AGRONOMIC, MORPHOLOGICAL, AND MOLECULAR (PROTEIN AND ISOENZYMES) CHARACTERISATION OF RECOMBINANT INBRED LINES FROM INTRA- AND INTERGENEPOOL POPULATIONS OF COMMON BEAN (*Phaseolus vulgaris* L.)

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AGRONOMIC, MORPHOLOGICAL, AND MOLECULAR (Protein and Isoenzymes) CHARACTERISATION OF RECOMBINANT INBRED LINES FROM INTRA- AND INTERGENEPOOL POPULATIONS OF COMMON BEAN (*Phaseolus vulgaris* L.)

BY

WILLIAM DOUGLAS WELSH

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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"An army marches on its stomach" - Mao Tse-Tung

ABSTRACT

The value of intra- and interracial populations in common bean (*Phaseolus vulgaris* L.) needs to be determined for creating useful genetic variation to maximize gains from selection, broaden the genetic base of commercial cultivars, and make efficient use of available resources. Objectives of this study were to (1) characterize and compare variation for marker and agronomic traits generated by intraracial versus interracial populations, (2) measure heritability and gains from selection for seed yield and other agronomic traits, and (3) identify marker traits that could be used as indirect selection criteria for yield and other agronomic traits. Five large-seeded determinate parents of Andean origin (race Nueva Granada) and three small- or medium-seeded determinate or indeterminate parents of Middle America (races Mesoamerica and Durango) were hybridized to produce one intraracial (Nueva Granada x Nueva Granada) and three interracial (crosses of Nueva Granada with Mesoamerica and Durango) populations. Seventy-nine F_2-derived F_6 recombinant inbred lines randomly taken from each population along with their respective parents were evaluated in two contrasting environments (Palmira and Popayán, Colombia) for two years. A reps-in-set design with two replications was used. Plot size in 1990 was a single row, 3 m long. In 1991, each plot consisted of 4 rows, 5 m long.

Spacing between rows at Palmira was 0.6 m and at Popayán 0.5 m. Nonetheless, a population density of approximately 221,000 plants/ha was obtained at both locations. Data were recorded for seed yield, days to maturity, 100-seed weight, biomass, harvest index, fifth internode length, and pods/m^2. Also, growth habit, leaf shape, bracteole size and shape, and flower colour were recorded. Phaseolin, and total seed proteins, and seven polymorphic isoenzyme systems (diaphorase, malic enzyme, malic dehydrogenase, shikimic dehydrogenase, ribulose biphosphate carboxylase, glutamate oxaloacetate transaminase, and acid phosphatase) were also analysed for all entries.

Effects of location, year, population, recombinant lines within populations, and their interactions were significant for all agronomic traits including seed yield. Variation for morphological traits, proteins, isoenzymes, and agronomic traits including seed yield was larger in interracial populations compared with the intraracial populations. Mean seed yield of recombinant inbred lines as well as yield of the highest yielding lines from two interracial populations were significantly higher than those of the intraracial population. However, none of the lines from these two interracial populations outyielded their best parent. Only in the low-yielding interracial and intraracial populations did recombinant lines outyield their best parent.

Heritability values for seed yield ranged from 0.19 ± 0.17 to 0.50 ± 0.16, from 0.80 ± 0.15 to 0.94 ± 0.15 for 100-seed weight, from 0.26 ± 0.17 to 0.55 ± 0.16 for biomass, from 0.51 ± 0.15 to 0.72 ± 0.21 for harvest index, from 0.38 ± 0.16 to 0.61 ± 0.16 for pods/m^2, from 0.48 ± 0.16 to 0.89 ± 0.16 for days to maturity, and from 0.30 ± 0.16 to 0.91 ± 0.15 for fifth internode length. Ranges for gains from selection (at 20% selection pressure) for these same traits, respectively, were 3.9% to 11.4%, 11.1% to 26.6%, 4.6% to 12.5%, 3.0 % to 9.6%, 6.3% to 17.7%, 2.5% to 5.0%, and 4.4% to 51.5%.

