed protein and coefficients of variation based on single plants grown in hill plots, 1972. Four parents, F₁, F₂ generations and a standard cultivar, Hancock Experiment Station.

Parents and populations	n	% protein	CV (%)
713893 ^Z	23	24.7	4.4
713895 ^Z	33	25.1	7.3
713896 ^Z	48	28.1	6.6
713897 ^Z	24	31.9	5.6
F ₁ (713893 x 713896)	23	24.7	5.2
F ₂ (713893 x 713896)	145	25.8	9.4
F ₁ (713895 x 713896)	22	28.4	7.5
F ₂ (713895 x 713896)	106	26.8	8.6
F ₁ (713897 x 713896)	10	26.3	5.6
F ₂ (713897 x 713896)	73	29.4	10.4
Sanilac	9	26.3	6.7

^ZHomozygous breeding lines used as parents.

high and realized gain from single plant selection is therefore expected to be low. This suggests that the breeder should use a selection procedure based on selection among means of advanced families grown in replicated trials to improve these traits. Variability among family means was reduced substantially compared to variability among individuals in both 1973 and 1974 (Table 6).

The use of hill plots provides an additional tool for the plant breeder who wishes to select among individuals in early generations to improve traits of moderate to high heritability. If extensive evaluation of families is desired, hill plots require small inputs of land, labor, and seed, thus allowing more replications and selection based on family performance. Although the precision of hill plots appears to be slightly less than that for row plots when evaluating traits such as yield on a per-plot basis, both appear to be equally good for quality factors. This design offers the opportunity to study factors affecting the performance of beans grown in equally-spaced planting arrangements rather than in the traditional row plots, thus gaining information useful for high-density planting.

Table 6. Means and CV for pod number and pod wt per plant of snap bean parents and F₁'s based on individual plants grown in hill plots, Hancock Experiment Station, 1973 and 1974.

Trait	Population	No. of families ^Z	Means		CV			
			1973	1974	Indiv. plants		Family \bar{x}	
					1973	1974	1973	1974
Pod no./ plant	parents	5	18.7	22.2	34.5	31.1	25.8	16.7
	F ₁ 's	10	27.6	34.6	30.3	25.4	14.4	11.9
Pod wt./ plant	parents	5	111.7	118.9	43.9	32.4	25.2	6.2
	F ₁ 's	10	165.1	213.2	37.1	27.3	20.4	12.9

^ZThree 10-plant replications in each family.

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