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Genetic Relationships among Cultivars and Landraces of Lima Bean (*Phaseolus lunatus* L.) as Measured by RAPD Markers

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Abstract. **Knowledge of relative genetic distance among genotypes is useful in a breeding program because it permits organization of germplasm resources. Genetic distance (GD) was estimated among 65** *Phaselous lunatus* **L.. accessions, which included 4 large-seeded and 7 small-seeded cultivars and 54 germplasm accessions (landrace's) from the Caribbean and North, Central, and South America. Based on 125 polymorphic random amplification polymorphic DNA (RAPD) bands, two major clusters, which generally correspond in seed size and geographic region to [be Mesoamerican and Andean gene pools, were observed among the landraces (GD = 0.726 ± 0.041). Four Fordhook cultivars and a landrace from the United States formed a separate cluster that is more distantly related to the small- (GD) = 0.561 ± 0.039) than to the large-seeded cluster (GD = 0.303 ± 0.022). The mean GD between the Andean and Mesoamerican (0.726), Mesoamerican and Fordhook (0.561), and Andean and Fordhook (0.303) clusters were all significant. The significant GD between the Andean and Mesoamerican groups supports the hypothesized existence of two major gene pools in lima bean. The RAPD marker diversity of the Mesoamerican group was the largest (0.1 10), followed by the Andean (0.097) and Ford hook (0.062) groups. The plot of the relationship between the coefficient of variation (cv) and sample size (number of bands) indicates that cvs as low as 10% for estimating CD between Andean and Mesoamerican lima bean accessions can be achieved by sampling as few as 100 bands.**

Cultivars and landraces of a crop species represent the primary gene pool available to plant breeders for hybridization and improvement of crop plants (Harlan and De Wet, 1971). Knowledge of the genetic relationships among cultivars and between cultivars and landraces is useful to the plant breeder because it permits organization of germplasm resources and allows for more efficient sampling of available genetic diversity.

Patterns of genetic diversity have been studied in crop species using a variety of molecular, chemical, and morphological descriptors. The most commonly used molecular tools for measuring genetic relationships have been isozymes, seed proteins, and molecular markers. Although informative and practical. the use of' variable protein and isozyme markers has often been limited by their low frequency in many crop species (Goodman and Stuber, 1980). Molecular markers provide an opportunity to measure genetic relationships more precisely than morphological and biochemical markers because they 1) are potentially unlimited in number, 2) are not affected by the environment, and 3) can be organized into linkage maps (Soiler and Beckmann. l983; Helentjaris et al., 1986). Estimates of genetic relationships based on restriction fragment length polymorphisms (RFLPs) have been shown to be consistent with expectations based on known breeding behavior and pedigrees in numerous crop species, including maize (Dudley, 1994; Smith et al., 1990). More recently, scientists have used random amplified polymorphic DNA (RAPD) molecular markers as a tool for measuring genetic relationships (Skroch et al., 1992; Welsh and McClelland, 1990, Williams et a1., 1990). RAPDs are technically simpler and cost less than RFLPs; however, reproducibility of banding patterns can be affected by different concentrations of reaction components and cycling conditions (Weeden et al., 1992). Nevertheless, in a study comparing molecular markers, the sampling variances associated with RAPDs and RFLPs were found to be similar for estimation of genetic relationships among *Brassica oleracea* genotypes (dos Santos et al., 1994).

Based primarily on the size, shape, and color of seeds, lima bean diversity was originally organized into three main gene pools: 1) the Hopi blanch, extending northward from Central America into the United States; 2) the Carib branch, including the islands in the West Indies and Amazon basin of Brazil; and 3) the Inca branch. extending southward from Central America to Peru (Mackie, 1943). More recent evidence, based on patterns of isozyme variation, indicates the existence of only two gene pools in lima beans, Mesoamerican and Andean (Baudoin, 1988; Debouck et al., 1989). The Meso american lima bean gene pool extends from the southwestern, United States to Argentina and is characterized by small seed size (0.24 to 0.70 g/seed) (Debouck et al., 1989: Maquet et al., 1990). The Andean lima bean gene pool extends from the equator to north of Peru for wild forms and from Colombia to southern Brazil for cultivated forms and is characterized by large seed size (>0.54 g/seed) (Debouck et al., 1989; Maquet et al., 1990; Maquet et al., 1993).