Seed yield was positively associated with biomass yield, pods/m^2, and days to maturity. Harvest index was negatively correlated with biomass, pods/m^2, and days to maturity. Biomass, pods/m^2, and days to maturity were positively associated among each other. Correlations of 100-seed weight with harvest index and fifth internode length were positive and those with pods/m^2 and days to maturity were negative. Fifth internode length was also negatively associated with pods/m^2 and days to maturity.

Polymorphism for phaseolin, lectins, protein Group 1 fraction, protein Group 2 fraction, and six isoenzyme systems (ME, MDH, SKDH, RBSC, GOT, and ACP) at a single locus and for one isoenzyme system (DIAP) at two independent loci was

recorded, mostly in interracial populations.

Recombinant inbred lines with indeterminate growth habit had significantly ($P < 0.01$) higher seed yield than their determinate counterpart (Redkloud x MAM 4). Also, lines with T phaseolin, *Diap1*$^{\underline{2}}$ allele, and lilac flower colour tended to possess higher seed weight. Similarly, lines with indeterminate growth habit, lanceolate leaf shape, white flower, *Acp*$^{\underline{96}}$ allele, and T phaseolin had a higher biomass yield. Lines with determinate growth habit, *Mdh*$^{\underline{98}}$, *Rbsc*$^{\underline{98}}$, and S phaseolin had a higher harvest index. The S phaseolin, *Me*$^{\underline{100}}$, *Skdh*$^{\underline{100}}$, and large bracteole size were associated with a higher number of pods/m^2. Indeterminate growth habit, lilac flower colour in one population and white in the other, *Diap1*$^{\underline{2}}$, and T phaseolin were associated with delayed maturity. Similarly, lines with determinate growth habit, cordate bracteole, *Rbsc*$^{\underline{100}}$, *Acp*$^{\underline{100}}$, and T phaseolin, possessed longer fifth internodes.

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I. INTRODUCTION

In many developing countries, food legumes are a major source of proteins and often play an important role in crop rotations. However, since the early 1970s, concern has been raised regarding the neglect of *Leguminosae* research necessary to increase their yield and correct certain nutritional and food-use qualities (PAG Statement No. 22, 1973).

Demand for and consumption of common bean (*Phaseolus vulgaris* L.) are expected to rise sharply into the next century. By the year 2000, bean production in Africa will have to be 72% above 1989-90 production levels in order to satisfy demand, while Latin American production will have to increase by 42% (Janssen, 1989). Presently, common bean is largely produced by small farmers who will progressively be challenged to increase food availability for a rapidly growing population with less access to land (Janssen, 1989). In summary, future common bean production will be predicated not on labour productivity but, rather, land productivity.

Common bean is a non-centric crop, originating in the two major genepools of Middle America and Andean America (Gentry, 1969; Kaplan, 1981; Gepts, 1984, 1988a, and 1988b; Gepts et al., 1986; Gepts and Debouck, 1991). Supportive evidence comes

from electrophoretic analysis of the major storage phaseolin protein, which determines two genepools according to phaseolin type: Middle American S phaseolin and Andean T, C, H and other phaseolin types (Brown et al., 1981b; Bliss and Brown, 1983; Gepts et al., 1986).

Isoenzyme characterization confirms the two genepools (Sprecher, 1988a and 1988b; Koenig and Gepts, 1989a and 1989b; Singh et al., 1990, 1991a, and 1991b). Additional isoenzyme analysis identifies gene flow from wild to cultivated germplasm and further stratifies the Middle American and Andean genepools into five and four cultivar subgroups, respectively (Singh et al., 1991a).

