

Improvement of large-seeded common bean cultivars under sustainable cropping systems in Spain

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Summary

Approaches are needed to broaden the genetic base and improve earliness and yield potential of large-seeded beans under sustainable cropping systems. The objective of this research was to develop adapted dwarf bean populations having a commercial seed quality and yield suitable for the production in the South of Europe. The original base populations were produced from crosses between genotypes within each Mesoamerica, Nueva Granada and Peru races, and between Peru and Nueva Granada, and Mesoamerica and Nueva Granada races. Visual mass selection for plant performance was practised in the F₂ and F₃ generations. In the F₄ and F₅, single plants were harvested under two cropping systems (sole cropping and intercropping with maize). From F₄, selection was based on precocity, combined with seed yield and seed commercial type. The F_{4:7} selected lines from each original population were compared with their parents and five checks at four environments and two cropping systems. Differences among environments, populations, parents and checks were observed for all traits. Under intercropping with maize, there was a 50% reduction in seed yield. Yield of parents and checks belonging to Andean South American races, intraracial (Nueva Granada × Nueva Granada) and interracial (Nueva Granada × Peru) populations, was higher than that of those of Middle American origin. Intraracial crosses within large-seeded Andean South American (Peru race) and Middle American gene pools (Mesoamerica race) did not produce lines yielding more than the highest yielding parent. Only two large-seeded lines selected from crosses between small- and large-seeded gene pools out-yielded the best parent and check cultivar.

Introduction

Large-seeded dry bean cultivars (*Phaseolus vulgaris* L.) in many parts of southern Europe tend to mature later than high-yielding breeding lines with small seeds. These large-seeded bean cultivars of various colours, though no black, permit growers to obtain high prices due to their good seed quality. They have become the most popular common bean cultivars in the South of Europe as a component of traditional recipes (Willet et al., 1995). Most of these cultivars contribute significantly to the sustainability of traditional cropping systems, and they are usually cultivated in association with maize (Santalla et al., 1994). The farmer's primary

objective under these traditional cropping systems is to produce a high yield of the maize crop which is mostly used for the local feed consumption, and the secondary objective is to produce a good bean yield. Seed yield of dry bean cultivars produced in the South of Europe is low, usually averaging less than 1,000 kg ha⁻¹ (FAO, 1999). The poor productivity of these bean cultivars is due, in part, to the low levels of inputs used to produce the crop; the growing season tends to be shortened by early rainfall patterns, the lack of cultivars adapted to intercropping systems, and the presence of diseases in the production areas.

Most large-seeded dry beans of Andean origin are grown in North America, South America, Europe, Asia

and Africa. This wide geographic distribution has generated new gene combinations that favour adaptation to diverse environments (Santalla et al., 2002). Thus, the seed size of the Andean cultivars ranges from 30 to 40 g 100⁻¹ seeds in warm climates such as the Caribbean, and from 50 to 100 g 100⁻¹ seeds in temperate and cool environments (Beaver, 1999). The large-seeded dry bean cultivars of major economic importance in the South of Europe are cranberry, white-kidney, favada, canellini and canela. Other small–medium-seeded dry beans such as great northern or small white are also grown for markets in the South of Europe (Singh et al., 1991; Santalla et al., 2001).

Most traits sought by large-seeded bean breeders can already be found within the Andean gene pool. However, significant genetic gain for some of the most important traits will require the development of populations from crosses between the Andean and Mesoamerican gene pools. Despite their partial reproductive isolation (Gepts & Bliss, 1985; Koinange & Gepts, 1992), viable and fertile progeny can be obtained, and therefore, genes can be transferred between the two pools, although the transfer of quantitative traits appears to be problematic. Attempts by breeders to recombine traits such as the large seed size of the Andean gene pool with the yield potential of the Mesoamerican gene pool, have generally failed (Nienhuis & Singh, 1986; Welsh et al., 1995), although there are notable exceptions. The success of Beaver and Kelly (1994), who employed recurrent selection strategies, and that of snap bean breeders (Skroch & Nienhuis, 1995), suggest that inter-gene pool hybridization could be a valuable resource for dry bean breeders. In addition, Middle American lines (e.g., ICA Pijao) that do not possess the *D1* genes can be used as a bridge in making crosses with beans of Andean origin. Moreover, Beaver (1993) obtained seed from dwarf F₁ hybrids by promoting adventitious root growth above the cotyledonary node.

Considerable progress has been achieved during the last years in the genetic improvement of large-seeded beans (Beaver, 1999; Singh, 2001). Significant gains in yield potential, disease resistance and other traits of economic value will require most bean breeding programs to broaden their genetic base. Breeding programs in the South of Europe have mentioned the need to produce improved early-maturing bean cultivars. Most large-seeded bean cultivars in these areas have an indeterminate growth habit and are of Andean origin. Indeterminate beans have usually greater seed yields than determinate genotypes although they usually also have a long cycle of maturity. The need to mechanize

and to reduce both production costs and pesticide use in most of these production regions due to the continuing abandon of the intercropping system demands the development of high yielding, upright cultivars that are resistant to a maximum number of diseases. Farmers often express a strong preference for early maturity among bean cultivars. Earliness may serve not only as a mechanism for avoiding late season stresses such as rainfall and an expected loss in yield potential, but also may have economic value depending on price fluctuations or the needs of farmers for rapid sources of food or marketable products. The bean cultivars should also combine yield and good acceptance by producers and consumers according to their seed quality. The objective of this study is to determine whether sufficient genetic variation exists within European Andean germplasm that would permit genetic gains in intra-gene pool crosses, and whether desirable genes from the Mesoamerican gene pool can be readily transferred to Andean types without altering traits that are important in determining farmer acceptance of new cultivars.

Material and methods

Three groups of breeding lines were chosen from the Misión Biológica de Galicia-Consejo Superior de Investigaciones Científicas (MBG-CSIC) bean breeding collection according to seed types and disease resistances. Two groups consisted of large-seeded races Nueva Granada (two white kidney, one red kidney and one favada accessions) and Peru (three bolon bayo, one purple caparron and one cranberry accessions) from the Andean American gene pool. The other group was formed by the small-seeded race Mesoamerica (two small white accessions) from the Middle American gene pool. Seed type and other characteristics of the 11 breeding lines used to develop intra, inter-racial and inter-gene pool populations are given in Table 1.