Lima bean cultivars harvested at the immature seed stage for processing as a vegetable are generally classified into two groups: Henderson (baby lima) and Fordhook. Henderson types correspond to the Mesoamerican gene pool and are characterized by broad environmental adaptation and small seed size (<0.50 g/seed). The original Henderson cultivar was apparently identified in 1883 in Virginia as a chance bush (determinate) plant in a landrace of lima beans cultivated by the Hopi Indians (Holland et al., 1953;

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Mackie, 1943). The Fordhook types correspond to the Andean gene pool and are characterized by narrow environmental adaptation and large seed size (>.50g/seed), Fordhook bush cultivars were derived from landraces that were adapted to the long, cool, dry summers in the Ica region on the coast south of Lima, Peru (Mackie, 1943). The objective of this research was to determine if RAPD molecular markers could be used to 1) estimate the genetic relationships among modern lima bean cultivars derived from the Fordhook and Henderson types and a random sample of accessions (landraces) from germplasm collections and 2) estimate the relative magnitudes of' genetic diversity (RAPD marker variance) among groups of accessions.

Materials and Methods

Germplasm. Sixty-five lima bean genotypes were used in this study (Table 1). Lima bean cultivars included four large-seeded (≥ 0.63 g/seed) Fordhook types and seven small-seeded (≤ 0.44 g/seed) Henderson (baby lima) types Fifty-four random lima bean accessions (landraces), which represented a broad range of lima bean diversity, were included in this study. Twenty-seven of the accessions were obtained from Steve Beebe, Genetic Resources Unit, C. I. A. T., Cali, Colombia (G preceding accession number) and the remaining twenty-seven accessions from Richard Hannan, U.S. Dept. of Agriculture-Agricultural research Service, Plant Introduction Station, Pullman. Wash. (PI preceding accession number).

Phenotypic characterization. Mean seed weight was calculated from a sample of 10 seeds from each entry (Table 1). Seed width was measured as the longest distance on the flattest surface of the seed bisecting the hylum, seed height was the longest distance between the two flattest surfaces of the seed, and seed length was measured as the longest distance on the flattest surface of the seed parallel to the hylum. The mean width, height, and length of a 10-seed sample were calculated for each entry. Five seeds of each entry were planted in June 1992 in unreplicated, 1-m-long plots at the Hancock (Wis.) Experiment Station, Hancock. using standard cultural practices. Each accession and cultivar was subjectively classified for flowering as follows: 1 = flowered and produced many seeds, 2 = flowered and produced few seeds, 3 = flowered but did not produce seeds, 4 = flowered late in season and, 5 = did not flower.

RAPD procedures. A sample of 10 seeds of each entry was germinated in a greenhouse, and 2 g of immature unifoliate leaves was used for extracting DNA, The fresh leaves were ground in 10 ml of extraction buffer at 65C using a mortar and pestle. The extraction buffer contained 2% CTAB, 100 mM tris (pH 8.0). 20 mM EDTA (pH 8.0). 1.4 M NaCl, and 1% PVP (polyvinylpyrolidone) (Rogers and Bendich, 1988). After grinding, the tissue was transferred to centrifuge tubes and allowed to soak for 30 min in a hot water bath at 65C. Following this soak, the mixture was extracted once with an equal volume of 24 chloroform : 1 isoamyl alcohol.

After organic and aqueous phases were separated by centrifugation, nucleic acids were precipitated by pipetting the aqueous phase into at least three volumes of a 6:1 mixture of 95% ethanol and ammonium acetate, Precipitating nucleic acids were allowed to stand overnight at –20C. Nucleic acids were transferred with a wide bore pipette into 1.5-ml microcentrifugc tubes. After spire ning for 15 to 30 sec at 14,000 rpm in a microcentrifuge, pellets were rinsed with a 70% ethanol solution and repelleted. The pellet was rehydrated in a dilute TE buffer (1 mM tris and 0.1 mM EDTA). RNA was removed by treatment with RNase A at 100 $\mu g \cdot ml^{-1}$ for 1 h at 37C. Samples were then spun and transferred to clean microcentrifuge tubes. DNA was precipitated by the addition of at least two volumes of a 20:1 mixture of 95% ethanol and sodium acetate. After precipitation and pelleting, DNA was rehydrated in a TE buffer and quantified using a Hoefer Scientific TKO 100 fluorometer.

RAPD reactions were mixed in volumes of 10 µl using the following reagents: 20 ng of genomic template, 100 µM of dNTP, 0.4 µM of primer, 0.6 units of *Taq* polymerase (Promega, Madison, Wis.), 2.0 mM of $MgCl_2$, 50mM tris pH 8.5, 2 mM $MgCl_2$, 20 mM KCl, 5 $\mu g \cdot \mu l^{-1}$ BSA, 2.5% of Ficoll 400, and 0.002 % xylene cyanole. The reactions were performed in glass capillary tubes in a thermal cycler (Idaho Technology, Idaho Falls, Idaho). The thermal cycler was programmed to cycle 40 times under the following conditions: for the first two cycles, denaturation for 60 sec at 91 C, annealing for 7 sec at 42C, and elongation for 70 sec at 72C; the subsequent 38 cycles were run with the denaturation time reduced to 1 sec at 91C. After amplification, the reaction products were separated by electrophesis in 1.5 % agarose gels, stained with ethidium bromide, and photographed under ultraviolet light with Polaroid 667 film.