Middle American germplasm is characterized as having smaller seed size (100-seed weight < 40 g) than its larger Andean counterpart (100-seed weight > 40 g) (Singh and Gutiérrez, 1984). Perhaps the most consequential agronomic factor separating the two germplasm types is their overall yielding abilities. Evidence from comparative trials shows that smaller seeded indeterminate Middle American genotypes with growth habits II and III have consistently outyielded larger seeded types with similar growth habits by an average of 0.4-0.6 t/ha, and even as high as 1.0 t/ha (Singh, 1988b and 1991; White and González, 1990; White et al., 1992).

There are several reasons for the growing interest in combining Andean and Middle American genotypes, not the least of which is to enlarge the genetic base for reliable and increased levels of resistance to both biotic and abiotic factors. Impetus for combining the higher yielding Middle American materials with their large-seeded Andean counterparts also stems from greater market demand for large-seeded materials in the Andes and Africa. There, the urgency to improve and stabilize yield is heightened because of limited resources available to farmers, diseases, low soil fertility, and drought.

Certain crosses of common bean cultivars between large-seeded Andean and small-seeded Middle American genotypes have resulted in F_1 hybrid weakness, growth abnormalities, and the appearance of dwarfing or crippling (York and Dickson, 1975; van Rheenen, 1979; Shii et al., 1980; Gutiérrez and Singh, 1982 and 1985; Singh and Gutiérrez, 1984; Gepts and Bliss, 1985; Vieira et al., 1989; Koinange and Gepts, 1992). Singh and Gutiérrez (1984) found that the frequency of F_1 hybrid dwarfism was approximately 2% in small-seeded (Middle American) by large-seeded (Andean) crosses.

There is a general tendency for bean breeders and geneticists to utilize intraracial crosses. As Singh (1989)

argues, breeders traditionally emphasize hybridization within bean genepools as there has been a history of poor adaptation of introduced germplasm, low frequencies of desirable interracial recombinants, and strict seed and/or pod quality requirements. The value of increased genetic diversity and selection criteria, whether molecular, morphological, and/or agronomic traits resulting from interracial crosses not demonstrating dwarfism or crippling, is not well understood. Likewise, the value of interracial populations for improvement of large-seeded germplasm is not presently known.

Most genetic studies and selection experiments focus on the use of early generation segregating populations and families and do not use recombinant inbred lines. These lines offer the advantages of providing a perpetual genetic material in which a high degree of homozygosity is firmly established, and they can be evaluated for multiple desirable traits over several environments (Burr et al., 1988). Often, it is not possible to obtain reliable estimates for quantitative traits such as yield from individual recombinant genotypes because of a reduced quantity of seed and their differential gene action in early generations.

For this study, 79 randomly chosen F_2-derived F_6 recombinant inbred lines and their parents for one intraracial

and three interracial populations were grown over two years at two locations in Colombia. Seed material was also cultivated in a greenhouse in order to analyse isoenzymes and total proteins. Agronomic field data and biochemical data were then used to characterize the recombinant inbred lines for their agronomic value and other traits.

The objectives of this study were to (1) determine the potentials of intra- and interracial populations to improve seed yield and other agronomic traits in large-seeded Andean germplasm, (2) measure heritability and gains from selection for seed yield and other agronomic traits, and (3) identify potential morphological and molecular markers that could facilitate selection for seed yield and other agronomic traits.

II. LITERATURE REVIEW

A. Common bean (*Phaseolus vulgaris* L.) as a non-centric crop

1. The existence of two domestication centres and two major groups of common bean germplasm

Wild common bean is the immediate ancestor of the two major groups of present-day cultigens that possess morphological differences corresponding to their geographic origin (Burkart and Brücher, 1953; Kaplan 1956; Miranda, 1967; Gentry, 1969; Gepts et al., 1986; Vanderborght 1986). A comparison of cultivated and wild common bean from both the southern Andes (of South America) and Mexico reflects differences between the two groups of germplasm. Beans from Mexico possess shorter raceme peduncles, larger flower bracteoles, and a greater quantity of flower nodes per raceme (Gepts and Debouck, 1991). Most significantly, wild and cultivated common beans from Mexico and parts of Central America possess smaller sized seeds than those originating in the Andes.