Three intraracial crosses were made within each Mesoamerica (PHA20/159), Nueva Granada (PHA267/257) and Peru (PHA338/306) races, five interracial crosses were carried out between Nueva Granada and Peru (PHA257/272, PHA257/306, PHA257/323, PHA267/338 and PHA452/119) races, and three inter-gene pool crosses were carried out between Nueva Granada and Mesoamerica (PHA257/159, PHA267/159 and PHA269/159) races. Part of the seed of each F₁, together with the respective parents, was grown in the field to check its hybrid origin and produce F₂ seed.

Table 1. Some characteristics of the genotypes of common bean of different races used as parents in intraracial, interracial and inter-gene pool populations

Identification	Race	Growth habit ^a	Seed		Observations
			Color	Size	
PHA-0020-07	Mesoamerica	III	White	Medium	HBB tolerance and rust race 38 resistance
PHA-0119-01	Peru	II	Beige	Large	HBB tolerance and high yield potential
PHA-0159-00	Mesoamerica	II	White	Small	HBB and rust race 38 resistance
PHA-0257-00	Nueva Granada	I	White	Large	Anthracnose race 17 resistance and commercial quality
PHA-0267-00	Nueva Granada	I	White	Large	Anthracnose race 17 resistance and commercial quality
PHA-0269-00	Nueva Granada	I	Red	Large	HBB tolerance
PHA-0272-00	Peru	IV	Purple	Medium	HBB tolerance and high yield potential
PHA-0306-00	Peru	I	Beige	Large	HBB tolerance and rust race 53 resistance
PHA-0323-02	Peru	IV	Beige	Large	BCMV and BCMNV resistance and HBB and rust races 38 and 53 tolerance
PHA-0338-00	Peru	I	Beige	Large	HBB tolerance and rust race 53 resistance
PHA-0452-01	Nueva Granada	IV	White	Large	BCMV, anthracnose race 17 and CBB resistance, rust races 38 and 53 tolerance and commercial quality

^aI: determinate erect, II: indeterminate erect, III: indeterminate prostrate, and IV: indeterminate climbing.

The 11 populations were advanced by mass selection for plant performance from F₂ to F₄ generation. For each F₂, two rows, 5 m long, were sown in 1993 at MBG-CSIC farms at Salcedo (42°24' N latitude, 8°38' W longitude, 40 m altitude, 14° C mean temperature and average rainfall 1600 mm), and spacing between rows was 0.5 m. A density of about 200,000 plants ha⁻¹ was obtained. Each F₃ plot consisted of two rows, 5 m long, which were sown in 1993–1994 under greenhouse conditions. Spacing between and within rows, agronomic management, and data recordings were similar to those of the above experiment. This was followed by single plant harvests selected for precocity combined with seed yield and seed commercial quality from F₄ to F₅ under two cropping systems (intercropping with field maize and sole cropping). They were grown in plant-to-progeny rows in the F₆ and scored for agronomic data. All plants within each plot were bulk-harvested.

A sample of 95 F_{4:7} lines in sole cropping and in intercropping with maize (commercial hybrid 'Dea' with a maturity rating about FAO 200 which is widely used in this area), the two parents from each population, and cultivars of known economic value in the South of Europe were evaluated in field trials in Salcedo and Soutomaior (43°14' N latitude, 8°16' W longitude,

20 m altitude, 13°C mean temperature, average annual rainfall 1000 mm) for 2 years (1999 and 2000). They were evaluated in a randomized complete block with two replications per trial. Each experimental plot consisted of a single 3.5 m row, with row spacing of 0.80 m and plant spacing of 0.25 m. Seeds were hand-sown but over planted by 100% and thinned to 15 plants per plot after emergence. Plots were kept clean of weeds during the entire cropping season. Data were recorded for days to first flower, days to physiological maturity and growth habit. Seed yield (expressed in kilograms per hectare, at 140 g kg⁻¹ moisture) and seed weight (expressed as g 100 seed⁻¹) were measured at harvest. Commercial seed quality data such as proportion of seed coat and water absorption (Santalla et al., 1995) and crude protein which was determined using Near Infrared Transmittance, were recorded after 2 months from harvest.

A separate nursery for each anthracnose (ANT), bean common mosaic virus (BCMV), common bacterial blight (CBB), halo bacterial blight (HBB) and rust disease was prepared to evaluate 95 F_{4:7} lines, parents, checks and susceptible (e.g., Common Pinto) and resistant (e.g., VAX-3) cultivars to CBB, susceptible (e.g., Common Pinto) and resistant (e.g., Jules) cultivars to HBB, susceptible (e.g., Common Pinto) and resistant

(e.g., USPT-ANT-1) cultivars to ANT, susceptible (e.g., Common Pinto) and resistant (e.g., Matterhorn) cultivars to BCMV and BCMNV, and susceptible (e.g., Othello) and resistant (e.g., Chase) cultivars to rust. Plants in the MBG-CSIC greenhouse were inoculated for ANT, using a power-driven, backpack sprayer when primary leaves were expanded. Two plants were inoculated with race 23 and the rest of the plants were inoculated with the race 17 of the pathogen. Disease reaction was recorded at 7 to 10 days after inoculation, and following a scale from susceptible (interveinal lesions on lower leaves surface), intermediate (venal lesions on lower leaves surface) to immune (without symptoms). For rust, plants in the MBG-CSIC greenhouse were inoculated with races 38 (Andean origin) and 53 (Mesoamerican origin) when primary leaves were expanded. Each pathogen was sprayed on one primary leaf. Disease reaction was recorded at 20 days after inoculation, and following a scale from susceptible (big pustules on the lower leaf surface), small pustules (very little pustules on lower leaf surface), necrotic pustules (small pustules with necrotic tissue and without spores) to resistant (without symptoms).

Plants in the MBG-CSIC greenhouse were mechanically inoculated for BCMV with the US6 and the NL3 necrosis-inducing strains, using infected bean leaf extracts and a sterile cotton tipped stick when primary leaves were expanded. Each primary leaf per plant was inoculated with a different strain. Disease reaction was recorded at 20 days after inoculation, and following a scale based on the presence or absence of mosaic symptoms (leaf curling, rugosity, vein banding, etc.). Plants presented different types of disease reactions with US6 from susceptible (presence of mosaic symptoms) to resistant (without symptoms) and with NL3 from susceptible (mosaic symptoms and local, venal or top necrosis) to resistant (symptomless).