The number of polymorphic primers used from sets A, B, C, D, E, F, and L (Operon Technologies, Alameda, Calif.) were 3,5, 5, 6, 4, 1, and 1, respectively. Information on specific primers is available from the authors. Polymorphic bands were classified as intense, medium, or faint, based on resolution and degree of' amplification (Weeden et al., 1992). Only bands classified as intense or medium were included in the analysis. From 2 to 8 bands, which were polymorphic among the genotypes included in this study, were scored for each of the 25 primers resulting in a total of 125 scored bands.

Genetic distance estimates and MDS plots. Each polymorphic RAPD band across all accessions was assigned a number (1, 2, 3 ...n) according to decreasing molecular weights. Each band was treated as a unit character, and the accession was scored for the presence or absence of a band and coded as 1 or 0, respectively. Genetic distances were calculated between all 2080 pairs of accessions based on the following formula, which is the complement to the simple matching coefficient (Gower, 1985):

$$GD(i,j) = S^N(i \neq j) / [S^N(i \neq j) + S^N(i \neq j)]$$

where GD(i,j) is the measure of genetic distance between accessions i and j, $S^N(i \neq j)$, and $S^N(i \neq j)$ are the total number of discordant and concordant scores between accessions i and j, respectively. GD values of 0.0 and 1.0 indicate no and maximum difference between two accessions, respectively.

The 65 × 65 triangular matrix of GD values is too lengthy to be presented here, but is available upon request from J. Nienhuis. The 65 × 65 matrix of GD estimates was reduced to two dimensions and displayed as a multidimensional scaling (MDS) plot (Wilkinson, 1989). Based on the MDS plot, the accessions were classified into two groups , Mesoamerican and Andean, which generally corresponded with seed size and geographic origin. The Andean group included 25 entries, excluding the Fordhook-type cultivars; the Mesoamerican group included the seven Henderson-type cultivars and 28 accessions. Separate MDS analyses were performed on the GD matrices corresponding to the Andean and Mesoamerican groups.

Mean CD was calculated between the three groups observed in the MDS plot: Mesoamerican, Andean, and Fordhook types. An F test modified from the analysis of molecular variance (AMOVA) was used to test the significance of differences between the groups of accessions (Excoffier et al., 1992). The relevant F value to test the significance of differences between groups of accessions was

Table 1. Lima bean cultivars and accession included in genetic distance study.