Gepts et al. (1986), Gepts and Bliss (1986), Koenig et al. (1990), and Singh et al. (1991b) found several phaseolin types-each one typical of its respective region of origin in

studies of both wild and cultivated common bean accessions. Wild beans from Middle America displayed electrophoregrams that resembled those of the cultivar Sanilac, thereby denoted as "S" phaseolin phenotype (Brown et al., 1981b). Similarly, wild forms from the Southern Andes had phaseolin electrophoregrams that resembled the cultivar Tendergreen phaseolin pattern and were denoted as "T" types (Brown et al., 1981b; Bliss and Brown, 1983). A large group of other phaseolin types found only in wild beans from Middle America has been grouped as "M" (Mexico). Likewise, other phaseolin types, e.g., "C" (Contender), "H" (Huevo de Huanchaco), "A" (Ayacucho), "I" (Inca), and "J" (Jujuy), were reported from the Andes (Gepts et al., 1986; Gepts and Bliss, 1986; Gepts, 1988a and 1988b). Phaseolin types from the wild beans are preserved in their cultivars from the respective region (Gepts et al., 1986).

Studies involving isoenzyme-allozyme analysis have provided further proof of the existence of the Middle American and Andean genepools (Schinkel et al., 1988; Sprecher, 1988a and 1988b; Koenig and Gepts, 1989a and 1989b; Koenig et al., 1990; Singh et al., 1991b). Similarly, using mitochondrial RFLPs, Khairallah et al. (1990 and 1992) have clustered bean lines corresponding to the two genepools. Using mitochondrial DNA (mtDNA), they were able to identify five restriction

fragment length polymorphism (RFLP) systems, three of which were used to group their experimental bean lines into two groups based on seed size, isoenzyme patterns, and phaseolin type.

B. Problematic crosses affecting growth, development, and yield in *Phaseolus vulgaris* L.

Whereas two primary centres of origin have been determined for common bean of all growth habits, Evans (1973) determined five subgroups, or races, for the two genepools. More recently, Singh (1988a and 1989) and Singh et al. (1991b) classified germplasm from the two domestication centres into six races according to seed size, phaseolin type, allozyme pattern, growth habit, adaptation habitat, yield, maturity, and other traits.

Certain crosses involving large-seeded (Andean) and small-seeded (Middle American) landraces have resulted in growth abnormalities, and/or the appearance of F_1 hybrid dwarfing phenomena or crippling symptoms such as chlorotic primary leaves, the absence of trifoliolate leaves, tap root degeneration, and adventitious root development on the stem just above the soil line (Davis and Frazier, 1964; Coyne, 1965; Provvidenti and Schroeder, 1969; York and Dickson, 1975;

van Rheenen, 1979; Shii et al., 1980; Gutiérrez and Singh, 1982 and 1985; Singh and Gutiérrez, 1984; Gepts and Bliss, 1985; Vieira et al., 1989). The occurrence of F_1 dwarfness has suggested the existence of some form of genetic barrier or isolation mechanism that interferes with genetic recombination between the two major genepools (Singh and Gutiérrez, 1984). This is further supported by distorted segregation ratios of specific loci expressions (Koenig and Gepts, 1989b). Moreover, it is known that common bean and the pathogens affecting it have undergone coevolution, resulting in different resistance mechanisms and genes in germplasm from each genepool (M. Pastor Corrales, pers. comm., 1992). In turn, this results in differential levels of response to diseases and pests caused by genetically variable pathogen populations.