For CBB, plants in the MBG-CSIC greenhouse were manually inoculated using the multiple-needle method on one of the primary leaves per plant and 3 to 10 days after the inoculation the pathogen was also sprayed. Disease reaction was recorded at 20 days after inoculation, and following a 1 to 9 scale, where 1 = immune (without symptoms) and 9 = highly susceptible (extended damaged areas on inoculated leaf). For HBB, plants in the MBG-CSIC greenhouse were mechanically inoculated with race 2 when primary leaves were unfolded and just beginning to expand, using the multiple-needle method. Disease reaction was recorded at 20 days after inoculation, and following a 1 to 9 scale, where 1 = immune (without symptoms) and 9 =

highly susceptible (extended water-soaked lesions on inoculated leaf) (Schoonhoven van & Pastor-Corrales, 1987).

Combined analysis of variance appropriate for a randomized complete block was conducted using the general lineal model (GLM) procedure of the SAS statistical package (SAS Institute, 2000). Data were analyzed, using a mixed model in which $F_{4;7}$ lines, parents and cropping systems were fixed and test environments (locations and years) were random effects.

Results

Significant differences for test environments, populations, parents and checks were found for first flowering and dry pod, seed yield and weight and seed quality traits (Table 2), and suggest contrasting differences among the genetic material, as well as among the four test environments. Mean seed yield, when pooled over 2 years for all 95 $F_{4;7}$ lines was higher at Salcedo (1,068 kg ha⁻¹) as than at Soutomaioir (463 kg ha⁻¹). The difference between years was much smaller when yield was averaged across locations (616 in 1999 vs. 932 kg ha⁻¹ in 2000). Common bean genotypes, on an average, took a week longer to mature at Soutomaioir (95 days) as compared to Salcedo (88 days). Moreover, significant interaction between populations and environments for seed yield, coat proportion and crude protein indicated that the performance of populations was not consistent across all environments. A significant interaction between parents and environments for seed yield, seed weight and crude protein, and between checks and environments for seed yield and weight was found. Similarly, significant mean squares due to cropping systems for seed yield and crude protein indicated that cropping system changed the mean values for both traits although the presence of interaction with the test environments for first to dry pod, seed yield, crude protein and water absorption showed that its magnitude was not consistent across the range of environments. Mean yield for all genotypes was 50% higher in sole cropping than in intercropping with maize. However, significant interaction between checks and cropping systems for seed yield indicated that the yield performance of the checks differed with the cropping system although a non significant interaction was found for the populations developed and parents. Thus, while developing high-yielding large seeded germplasm for different cropping systems, evaluations can be conducted across representative cropping systems and mean seed yield

Table 2. Some mean squares from the analysis of variance for F_{4:7} lines selected from intraracial, interracial and inter-gene pool common bean populations, parents and checks, grown in two locations (Salcedo and Soutomaior), Spain, for 2 years (1999 and 2000), under sole cropping and intercropping

Source of variation	df ^a	Mean square						
		First flowering (days)	First dry pod (days)	Seed yield (kg ha ⁻¹)	Seed weight (g 100 seeds ⁻¹)	Coat proportion (%)	Crude protein (%)	Water absorption (%)
Environment (E)	3	1467.76**	2544.86**	2822459.8**	1734.73*	13.19**	201.45**	10248.95**
Replication/E	4	12.09	38.46	472159.2**	140.18**	0.19	3.34**	158.28
Systems (S)	1	15.35	12.31	3937944.9**	42.57	0.10	7.64**	1189.19
E × S	3	26.41	208.75**	3564124.3**	41.27	0.98	4.04**	5825.77**
Replication × S/E	4	13.08	71.03	600940.6	98.36	1.02	6.07	1328.97
Populations (P)	10	259.43**	447.12**	2879427.8**	3566.26**	9.14**	30.09**	1682.97**
Parents	10	528.79**	516.42**	1082701.3*	2179.57**	6.45**	24.51**	6603.51**
Checks (C)	4	477.61**	784.86**	1123308.9**	1547.08**	6.07**	7.09*	1694.82**
E × P	30	15.21	20.16	851150.9**	56.89	1.22*	2.57**	455.56
E × Parents	30	25.88	83.27	367652.3*	64.14*	0.78	2.51*	749.23
E × C	12	12.47	24.25	165863.3**	59.89**	0.54	1.98*	220.57
S × P	10	81.32	167.68	677261.8	960.79	6.71	5.26	698.88
S × Parents	10	24.89	61.41	241464.2	48.34	0.79	0.98	1321.56
S × C	4	7.23	30.82	170708.3**	39.26	0.34	1.15	121.54
E × S × P	30	12.65	22.31	451150.9**	42.82	0.57	1.69	730.10*
E × S × Parents	30	27.28	42.44	323322.8	25.92	0.51	0.75	1037.77
E × S × C	12	15.09	49.09*	263444.4**	27.06	0.61	1.65*	286.67
Error	627	13.29	25.92	115004.4	38.81	0.65	0.81	499.53

^adf: Degrees of freedom.

*, **: significant at $P \leq 0.05$, $P \leq 0.01$, respectively.

can be used as a selection criterion. However, to maximize gains from selection for a given environment, a breeder must conduct evaluations for different bean-growing environments, provided that gains will be large enough to compensate for the additional research cost.

When F_{4:7} lines were separated into three within, one between race crosses and one between gene pool crosses, those intraracial populations in which all parents were of Nueva Granada origin, followed by those interracial populations (crosses of race Peru with Nueva Granada) gave the highest yielding lines on average (Table 3). By contrast, intraracial populations in which all parents were of race Mesoamerica and rather broad-based inter-gene pool populations (between the small-seeded race Mesoamerica and the large-seeded race Nueva Granada) gave low-yielding lines on average. Part of the reason for the high yield of the N × N intra- and N × P inter-racial crosses could be that they included high-yielding parents. High-yielding parents tended to give rise to high-yielding F₁ crosses.