Entry no.	Name or[z] accession	Origin	Seed[x] Color[y]	Wt (g)	Width (mm)	Ht (mm)	Length (mm)	Flowering[w] classification
			Cultivar					
1	Burpee Improved	U.S.	White	1.19	16.3	13.4	20.3	1
2	Fordhook 242	U.S.	White	1.14	8.0	12.6	16.2	1
3	King of Garden	U.S.	White	1.00	5.9	12.0	21.0	1
4	Fordhook 169	U.S.	Green	0.63	6.0	10.4	14.9	1
5	Maffei 15	U.S.	White	0.44	5.3	10.8	14.4	1
6	Jackson Wonder	U.S.	Brown/light	0.40	4.4	9.9	15.0	1
7	Early Thorogreen	U.S.	Green	0.39	4.6	10.4	13.4	1
8	New Kingston	U.S.	White	0.38	6.2	9.9	11.1	1
9	Packer	U.S.	White	0.36	4.8	9.2	12.6	1
10	EastLand	U.S.	Green	0.33	4.3	10.4	12.5	1
11	PSBG1-bridgeton	U.S.	Green	0.29	5.0	9.0	11.2	1
			Germplasm bank accessions (landraces)					
12	PI306196	Peru	White	2.06	6.0	18.0	28.0	4
13	PI241791	Peru	White	1.50	7.3	16.3	25.4	3
14	PI256872	Peru	White	1.43	5.8	14.2	23.3	5
15	PI256883	Peru	White	1.41	4.6	14.4	21.8	4
16	PI306197	Peru	White	1.39	5.8	15.2	25.1	4
17	PI241784	Peru	Brown/dark	1.38	6.2	14.7	24.5	5
18	PI358288	Ethiopia	White/red dots	1.32	6.8	13.3	23.3	5
19	PI256904	Peru	White	1.26	5.0	14.4	24.0	5
20	PI256909	Peru	White/red dots	1.24	5.9	14.5	23.3	4
21	G25300 (Pallar)	Peru	White	1.22	6.2	13.6	25.5	---
22	G25981 (Pallar or Chuies)	Boliva	Grey	1.20	5.5	14.7	24.8	---
23	PI256881	Peru	White	1.17	6.2	14.9	24.0	4
24	G25985	Boliva	Black/white dots	1.11	4.3	14.2	23.1	5
25	G25980	Boliva	White/brown dots	1.08	4.7	13.7	24.0	5
26	G25989	Boliva	Red/white dots	1.05	4.4	15.2	25.0	5
27	PI257393	Colombia	White	1.05	5.4	13.4	23.3	5
28	G25517	Colombia	Gray/red	1.04	9.0	13.0	16.5	4
29	PI256870	Peru	White	1.04	6.8	13.8	17.9	3
30	G25267	El Salvador	White	0.99	4.9	12.4	20.3	1
31	G25579	Boliva	Black/white dots	0.98	4.8	14.9	24.2	5
32	PI256913	Peru	Gray	0.78	7.0	11.8	16.2	3
33	PI477041	U.S.	Brown	0.78	5.0	11.9	19.3	3
34	PI256846	Peru	White	0.77	6.4	12.2	16.5	3
35	PI256845	Peru	White	0.75	7.2	13.4	14.9	3
36	PI257416	Argentina	Gray	0.73	4.5	12.4	17.6	3
37	G25821	Colombia	Gray/purple dots	0.69	7.7	10.2	15.9	4
38	PI256823	Peru	Gray	0.63	8.2	10.8	11.3	3
39	PI427216	U.S.	White	0.62	5.8	11.6	15.0	3
40	PI451715	U.S.	Red	0.54	4.7	10.7	15.6	4
41	G25296	Cuba	Gray	0.51	5.5	10.1	14.4	5
42	PI440808	U.S.	Brown	0.43	5.3	8.9	14.1	4
43	G25547	Argentina	White	0.42	3.7	9.8	13.5	3
44	G25623	U.S.	White	0.42	4.7	9.3	13.3	2
45	G25626	U.S.	White	0.42	5.3	9.7	12.1	3
46	G25176 (Jackson Wonder)	U.S.	Brown/purple dots	0.41	4.0	9.5	14.5	1
47	G26222	Caribe	White	0.40	4.9	9.7	15.1	2
48	PI257375	Colombia	White	0.40	4.7	9.6	12.5	2
49	G25137 (Jackson Wonder)	U.S.	Brown/purple dots	0.40	4.1	9.2	13.8	1
50	G25545	Argentina	White	0.39	4.6	10.0	13.9	4
51	G25107	Brazil	White	0.39	4.4	8.7	13.7	3
52	G26085	Brazil	Purple	0.39	6.2	8.0	10.0	4
53	G25193	Mexico	White	0.38	5.1	10.0	13.0	2
54	G25515	Colombia	White	0.38	4.7	9.6	13.3	3
55	PI257355	Colombia	White	0.36	5.4	9.8	12.2	3
56	PI198092	---	White	0.36	5.1	10.0	11.5	3
57	G25138 (Allgreen)	U.S.	White	0.35	4.6	8.6	12.1	2
58	PI451782	U.S.	Brown/black dots	0.35	4.6	10.8	14.7	3
59	G25667	U.S.	Brown/purple dots	0.35	4.3	9.6	15.3	2
60	G25521	Colombia	White	0.34	4.4	9.0	13.0	3
61	G25629 (Hopi 156)	U.S.	White	0.33	3.9	8.9	11.2	3
62	PI433604	U.S.	White	0.32	4.5	8.90	11.7	1
63	G25624	U.S.	White	0.31	4.4	9.00	13.6	3
64	G25556	Mexico	Brown/light	0.28	5.1	8.40	12.3	5
65	PI502185	---	Red	0.27	3.9	9.10	12.5	3

[z]Accessions names begining with PI were obtained from Richard Hannan, U.S. Dept. of Agriculture-Agricultural Research Service, Pullman, Wash., and accessions beginning with G were obtained from Steve Beebe, CIAT, Cali, Colombia. Local names of accessions are written in paretheses.

[y]The first color indicated is the solid or background color, the second is mottled over the first.

[x]Mean of 10 seeds.

[w]Classification scale 1 = produced many seeds, 2 = produced few seeds, 3 = produced no seeds, but flowered, 4 = produced flowers late in season, 5 = did not flower when planted in early June 1992 at Hancock, Wis.

the ratio of the mean square for the interaction between marker frequency and group relative to the interaction between marker frequency and individual accessions nested within each group.

Sampling variance. Bootstrap samples were drawn from the full data set and from subsets corresponding to the Andean and Mesoamerican groups. One hundred bootstrap samples each of size n (n = 10,20, 30...n) were drawn independently from each data set (Tivang et al., 1994). The GD between all pairs of accessions was calculated for each bootstrap sample. The variance among the 100 bootstrap samples for each pair of accessions was standardized to the coefficient of variance (CV) by dividing the variance by the bootstrap sample mean.