Shii et al. (1980 and 1981) reported that the dwarfing trait is controlled by two complementary dominant dosage-dependent lethal genes-Middle American $\underline{Dl_1}$ and Andean $\underline{Dl_2}$. An F_1 hybrid of such a cross will result in a lethal $\underline{Dl_1}dl_1\underline{Dl_2}dl_2$ genotype. On the other hand, segregation data provided by Coyne (1965) suggest that the virus-like crippling trait observed in segregating generations is controlled by two complementary recessive genes (i.e., the parental combinations of Mesoamerican $\underline{cr_1}$ allele with Andean alleles $\underline{cr_2}$ and/or $\underline{cr_3}$). The relationship, if any, between these two phenomena is not

known.

C. Yield differences between Andean and Middle American common beans

Evidence from comparative trials has shown that smaller seeded Mesoamerican materials of indeterminate bush beans of growth habits II and III have consistently outyielded larger seeded cultivars of the same growth habit by an average of 0.4-0.6 t/ha and as high as 1.0 t/ha (CIAT, 1984; Beaver et al., 1985; Gutiérrez and Singh, 1985; Kelly et al., 1987). Negative relationships between seed size and yield have also been described by White et al. (1992), White and González (1990), and White and Izquierdo (1991). The seed-size effect accounts for a 280 kg/ha reduction in yield per 100 mg increase in cultivar seed weight (White and González, 1990). The relationship between seed size and yield also depends on environment. For example, positive relationships between seed size and yield were recorded in trials situated at high elevations with low mean temperature (White and González 1990; White and Izquierdo, 1991).

D. Morphological traits

Morphological traits such as bracteoles, flower colour, leaf shape and fifth internode length, among others, have been

used to classify germplasm. However, their value as marker-based selection criteria is unknown.

Selection for morphological markers and overall architectural plant characteristics is a rapid and straightforward process because of easy visualisation, higher heritability, and additive genetic control (Ghaderi and Adams, 1981; Nienhuis and Singh, 1986; Singh, 1991). With *Phaseolus vulgaris*, morphological traits combined with correlation studies have shown that node number, plant height, and the number of leaves per plant are positively associated with seed yield via pod number, but that seed weight is negatively correlated with node number (Duarte and Adams, 1972; Denis and Adams, 1978; Adams, 1982; Nienhuis and Singh, 1985). Nienhuis and Singh (1985) found that internode length is an important architectural factor as it is positively correlated with yield and seed size. Furthermore, Singh et al. (1991a) reported that fifth internode length was helpful in distinguishing Andean from Middle American germplasm, with greater lengths indicating Andean germplasm.

Conflicting studies involving the enhanced expression effects of plant architectural traits, especially those showing developmental associations, on yield have led Singh (1991) to summarize that architectural traits may or may not

be useful, depending on such variables as environment, agronomic management, or yield component compensation.

E. The use of intergenepool populations and recombinant inbreds

There has been a general tendency for breeders to implement hybridization within bean genepools because of a history of poor adaptation of introduced germplasm as well as low frequencies of appropriate desirable recombinants (Singh, pers. comm., 1992). However, small or no yield gains are expected from intragenepool and intraracial populations because of insufficient genetic variation (Singh et al., 1989; Singh and Gutiérrez, 1990). Lines derived from interracial and intergenepool populations have outyielded those from intragenepool populations (Singh et al., 1989 and 1992b; Singh and Gutiérrez, 1990). Apparently, the higher yield gains obtained in intergenepool populations reflect a larger degree of useful genetic variation.

Previous genetic and selection studies in common bean have used early segregating generations of hybrid populations, for which heterozygosity and heterogeneity for desirable attributes is high. This often does not permit a reliable evaluation of individual genotypes for such important traits

as seed yield, because a larger quantity of seed is required. Moreover, the relative importance and proportion of different genotypic frequencies and types of gene action change considerably from one generation to another. Few studies of common bean have used recombinant inbred lines, which offer several advantages over commonly used F_2 and backcross populations. Recombinant inbreds constitute a perpetual population (i.e., they are genetically stable, hence no changes in genotypic frequencies and gene action are expected) and source of marker alleles (Burr et al., 1988). Also, as reported by these authors, recombinant inbred populations are more efficient than backcross populations for calculating recombination frequencies and map distances. All genetic variation for quantitative traits is additive and is distributed among lines instead of within lines. Moreover, recombinant inbred lines permit reliable evaluation for morphological, molecular, agronomic, and adaptive marker traits at contrasting sites and over growing seasons.