F_{4:7} lines in N × N (PHA267/257) intraracial crosses significantly out-yielded ($p \leq 0.05$) the highest yielding Nueva Granada parent (Table 4). P × P and M × M F_{4:7} lines did not significantly out-yielded ($p \leq 0.05$) the highest yielding Mesoamerica and Peru parent. N × P interracial crosses significantly out-yielded ($P \leq 0.05$) the highest yielding parent except for two populations (PHA257/272 and PHA257/323). Seed yield of 19 F_{4:7} lines in N × M inter-gene pool crosses did not significantly out-yielded ($p \leq 0.05$) the highest yielding parent except for PHA257/159 population.

In N × N intraracial populations, six white kidney lines (PHA257/267-12, PHA257/267-13, PHA257/267-19, PHA257/267-20, PHA257/267-21 and PHA257/267-76) yielded significantly higher ($p \leq 0.05$) than the highest yielding large-seeded Nueva Granada parent and checks (Table 5). No lines were selected from M × M and P × P populations, whereas none of the lines significant ($p \leq 0.05$)

Table 3. Mean for the F_{4:7} lines selected from intraracial, interracial and inter-gene pool common bean populations and parents for days to flowering and maturity, seed yield, seed weight and seed quality averaged across four environments and two cropping systems

Race combination ^a	No	First flowering (days)		First dry pod (days)		Seed yield (kg ha ⁻¹)		Seed weight (g 100 seeds ⁻¹)		Coat proportion (%)		Crude protein (%)		Water absorption (%)	
		Parents	F _{4:7}	Parents	F _{4:7}	Parents	F _{4:7}	Parents	F _{4:7}	Parents	F _{4:7}	Parents	F _{4:7}	Parents	F _{4:7}
Intraracial															
N × N	1	42.9	41.5	90.3	88.2	579.9	982.4	53.8	55.4	7.3	7.1	24.9	25.0	111.4	116.3
P × P	1	48.1	46.4	93.5	91.9	415.4	508.8	59.1	54.1	7.6	7.9	28.9	27.9	103.9	99.0
M × M	1	48.2	48.2	99.0	95.9	620.9	559.1	33.3	30.1	7.8	7.9	26.1	25.4	104.8	110.9
Mean		46.2	44.1	93.3	90.3	528.5	770.2	52.3	52.1	7.5	7.5	2.5	26.0	107.3	109.8
Interracial															
N × P	5	46.7	43.6	92.9	91.0	700.1	822.9	57.4	58.7	7.6	7.5	25.9	25.8	105.7	111.9
Inter-gene pool															
N × M	3	47.7	45.2	95.2	93.6	770.6	509.3	51.9	39.3	7.8	8.1	25.4	25.8	122.1	115.9
LSD (0.05) ^b		2.7	2.4	5.3	2.1	ns	354.8	7.2	3.9	ns	0.4	1.2	0.9	25.1	10.3
LSD (0.05) ^c		ns	1.7	ns	0.8	ns	ns	ns	2.9	ns	0.3	0.4	ns	ns	ns

^aMiddle American races: M: Mesoamerica; Andean races: N: Nueva Granada, and P: Peru.

^bFor comparison among populations for different races.

^cFor comparison among intra and interracial, and inter-gene pool populations.

out-yielded its highest yielding parent. In N × P interracial populations, eleven white-kidney (PHA257/306-4, PHA257/306-5, PHA257/306-6, PHA257/306-7, PHA257/306-25, PHA257/306-31, PHA257/306-56, PHA257/306-60, PHA267/338-42, PHA267/338-109 and PHA452/119-34) and two cream bolon bayo (PHA267/338-40 and PHA267/338-41) lines yielded significantly more than their highest yielding Nueva Granada and Peru parents, and checks. Moreover, two white kidney lines (PHA267/159-2 and PHA267/159-24) from N × M inter-gene pool populations significantly outyielded ($p \leq 0.05$) the highest yielding parents. These N × M lines had a seed commercial type as the Nueva Granada parent.

Although no disease selection was made from F₄ to F₆, a high number of F_{4:7} lines possessed highly resistance to race 17 of ANT (Table 6). No F_{4:7} lines were highly resistant (i.e., receiving scores ≤ 3) to CBB, HBB and rust except for one N × M line (PHA267/159-24) that was moderate resistant (i.e., receiving scores ≤ 6) to HBB. Moreover, three of the eleven parents used to develop the intra, interracial and inter-gene pool populations possessed resistance to race 17 of ANT, two parents were resistant to BCMV (resistance to BCMV is controlled by major dominant (*J*) and recessive genes in PHA-0323-02), one parent (PHA-0452-01) was resistant to CBB (i.e., receiving scores ≤ 3), one parent (PHA-0159-00) was resistant to HBB (i.e., receiving scores ≤ 3) and to race 38 of rust, and two parents

(PHA-0306-00 and PHA-0338-00) were resistant to race 53 of rust. Most of the checks were resistant to race 17 of ANT except for the white kidney-PHA-0838 cultivar, one check (canellini-PHA-0930) possessed recessive genes of resistance to BCMV, and other check (white kidney-PHA-0838) had a moderate resistance to rust.

Discussion

Yield differences in common bean genotypes between the environments evaluated could be due to a slower crop growth and delayed maturity which favoured losses of seed yields at Soutomaioir. Total rainfall was more scarce in Salcedo at the onset of the reproductive phase (20 mm) compared to Soutomaioir (39 mm). Thung & Rao (1999) showed that differences in rainfall pattern and temperature during the reproductive period may be the most important factors contributing to the changes detected in the duration of the reproductive phase and seed yield. Seed yield reductions in intercropping with maize have been widely documented (Francis et al., 1978). Moreover, in most bean-growing environments, large fluctuations often occur in abiotic factors from year to year and among locations, even at relatively short distances, as shown by these data. Yield performance of F_{4:7} lines selected in specific environments varied at different locations and one year

Table 4. Range and mean for the F_{4:7} lines selected from intraracial, interracial and inter-gene pool common bean populations, parents and checks for days to flowering and maturity, seed yield, seed weight and seed quality averaged across four environments and two cropping systems