Genetic diversity. Genetic diversity (RAPD marker diversity) was estimated within each of the three groups (Andean, Mesoamerican, and Fordhook) as Nei's gene diversity at a locus, $h = (1 - \sim x_i^2)n/n - 1) = 2pqn/(n - 1)$, where p is the frequency of absence of RAPD amplification at a locus and n is the number of individuals evaluated, averaged over all 125 loci (Nei, 1987). Comparison of populations was done using t tests. Standard errors fort tests were computed using the bootstrap (Efron and Tibshirani, 1987).

Results and Discussion

Relationships revealed by the MDS plots. Inspection of the MDS plot indicated three distinct clusters of accessions with no overlap (Fig. 1). The accessions within the two main clusters generally corresponded in seed size and geographic region to the Mesoamerican and Andean gene pools described by Debouck (1989) (Table 1). Within the large-seeded accessions, the Fordhook cultivars formed a third distinct cluster. The mean GD between the Andean and Mesoamerican (0.726), Mesoamerican and Fordhook (0.561), and Andean and Fordhook (0.303) clusters were all significant (Table 2). The significant GD between the Andean and Mesoamerican groups supports the proposal by Debouck et al. (1989) of the existence of two major gene pools in lima bean.

Entry 30 has large, white seeds, a determinate (bush) growth habit, and seed shape and flowering characteristics very similar to the Fordhook-type cultivars included in this study (Table 1). Based on its phenotypic characteristics and clustering in close proximity to the Fordhook cultivars, entry 30 likely represents a Fordhook type, which is either feral or cultivated in El Salvador. Thus, the Fordhook cluster includes only cultivars and cannot be considered a third gene pool. The significant distance of the Fordhook cultivars from the other large-seeded, mostly indeterminate growth habit types suggests that the development of Fordhook cultivars was not a simple mutation to a determinate growth habit, but rather involved changes at many loci (Fig. 1 and Table 2).

The MDS plot revealed that two entries, 33 and 39, which had mean seed weights of 0.78 g and 0.62 g, respectively, clustered with the Mesoamerican group (Fig. 2). Both of these large-seeded accessions had white seed and were collected in the United States. Entry 6 was known to be the cultivar Jackson Wonder. Two accessions, which were collected in the United States and which had similar seed color and size (entries 46 and 49), were described by their collectors as having a local name of 'Jackson Wonder'. These three entries grouped in close proximity in the MDS plot (Fig. 2). All the small-seed Henderson-type culitvars (entries 4