F. Marker traits

The value of marker-assisted selection for agronomic traits in common bean is not known except for resistance to bruchids (*Zabrotes* spp.) in bean lines possessing the arcelin protein. Other molecular traits, such as phaseolin, allozymes,

random amplified polymorphic DNA (RAPD), and RFLP applications, could very well serve as markers for agronomic traits.

1. The phaseolin protein

Phaseolin is the major storage protein of *Phaseolus vulgaris* L. and possesses between 35 and 50% of total seed nitrogen (Ma and Bliss, 1978; Gepts and Bliss, 1986).

Two-dimensional IEF/SDS-PAGE analysis has revealed that T and S phenotypes have no common structural polypeptides (Brown et al., 1981b). Brown et al. (1981b and 1982a) found that the T phaseolin is composed of five polypeptides, whereas the S and C phenotypes comprise eight polypeptides each. The C phenotype is unique and contains all five of the protein subunits of the T pattern, two of the S pattern, and a unique polypeptide (Brown et al., 1981b; Talbot et al., 1984; Gepts et al., 1986; Gepts, 1988a).

The phaseolin polypeptides are coded by a group, or family, of co-dominant, tightly linked alleles operating as a singular heritable unit (Brown et al., 1981a). Tight linkage between genes reduces the probability of crossing over amongst the chromosomal homologues, thereby lowering phaseolin

recombination probability.

G. Linkage studies in *Phaseolus vulgaris* L. using morphological, protein, isoenzyme, RFLP, RAPD, and agronomic marker traits

In comparison with that of other crops, the linkage map of common bean is not well developed. One of the major challenges in bean genetics is to produce a linkage map that will integrate both molecular/biochemical and morphological markers with agronomic and adaptive traits. A study of recent literature on quantitative traits in *P. vulgaris* suggests that a multifactorial approach involving phaseolins, isozymes, RAPDs, and RFLPs is needed. Individually, neither morphological and agronomic traits nor molecular markers (phaseolins, isozymes, RAPDs and RFLPs) will suffice to provide complete information on patterns of variation to be found in any crop. However, these traits and markers taken together could serve as satisfactory selection criteria (Stuber, 1989; Wendel and Weeden, 1989; Singh et al., 1991b). A combination of these methodologies and approaches covering a wide range of traits would provide the most credible strategy towards addressing these problems.

Mok (1989) argues that more information on morphological

markers, proteins, and isoenzymes is needed to effect accurate genomic mapping. It has also been suggested that molecular tools such as RFLP techniques can be used to generate a saturated map for *P. vulgaris* allowing a genetic analysis of the quantitative trait loci (QTL) that control yield, maturity, and plant height (Kelly et al., 1991). As Kelly et al. (1991) state, the use of RFLPs would not be essential to study simply inherited traits, but would be useful in studying those traits that require tedious and costly measurement techniques as well as those traits that are unevenly and non-reproducibly expressed in specific environmental conditions, such as yield, drought, and low soil fertility tolerance.

Recently, several researchers have begun to develop linkage maps for *P. vulgaris* and have established several linkage groups. For example, Weeden and Liang (1985) reported a linkage between the white flower colour of *P. vulgaris* and the allozyme allele Est-2. Koenig and Gepts (1989b) have confirmed a linkage between the gene system coding for phaseolin protein (Phs) and the gene coding for seed shininess (J). Vallejos and Chase (1991) have employed RFLP techniques to uncover polymorphism in common bean. Polymorphism for RFLP, phaseolin, and isozymes has been used to develop a linkage map. One of the isozyme linkages (Adh-1 to Got-2 segment) seems to be linked with a locus (Ssz_1) that affects seed size.