Populations and checks	No	First flowering (days)		First dry pod (days)		Seed yield (kg ha ⁻¹)		Seed weight (g 100 seeds ⁻¹)		Coat proportion (%)		Crude protein (%)		Water absorption (%)									
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean								
PHA267/257 F _{4:7}	14	39.8	47.9	41.5	86.3	95.7	88.2	569.8	1653.8	982.4	52.8	65.4	55.4	6.7	8.4	7.1	24.4	25.8	25.0	104.6	139.5	116.3	
Parents		40.9	45.9	42.9	87.8	93.8	90.2	216.4	700.7	758.9	53.6	55.1	53.8	7.2	7.3	7.2	24.7	25.6	24.9	111.4	112.1	118.0	
PHA338/306 F _{4:7}	12	42.8	50.0	46.4	86.7	98.9	91.9	308.8	895.2	508.8	35.5	57.4	54.1	7.5	8.7	7.9	26.9	28.8	27.9	93.3	109.4	99.0	
Parents		45.6	50.5	48.5	89.6	98.3	95.5	123.7	532.1	346.2	58.0	60.4	58.0	7.5	7.8	7.8	28.8	29.2	28.7	101.9	104.9	108.9	
PHA20/159 F _{4:7}	3	46.3	50.0	48.2	93.7	97.3	95.9	464.2	618.6	559.1	27.8	33.4	30.1	7.9	8.1	7.9	24.6	25.7	25.4	107.2	117.9	110.9	
Parents		45.4	51.0	50.1	96.3	101.7	99.2	209.1	826.8	511.2	25.1	40.1	33.4	7.7	7.8	8.3	25.6	26.3	26.0	87.8	126.0	106.6	
PHA257/272 F _{4:7}	5	41.6	45.5	43.6	89.8	95.8	92.3	184.2	666.7	412.8	34.3	54.5	46.1	7.0	10.8	8.6	26.3	29.5	27.3	112.4	126.7	118.5	
Parents		49.0	53.7	49.1	92.5	101.3	97.1	483.9	817.4	745.6	45.8	58.9	51.0	6.8	7.7	7.5	23.9	27.7	26.3	48.5	107.2	83.6	
PHA257/306 F _{4:7}	24	40.1	48.4	42.4	83.9	99.1	89.7	370.4	1533.3	816.6	46.1	70.5	59.9	6.3	8.5	7.3	24.3	27.3	25.8	98.3	132.3	112.8	
Parents		41.3	47.1	45.5	89.2	95.7	92.8	596.7	788.7	621.2	53.4	57.8	55.9	7.5	8.1	7.6	24.7	27.7	26.5	109.7	126.9	117.4	
PHA257/323 F _{4:7}	4	43.0	45.5	44.5	86.3	95.1	91.3	350.6	995.1	650.3	62.8	64.4	62.8	7.1	7.2	7.1	24.6	25.8	25.3	111.9	131.6	119.6	
Parents		41.2	49.5	47.0	87.5	96.9	94.9	485.7	703.1	579.8	52.0	57.7	56.9	7.3	9.3	8.3	24.6	26.5	25.7	50.9	125.8	84.8	
PHA267/338 F _{4:7}	13	40.3	58.8	45.0	87.5	108.0	91.7	634.8	1469.6	916.8	44.5	65.9	58.9	6.9	9.4	7.8	24.6	27.3	25.8	33.2	124.4	106.4	
Parents		42.0	50.5	45.9	86.5	98.3	92.9	123.7	638.2	483.9	59.7	60.4	62.9	7.0	7.7	7.4	25.4	29.2	27.1	101.9	105.9	109.6	
PHA452/119 F _{4:7}	2	44.8	51.5	50.5	92.6	114.8	104.8	606.1	2149.2	2149.2	54.8	57.5	57.5	7.8	7.9	7.9	24.2	26.2	24.2	111.1	114.5	111.0	
Parents		46.9	59.0	52.9	91.3	95.0	93.2	258.9	1521.0	889.9	55.9	73.1	64.5	7.2	7.3	7.3	25.1	27.2	26.2	107.6	126.1	116.9	
PHA257/159 F _{4:7}	7	42.5	49.8	45.9	87.1	98.1	93.7	151.4	568.3	312.3	24.3	58.1	35.8	7.9	9.1	8.5	24.6	26.8	25.6	107.2	121.7	115.5	
Parents		47.5	52.1	46.9	96.1	98.1	94.8	221.2	736.8	434.7	26.9	58.8	41.4	7.3	8.5	8.1	24.9	25.6	25.3	126.6	136.7	122.1	
PHA267/159 F _{4:7}	8	39.9	52.4	44.1	86.5	105.6	93.1	137.4	1477.7	706.5	26.3	69.1	43.7	6.8	8.7	7.7	24.6	26.3	25.6	84.9	144.9	113.3	
Parents		41.5	47.8	45.3	87.7	97.2	92.1	141.1	1016.6	519.8	23.4	69.5	46.0	6.9	8.8	7.9	25.1	27.1	25.4	106.5	119.9	121.3	
PHA269/159 F _{4:7}	4	38.8	55.4	45.9	90.4	99.5	94.8	297.5	993.7	490.7	27.9	44.2	35.7	7.6	9.0	8.4	26.2	29.7	27.1	71.2	131.4	123.2	
Parents		48.4	52.8	51.0	97.5	101.1	98.9	95.2	646.3	420.9	37.6	50.8	38.7	8.4	11.4	8.7	24.7	26.5	26.1	112.7	131.5	119.0	
Checks	5	41.3	48.9	44.8	86.4	103.5	92.6	298.9	985.9	606.1	43.9	69.1	54.8	7.1	9.1	7.9	25.3	27.6	26.2	102.8	128.1	114.5	
LSD(0.05) ^a				3.1			5.2		809.4		6.1			0.8						1.4			18.5

^aFor comparison among populations for different races, parents and checks.