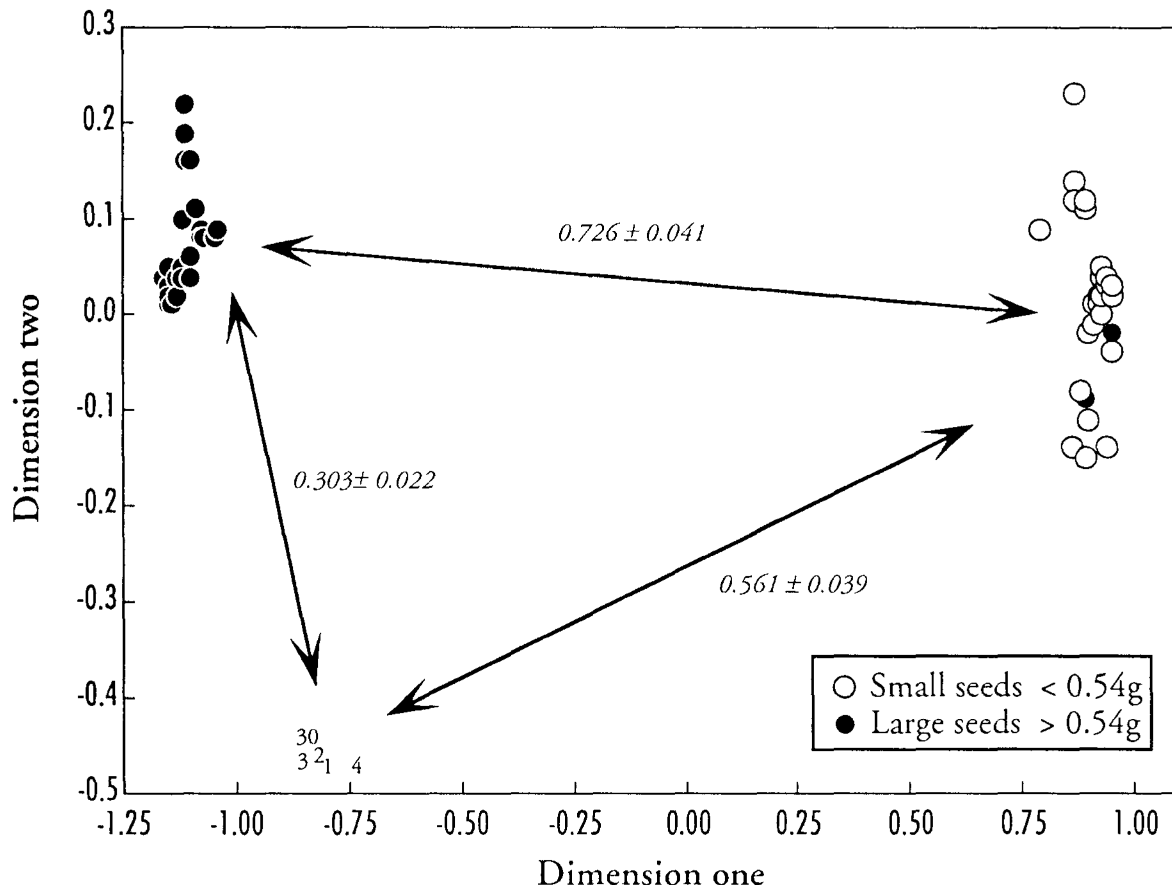

Fig. 1. Plot of first and second dimensions of MDS analysis of genetic distance matrix of 65 *Phaseolus fanatus* accessions. Mean genetic distance between groups was estimated based on 125 polymorphic RAPD bands. Numbered accessions are listed in Table I and represent Fordhook-type cultivars or accessions.

Table 2. Comparisons of mean genetic distance among Andean, Mesoamerican, and Fordhook groups of *Phaseolus lunatus.*

Comparison	No. of comparisons[z]	Mean geneticy distance	F value[x]
Mesoamerican vs. Andean	875	0.726	12.13***
Mesoamerican vs. Fordhook	175	0.561	10.04***
Andean vs. Fordhook	125	0.303	1.71**

[z](m*n) where m and n = the number of accessions in each group respectively.
'Calculated as the mean genetic distance between all m*n comparisons.
'F values were calculated from [he analysis of molecular variance comparing the mean squares for genotype x band frequency between populations vs. the mean square for genotypes nested within population × band frequency.
***,**Significant at $P = 0.001$ or 0.01. respectively.

through 11) included in this study tended to cluster in the MDS plot (Fig. 2). 'New Kingston' (entry 8), a cultivar originally developed for adaptation to high temperature conditions in Utah, appears the most distant from the other small-seeded cultivars (Pollard and Hamson, 1970). In contrast, 'Early Thorogreen', 'East land'. and 'Bridgeton' (entries 7, 10, and 11, respectively) appear to form a separate cluster of cultivars related to one another and to entry 56, a white-seeded accession of unknown origin. Three other cultivars, Maffei 15, Jackson Wonder, and Packer (entries 5.6. and 9, respectively), appear to be more closely related to the other small-seeded accessions.

All large-seeded accessions from Bolivia (entries 22, 24, 25, 26, and 31) clustered in close proximity in the MDS plot of Andean accessions (Fig. 2). All remaining large-seeded accessions from Peru and Colombia formed a separate cluster.