Studies by Nodari et al. (1992 and in press) have concentrated on polymorphism between and within each of the two major genepools using genomic DNA probes and RFLP applications with a number of evolutionary divergent genotypes. They argue that mapping over several populations will result in a denser map and increased likelihood of detecting polymorphic markers in the given chromosomal region.

The above findings indicate established efforts directed at the genomic mapping of *Phaseolus vulgaris* L. using a variety of experimental techniques as Mok (1989) has suggested. However, little is known regarding the use of isoenzyme and protein markers in relation to reliable agronomic and morphological markers in bean improvement programs and how such relationships would contribute toward the linkage map of *Phaseolus vulgaris* L.

III. MATERIALS AND METHODS

A. Parental material

Eight common bean cultivars or lines of contrasting characteristics were used to develop four single-cross populations. Both parents of population Canadian Wonder x A486 had determinate type I growth habit, large seed size, and characteristics of Andean race Nueva Granada (Singh et el., 1991a). The second population was a cross between two parental types from differing geographic origins but possessing the same type I growth habit. Large-seeded Andean material ICA L23 of race Nueva Granada was crossed with small-seeded Middle American material Brasil 2 of race Mesoamerica. Population Rio Tibagi x ABA 58 was a cross between a widely used small-seeded Brazilian cultivar of type II growth habit and black seed coat colour belonging to race Mesoamerica. ABA 58 possessed large white seeds and type I growth habit and other characteristics of Andean race Nueva Granada. The fourth population was obtained by crossing Redkloud with MAM 4. The former parent was of Andean origin (race Nueva Granada) and possessed a type I growth habit, whereas the latter parent was of Middle American race Durango with indeterminate type III growth habit. One population was thus intraracial and the other three were interracial.

Using hand emasculation and pollination, the F_1 of all crosses were produced in late 1987. The F_1, along with the parents, were planted in the first trimester of 1988 to verify their hybrid origin and produce selfed (F_2) seeds. The F_2 populations were space-planted. More than 300 single plants, taken randomly, were harvested from each population to develop F_2-derived F_5 or F_6 recombinant inbred lines. A random sample of 79 lines and two parents from each population was then used to analyse phaseolin proteins and isoenzymes. Data on morphological and agronomic traits were collected in the F_6 or F_7 (first year) and F_7 or F_8 (second year).

B. Field trials

Seventy-nine recombinant inbred lines, along with two parents from each of the four populations, were planted at CIAT farms at Popayán (1750 metres above sea level) and Palmira (1000 m.a.s.l.), Colombia. The soil in Palmira is a fine/sulty mixed, isohyperthermic Aquic Hapludoll type (pH 7.0) and Popayán has medial, isothermic Typic Dystrandept soil (pH 5.3). These two sites were used in order to study environmental effects on development and growth. At both locations, a 1 ha plot was subdivided into four subplots (one for each of the four populations). In 1990, each plot consisted of a single row with two replications (reps-in-set

design). Each row was 3 m long with spacing of 0.6 m between rows at Palmira and 0.5 m at Popayán. A population density of 21 plants/m^2 for Popayán and Palmira was used. Of the 3 m sown for each row, the outer 0.5 m on both ends (i.e., border region) was not harvested, leaving the central 2 m for harvesting. In 1991, the field trials were repeated at both locations, using a similar experimental design. However, each plot consisted of four rows, 5 m long. In both years, trials were protected from diseases and pests. Fields were kept free from weeds and agronomic management was according to recommended practices to assure good crop growth and development. Data were recorded on the two central rows, leaving head borders of 0.5 m on both ends. Data were recorded for the following traits:

1. Leaf shape. The shape of the central leaflet of fully developed trifoliolate leaves was noted. It can be cordiform or heart-shaped, ovate, rhombohedric, lanceolate, or hastate.