Table 5. Means for maturity, seed yield, seed weight and seed quality for twenty-one common bean lines selected, eight parents and five checks, averaged across four environments and two cropping systems

Identification	First flowering (days)	First dry pod (days)	Seed yield (kg ha ⁻¹)	Seed weight (g 100 seeds ⁻¹)	Coat proportion (%)	Crude protein (%)	Water absorption (%)
Selected lines							
PHA267/159-2	41.8	89.6	1290.5	69.1	6.9	24.6	119.6
PHA267/159-24	40.0	87.4	1477.7	54.9	6.8	25.2	119.9
PHA257/267-12	40.0	86.3	1585.8	54.4	7.0	25.8	139.5
PHA257/267-13	41.0	87.0	1428.0	56.6	6.7	25.0	119.6
PHA257/267-19	40.0	88.1	1258.2	58.7	6.8	25.4	114.7
PHA257/267-20	40.1	86.8	1653.8	56.3	6.9	24.8	118.4
PHA257/267-21	39.9	85.4	1012.8	54.7	6.8	25.2	118.0
PHA257/267-76	41.5	90.1	1050.0	54.5	8.4	24.8	117.9
PHA257/306-4	41.5	90.5	1214.0	67.4	6.7	24.8	109.9
PHA257/306-5	42.8	88.9	1087.3	66.8	6.8	25.1	110.2
PHA257/306-6	40.5	87.4	1533.3	70.5	6.9	24.7	116.4
PHA257/306-7	42.8	90.4	1289.8	66.3	6.7	24.9	128.9
PHA257/306-25	42.4	90.0	1070.3	59.6	7.1	26.3	99.3
PHA257/306-31	39.8	85.4	1201.1	56.3	7.0	25.2	110.1
PHA257/306-56	41.1	90.0	1043.3	62.3	7.1	25.3	121.1
PHA257/323-60	43.0	86.3	995.1	64.3	7.1	25.2	111.9
PHA267/338-40	44.8	88.7	1469.6	63.2	8.9	26.1	120.4
PHA267/338-41	40.3	87.5	1397.4	58.4	7.9	27.2	100.7
PHA267/338-42	41.3	87.5	1020.6	60.8	6.9	25.9	119.8
PHA267/338-109	43.5	90.1	1085.2	63.1	6.8	24.6	124.4
PHA452/119-34	50.5	104.8	2149.2	57.5	7.9	24.2	111.0
Mean (21 lines)	41.8	88.9	1299.2	60.8	7.1	25.2	116.8
Mean (95 lines)	44.4	91.4	797.6	52.0	7.6	26.0	115.8
Parents							
PHA119	46.9	91.3	1521.0	55.9	7.3	27.2	107.6
PHA159	47.8	97.2	141.1	23.4	8.8	27.5	106.5
PHA257	42.7	90.1	650.9	54.6	7.3	24.8	116.1
PHA267	41.4	87.5	844.0	65.4	7.0	25.0	117.3
PHA306	47.1	95.7	596.7	53.4	8.1	27.7	126.9
PHA323	49.5	96.9	485.7	57.7	9.3	26.5	50.9
PHA338	50.5	98.3	123.7	60.4	7.8	29.2	101.9
PHA452	59.0	95.0	258.9	73.1	7.3	25.1	126.1
Mean (8 parents)	44.5	90.9	739.9	57.4	7.5	25.5	114.0
Mean (11 parents)	48.0	96.0	684.3	48.3	7.5	26.1	111.6
Checks							
Canela-PHA-0135	48.9	94.5	985.9	64.4	8.1	27.6	110.1
Red Pinto-PHA-0601	39.6	86.4	605.8	44.0	7.8	26.1	102.8
White kidney-PHA-0624	41.3	87.6	441.1	53.0	7.1	26.6	128.0
White kidney-PHA-0838	52.2	103.5	729.0	69.1	7.4	25.3	122.6
Canellini-PHA-0930	42.0	91.8	298.9	45.8	8.1	25.3	114.4
Mean	44.8	92.8	633.9	55.3	8.1	26.2	114.5
LSD (0.05) ^a	3.5	6.1	617.4	5.9	1.2	1.4	25.7
LSD (0.05) ^b	2.9	5.8	1056.2	6.6	1.3	1.3	27.9

^aFor comparison of individual values for lines, parents and checks.

^bFor comparison of mean values of twenty-one lines, eight parents and checks.

Table 6. Growth habit, seed type and reaction to five diseases for twenty-one common bean lines selected, eight parents and five checks

Identification	Growth habit ^a	Seed Type	BCMV	Score ^b					
				ANT-17	ANT-23	CBB	HBB	RUST-38	RUST-53
Selected lines									
PHA267/159-2	I	White kidney	8.0	1.2	8.0	8.2	9.0	8.2	8.0
PHA267/159-24	I	White kidney	8.3	1.0	8.3	8.3	5.5	8.1	8.3
PHA257/267-12	I	White kidney	8.2	1.0	8.2	8.2	8.2	8.2	8.2
PHA257/267-13	I	White kidney	8.2	1.0	8.2	8.2	8.2	8.2	8.2
PHA257/267-19	I	White kidney	8.2	1.0	8.2	8.2	8.2	8.2	8.2
PHA257/267-20	I	White kidney	8.2	1.0	8.2	8.2	8.2	8.2	8.2
PHA257/267-21	I	White kidney	8.2	1.0	8.2	8.2	8.2	8.2	8.2
PHA257/267-76	I	White kidney	8.2	1.0	8.2	8.2	8.2	8.2	8.2
PHA257/306-4	I	White kidney	8.3	2.0	8.3	8.3	8.3	8.3	8.0
PHA257/306-5	I	White kidney	8.2	1.7	8.2	8.2	8.2	8.2	8.1
PHA257/306-6	I	White kidney	8.2	1.7	8.2	8.2	8.2	8.2	8.2
PHA257/306-7	I	White kidney	8.2	1.0	8.2	8.2	8.2	8.2	8.0
PHA257/306-25	I	White kidney	8.3	1.4	8.3	8.3	8.3	8.3	8.3
PHA257/306-31	I	White kidney	8.3	1.0	8.3	8.3	8.3	8.3	8.3
PHA257/306-56	I	White kidney	8.2	2.8	8.2	8.2	8.2	8.2	7.9
PHA257/323-60	II	White kidney	8.2	1.5	8.2	8.2	8.2	8.2	8.3
PHA267/338-40	I	Bolon Bayo	8.3	3.7	8.3	8.3	8.3	8.3	8.0
PHA267/338-41	I	Bolon Bayo	8.3	3.3	8.3	8.3	8.3	8.3	8.0
PHA267/338-42	I	White kidney	8.3	6.0	8.3	8.3	8.3	8.3	8.2
PHA267/338-109	I	White kidney	8.2	1.0	8.2	8.3	8.7	8.3	8.0
PHA452/119-34	I	White kidney	8.3	6.0	8.3	8.3	8.3	8.3	8.0
Mean (21 lines)	–	–	8.2	1.9	8.2	8.2	8.2	8.2	8.2
Mean (95 lines)	–	–	8.2	4.2	8.2	8.2	7.9	7.9	8.2
Parents									
PHA119	II	Bolon Bayo	8.0	8.0	8.0	9.0	6.0	6.6	8.0
PHA159	II	Small White	8.1	8.0	7.9	8.4	2.5	1.9	8.0
PHA257	I	White kidney	8.2	2.0	8.0	8.0	7.1	8.1	7.2
PHA267	I	White kidney	8.2	2.2	8.6	9.0	7.2	8.3	7.8
PHA306	I	Red Kidney	8.4	8.1	8.2	8.8	6.3	8.4	2.9
PHA323	IV	Cranberry	1.3	8.7	8.5	7.2	6.0	3.6	3.4
PHA338	I	Bolon Bayo	8.3	8.3	8.0	6.6	5.2	8.3	2.9
PHA452	IV	Favada	1.7	1.3	8.0	2.7	6.5	4.3	4.3
Mean (8 parents)	–	–	7.5	5.6	8.1	8.0	5.3	5.8	6.5
Mean (11 parents)	–	–	7.5	5.6	8.0	8.0	5.3	5.8	6.5
Checks									
PHA-0135	IV	Canela	8.5	2.5	7.2	8.2	8.0	7.8	8.0
PHA-0601	I	Red Pinto	8.5	1.5	7.9	8.0	8.2	8.2	8.2
PHA-0624	I	White kidney	4.8	1.3	7.8	8.2	8.0	8.3	8.0
PHA-0838	IV	White kidney	8.3	7.0	8.0	9.0	7.8	4.0	3.2
PHA-0930	I	Canellini	1.3	1.4	7.8	7.6	8.2	8.0	8.0
Mean	–	–	6.1	2.5	8.0	9.0	7.8	4.0	3.2
LSD (0.05) ^c	–	–	0.8	1.4	ns	0.8	1.0	0.8	1.1
LSD (0.05) ^d	–	–	0.9	1.1	ns	0.9	1.0	0.7	1.4