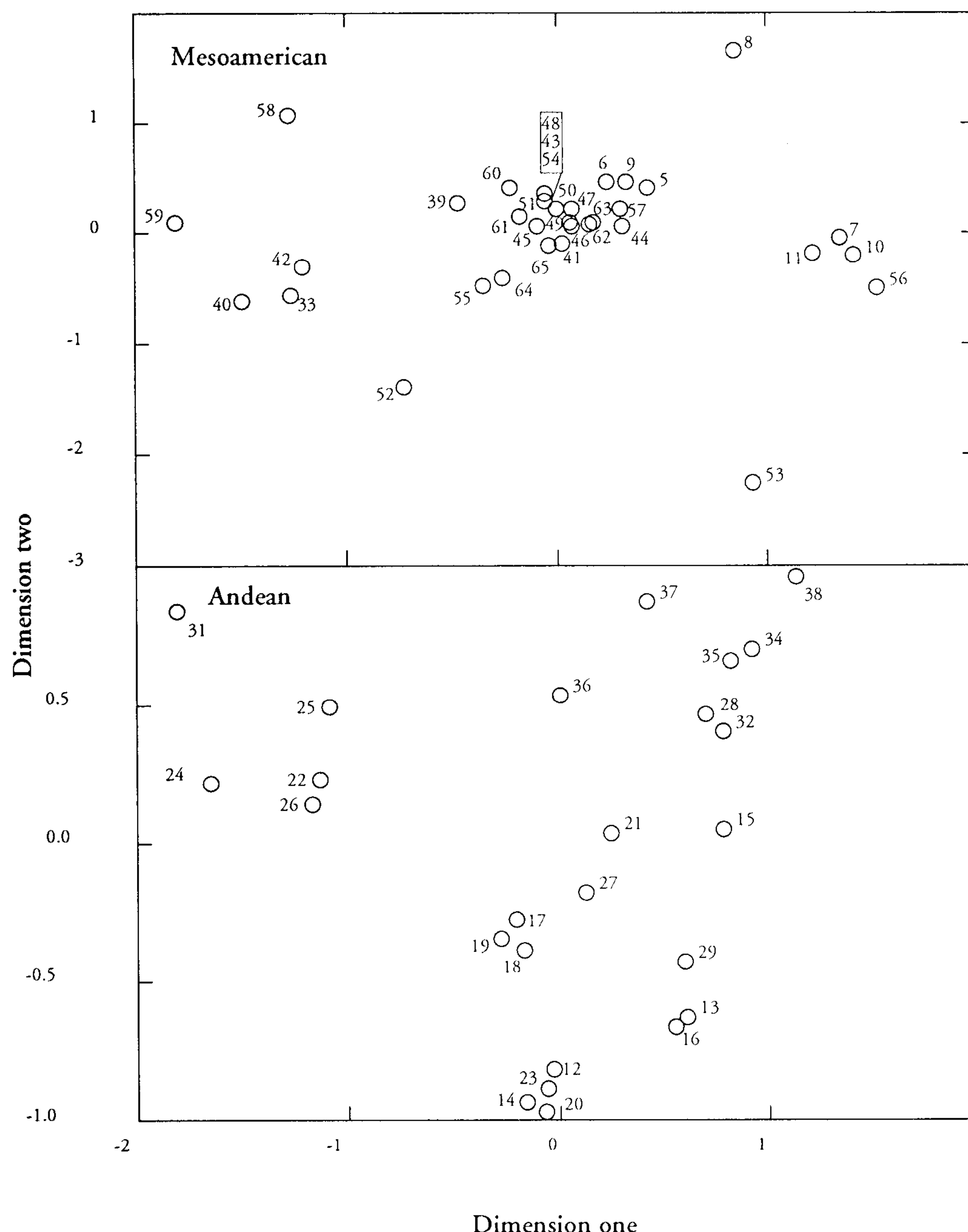

Fig. 2. Plots of first and second dimensions of MDS analysis of genetic distance matrices of lima bean accessions corresponding to the Mesoamerican and Andean gene pools. Accessions are listed in Table 1.

Sampling variance. Sampling variance in estimation of genetic relationships occurs when a random subset of marker bands does not equal the value obtained from all possible bands. Larger numbers of random marker bands will provide an increasingly more uniform distribution and will reduce the variance inestimation of genetic relationships clue to over- or undersampling certain regions of the genome (Tivang et al., 1994). The plot of the relationship between the cv and sample size (number of bands) indicates that cvs as low as 10% [or estimating CD between Andean and Mesoamerican lima bean accessions can be achieved by sampling as few as 100" bands (Fig. 3). However, fewer bands were polymorphic within the Andean and Mesoamerican gene pools than were polymorphic between them; thus, cvs were larger for comparisons of GD within compared to between gene pools. The cvs for estimating GD within the Andean and Mesoamerican gene pools were ≈ 25%, corresponding to ≈ 40 and 70 bands, respectively. Although the variance among bootstrap samples was similar within and between the Andean and Mesoamerican gene pools, the cv relationships do not follow the same curve because the mean GD (the divisor used for calculating the cv) was smaller within groups compared to between groups.

Genetic diversity. The basis of genetic diversity is sequence variation. RAPDs are molecular markers that sample and reveal sequence variation by the differential amplification of DNA fragments; thus, genetic diversity within groups of lima bean accessions was measured as RAPD marker diversity. Genetic diversity estimated by RAPD markers was the largest in the Mesoamerican group (0.110) followed by the Andean (0.097) and Fordhook (().()62) groups (Table 3). The Mesoamerican and Andean groups differed significantly from the Fordhook group but were not significantly different from each other.

Conclusions

Plant breeders can usc RAPD markers to organize genetic resources into related groups to make more informed decisions regarding choice of parents. This capacity is especially useful in this study because the effect of photoperiod on flowering of many

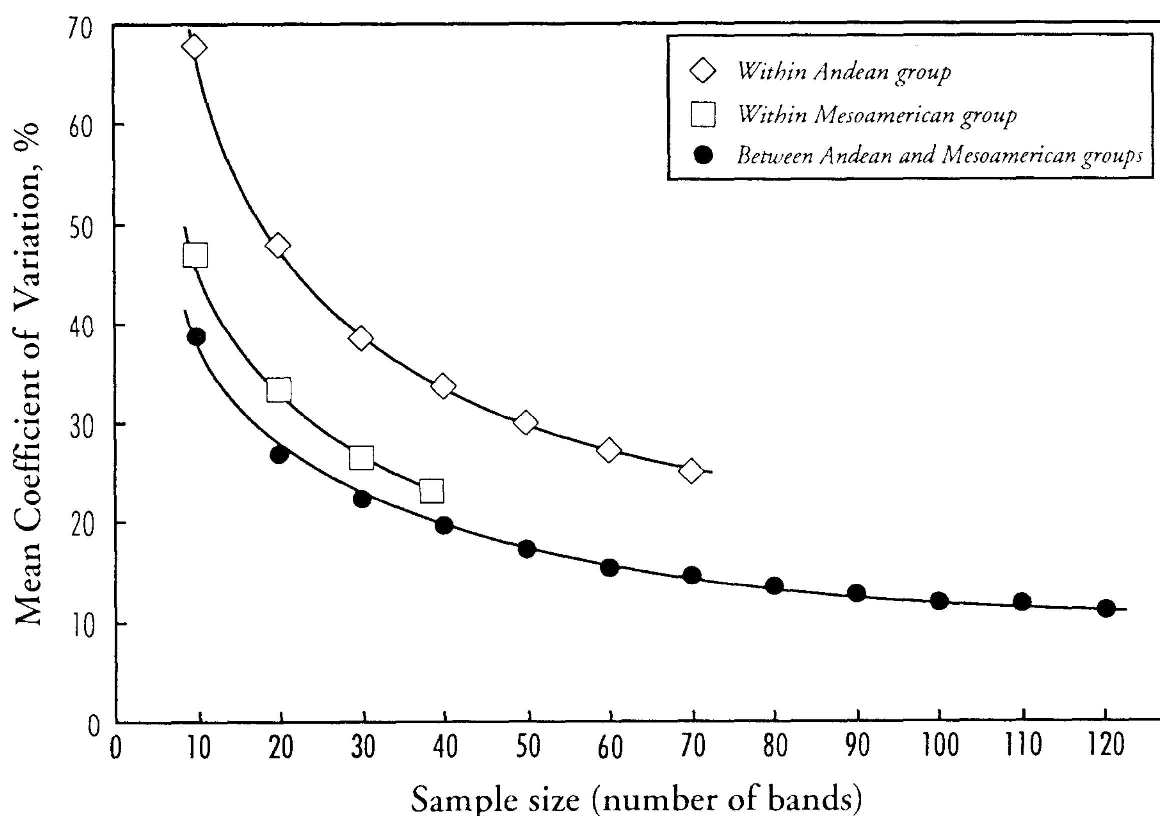

Fig. 3. Sampling variance of genetic distance displayed as a plot of the coefficient of various vs. simple size within and between lima bean acccessions corresponding to the Andean and Mesoamerican gene pools.

Table 3. RAPD marker diversity and *r* tests of differences among Andean, Mesoamerican, and Fordhook groups of *Phaseolus lunatus.*[z]

Group	No. of individuals	RAPD marker[y] diversity	*t* Tests: Mesoamerican	Andean	Fordhook
Mesoamerican	35	0.110	---	1.00[NS]	2.96**
Andean	25	0.097	1.00[NS]	---	2.42**
Fordhook	5	0.062	2.96**	2.42**	---

[z]Accessions that correspond to Mesoamerican and Andean gene pools and the Fordhook group that are cultivars or landraces phenotypically similar to the culitvar Fordhook.
[y]RAPD marker diversity is the mean over 125 RAPD loci scored for Nei's gene diversity at a locus (2pqn) (kn – 1], where n = number of individuals, and p and q = the frequency of presence or absence of a band, respectively.
NS,**Nonsignificant or significant ar *P* = 0.01.

of the exotic accessions included in this study precluded organization based on yield and quality characteristics (Hartmann, 1969). To evaluate the breeding value of unadapted lima bean accessions will first require the development of populations and selection for adaptation. Knowledge of the organization of these lima bean accessions permits prudent investment of time and resources in the choice of fewer, more-diverse parents to cross in the development of populations for selection for adaptation.

Knowledge of genetic relationships when complemented by phenotypic data can reveal sources of desirable characteristics in more closely related accessions. This combined knowledge may permit the recovery of the recurrent parents' phenotype in fewer backcross generations than would be required for a more-distantly related donor parent. For example, one of the objectives of our lima bean breeding program is the development of larger seed size in Henderson-type cultivars. The two large-seeded accessions that clustered with the otherwise small-seeded types may represent a useful source of genetic variation for increased seed size within the Mesoamerican gene pool.

To maximize genetic variation, populations derived from Mesoamerican × Andean crosses would be more variable than crosses made within either gene pool. The two gene pools are interfertile; however, crosses between them sometimes produce lethals among F_2 segregants (Erickson, 1982). Populations developed from crosses between cultivars in the Mesoamerican gene pool and Fordhook group would also produce greater variation than crosses made within the Mesoamerican gene pool. Moreover, crosses between Henderson and Fordhook types, which are commercial varieties, will likely maintain seed quality and horticultural characteristics.

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