^aI: determinate erect, II: indeterminate erect, III: indeterminate prostrate, and IV: indeterminate climbing.

^bANT: anthracnose; BCMV: bean common mosaic; CBB: common bacterial blight; HBB: halo blight. Disease scores: 1: immune to 9 highly susceptible.

^cFor comparison of individual values for lines, parents and checks.

^dFor comparison of mean values of twenty-one lines, eight parents and checks.

to another within locations as indicated by a significant interaction of the populations with environments for seed yield. This interaction was found for the parents and checks also. Selections made across all environments would be more stable than those selected in one environment.

Differences in seed weight are one of the morphological traits distinguishing the Andean and Mesoamerican gene pools (Singh et al., 1991). Debouck et al. (1993) presented evidence that the large seeded Andean common bean germplasm was better adapted to cooler, higher elevations than Mesoamerican germplasm. Under the environments studied, Andean American materials achieved higher or similar yields than their small-seeded Middle American counterparts. Nonetheless, the mean yield of 14 lines in Andean intraracial populations (PHA257/267) and 48 lines in Andean interracial populations (PHA257/272, PHA257/306, PHA257/323, PHA267/338 and PHA452/119) was higher than that of 19 lines in Andean \times Middle American inter-gene pool populations (PHA267/159, PHA257/159 and PHA269/159) and of 3 lines in Middle American intraracial populations (PHA20/159). Kornegay et al. (1992) also found that Middle American \times Andean inter-gene pool populations (between the Mesoamerica and Nueva Granada races) gave low-yielding lines using a pedigree breeding system. Welsh et al. (1995) did not find any high yielding F_2 -derived recombinant inbred lines using the single-seed-descent method and problems existed with mass selection (Singh et al., 1989). But, those of Beaver & Kelly (1994), Urrea & Singh (1995) and Singh et al. (1999), unequivocally indicate that recurrent selection or congruity backcrossing could be used to select for high yielding large-seeded Andean beans from Andean interracial and Andean \times Middle American inter-gene pool populations.

Because only two (PHA267/159-2 and PHA267/159-24) out of 19 lines from Andean \times Middle American populations out-yielded and weighted its highest yielding and weighting parent, should be a matter of concern for those interested in developing large-seeded, high yielding cultivars from such broad-base Andean \times Middle American common bean populations. Apparently, alleles from Middle American controlling small seed size were favoured above their Andean counterparts. An explanation could be that large-seeded recombinants were poor competitors and, hence, eliminated from the Andean \times Middle American populations. This could suggest that delaying yield selection within Andean \times Middle American

promising populations until a later generation may not be advisable, because having to advance a large number of low-yielding genotypes. Singh (1995) observed as one strategy to identify and increase the frequency of high-yielding lines is to adopt a modified pedigree method combined with a yield test of early generation population bulks (Singh, 1992), or the gamete selection (Singh, 1994) which involves yield evaluation of the F_1 -derived, F_2 and F_3 families. In addition, the low frequency of evaluated lines with resistance to diseases is because no selection within populations was practised for the biotic stresses from F_4 to F_6 , and gene recombination in such populations is unlikely to be random. Simultaneous recombination and selection for multiple traits can now be expeditiously accomplished traits by incorporating marker-assisted gamete selection (Singh, 1994) during population development and in each intervening recombination phase.

Seed yield of large-seeded indeterminate growth habit Andean cultivars of Nueva Granada and Peru races is often 40 to 60% lower than their small- and medium-seeded Middle American counterparts of Durango and Mesoamerican races in most growing environments (Singh et al., 2002). However, southern Europe seems to have well-adapted and high-yielding large-seeded cultivars, which are grown commercially on a fairly small scale, while many people grow their own varieties in intercropping systems. These cultivars have a great commercial value, their culinary qualities, especially the tenderness of the seed coat and the buttery texture, have allowed to withstand competition with other dry bean varieties and ensured their high market value. While some yield improvements were found from intraracial populations within Andean gene pool (e.g., PHA257/267), Andean interracial populations (e.g., PHA452/119) provided much larger gains from selection. The large base of variation in germplasm within Andean gene pool (Santalla et al., 2002), and particularly within race Nueva Granada permitted further progress in breeding for quantitative traits such as seed yield. No gains from selection were observed from Andean and Middle American intraracial crosses within race Peru and Mesoamerica, respectively, and between Andean and Middle American gene pools. The availability of genetic variation from inter-gene pool crosses was not completely successful in this work. Most of the lines were intermediate for seed yield to the values of the two parents in the inter-gene pool populations, although some larger transgressive segregants were present. The factors that could have discouraged inter-gene pool recombination

could be the difficulty of recovering the agronomic and seed characteristics of parents.

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References

- Beaver, J.S., 1993. A simple method for producing seed from hybrid dwarfs derived from crossed between Middle American and Andean gene pools. *Annu Rept Bean Improv Coop* 36: 28–29.
- Beaver, J.S., 1999. Improvement of large-seeded race Nueva Granada cultivars. In: S.P. Singh (Ed.), *Common bean improvement in the twenty-first century*, pp. 275–288. Kluwer Academic Publishers, The Netherlands.
- Beaver, J.S. & J.D. Kelly, 1994. Comparison of selection methods for dry bean populations derived from crosses between gene pools. *Crop Sci* 25: 923–926.
- Debouck, D.G., O. Toro, O.M. Paredes, W.C. Johnson & P. Gepts, 1993. Genetic diversity and ecological distribution of *Phaseolus vulgaris* in northwestern South Africa. *Econ Bot* 47: 408–423.
- FAO, 1999. Food and Agriculture Organization of the United Nations. Statistics series No. 156. Production Vol. 53. Rome, Italy. 2001.
- Francis, C.A., M. Prager, D.R. Laing & C.A. Flor, 1978. Genotype × environment interactions in bush bean cultivars in monoculture and associated with maize. *Crop Sci* 18: 237–241.
- Gepts, P. & F.A. Bliss, 1985. F1 hybrid weakness in the common bean: Differential geographic origin suggest two gene pools in cultivated bean germplasm. *J Hered* 76: 447–450.
- Koinange, E.M.K. & P. Gepts, 1992. Hybrid weakness in wild *Phaseolus vulgaris* L. *J Hered* 83: 135–159.
- Kornegay, J., J.W. White & O. Ortíz de la Cruz, 1992. Growth habit and gene pool effects on inheritance of yield in common bean. *Euphytica* 62: 171–180.
- Nienhuis, J. & S.P. Singh, 1986. Combining ability analysis and relationships among yield, yield components and architectural traits in dry beans. *Crop Sci* 26: 21–27.
- Santalla, M., A.M. De Ron & M.R. Escribano, 1994. Effect of intercropping bush bean populations with maize on agronomic traits and their implications for selection. *Field Crops Res* 36: 185–189.
- Santalla, M., A.M. De Ron & P.A. Casquero, 1995. Nutritional and culinary quality of bush bean populations intercropped with maize. *Euphytica* 84: 57–65.
- Santalla, M., A.M. De Ron & O. Voysest, 2001. European bean market classes. In: M. Amurrio, M. Santalla & A.M. De Ron (Eds.), *Catalogue of bean genetic resources*, pp. 77–94, FAIR5-PL97-3463 programme.
- Santalla, M., A.P. Rodiño & A.M. De Ron, 2002. Allozyme evidence supporting southwestern Europe as a secondary center of genetic diversity for common bean. *Theor Appl Genet* 104: 934–944.
- SAS Institute, 2000. The SAS System. SAS online Doc. HTML Format. Version eight. SAS Institute, Cary, NC, USA.
- Schoonhoven van, A. & M.A. Pastor-Corrales (comps), 1987. Standard system for the evaluation of bean germplasm. CIAT, Cali, Colombia.
- Singh, S.P., 1992. Common bean improvement in the tropics. *Plant Breed Rev* 10: 199–269.
- Singh, S.P., 1994. Gamete selection for simultaneous improvement of multiple traits in common bean. *Crop Sci* 34: 352–355.
- Singh, S.P., 1995. Selection for seed yield in Middle American versus Andean × Middle American interracial common bean populations. *Plant Breed* 114: 269–271.
- Singh, S.P., 2001. Broadening the genetic base of common bean cultivars: A review. *Crop Sci* 41: 1659–1675.
- Singh, S.P., C. Cajiao, J.A. Gutiérrez, J. García, 1989. Selection for seed yield in inter-gene pool crosses of common bean. *Crop Sci* 29: 1126–1131.
- Singh, S.P., P. Gepts & D.G. Debouck, 1991. Races of common bean (*Phaseolus vulgaris*, Fabaceae). *Econ Bot* 45: 379–396.
- Singh, S.P., H. Terán, C. Germán Muñoz & J.C. Takegami, 1999. Two cycles of recurrent selection for seed yield in common bean. *Crop Sci* 39: 391–397.
- Singh, S.P., H. Terán, C.G. Muñoz & J.M. Osorno, 2002. Selection for seed yield in Andean intra-gene pool and Andean × Middle American inter-gene pool of common bean. *Euphytica* 127: 437–444.
- Skroch, P.W. & J. Nienhuis, 1995. Qualitative and quantitative characterization of RAPD variation among snap bean (*Phaseolus vulgaris*) genotypes. *Theor Appl Genet* 91: 1078–1085.
- Thung, M. & I.M. Rao, 1999. Integrated management of abiotic stresses. In: S.P. Singh (Ed.), *Common bean improvement in the twenty-first century*, pp. 331–370. Kluwer Academic Publishers, The Netherlands.
- Urrea, C.A. & S.P. Singh, 1995. Comparison of recurrent and congruity backcrossing for interracial hybridization in common bean. *Euphytica* 81: 21–26.
- Welsh, W., W. Bushuk, W. Roca & S.P. Singh, 1995. Characterization of agronomic traits and markers of recombinant inbred lines from intra- and interracial populations of *Phaseolus vulgaris* L. *Theor Appl Genet* 91: 169–177.
- Willet, W.C., F. Sacks, A. Trichopoulou, G. Drescher, A. Ferro-Luzzi, E. Helsing & D. Trichopoulos, 1995. Mediterranean diet pyramid: A cultural model for healthy eating. *Am J Clin Nutr* 61: 1402–1406.