2. Bracteole shape and size. Bracteoles, in the flowers of *Phaseolus vulgaris* L., are located at the base of the flower, embracing the calyx. The overall shapes and sizes of the bracteoles were recorded for each recombinant inbred line and parent. Bracteole size was ranked as small, medium, or large. Lance-shaped and triangular bracteoles are of Andean origin.

In contrast, heart-shaped bracteoles are of Middle American origin.

3. Flower colour. Colour of flower petals was recorded for plants bearing fresh, new flowers. This was done in order to discern the yellow colour of senescing flowers from the white, purple, pink, or lilac colour of fresh flowers. In noting overall flower colour, both the standard and winged portions of each flower were observed.

4. Growth habit. Determinate type I, indeterminate erect type II, and indeterminate prostrate semi-climbing type III growth habits were recorded according to Singh (1982).

5. Seed yield (kg/ha). Seeds harvested from each plot were separated from plant material and sun-dried. Materials were weighed repeatedly as they dried over a period of time until a constant weight value was obtained.

6. Seed weight (g). One hundred seeds were randomly taken from the harvested bulk for each plot, and were subsequently dried and weighed on an analytical balance.

7. Plant dry weight (biomass) (kg/ha). Plant dry weight was recorded as a post-harvest trait. Harvested plant material

consisted of the seed, pod walls, the main stem, and branches, but excluded roots (below the point of the hypocotyl). Plants were sun-dried and repeatedly weighed until a constant weight value was obtained. Seed yield, seed weight, and plant dry weight were expressed on a 14% moisture basis.

8. Harvest index. The harvest index was recorded as the ratio of seed yield to plant dry weight:

$$\text{Harvest index} = \frac{\text{weight of seeds harvested/m}^2}{\text{weight of total plant material/m}^2}$$

9. Number of pods per plant. The total number of harvested pods from each plot was divided by the total number of plants harvested. The resulting value was the number of pods/plant.

10. Number of days to maturity. The number of days from date of planting to date of harvesting was recorded.

11. Internode length (cm). The distance between the fifth and sixth nodes on the main stem was recorded in cm at maturity.

C. Statistical analysis of agronomic traits

A pooled analysis of variance (ANOVA) was conducted

according to McIntosh (1983). Years and treatments (lines) were random and locations fixed. The Genstat package of statistical programs (Rothamsted Agricultural Experiment Station, United Kingdom) was used to analyse the data.

Narrow-sense heritability (h^2) was calculated according to Hallauer and Miranda (1981). Percentage genetic gain (20% selection pressure) was also calculated according to Frey and Horner (1957).

Simple correlation coefficients were calculated among agronomic traits using the Pearson correlation coefficient procedure on SAS (SAS Institute Inc., Cary, North Carolina, USA).

D. Protein extract preparation (phaseolin and total proteins)

Extraction and subsequent analysis of *Phaseolus vulgaris* L. total proteins were performed based on sodium dodecylsulphate polyacrylamide gel (SDS-PAG) electrophoresis protocols established by Brown et al. (1981a) and Gepts et al. (1986). Stacking and separation gels were 4.0% and 13.86% acrylamide, respectively. The electrophoretic separation conditions were 95 mA/250 v for seven to eight hours and the

stain used was Coomassie-G Blue (Table 1).

E. Seed material preparation (isoenzymes)

Five-day-old incubation room-germinated plants (25-27°C) were transferred to a greenhouse (30-32°C) for growth for 15 days, after which the roots and youngest leaves were removed and transferred to deep-freeze storage conditions (-80°C) until extraction. Leaves and stems weighing 0.3 g were ground-extracted with 0.6 ml of 0.1M Tris-malate buffer adjusted to pH 7.4 in a chilled mortar and pestle. Likewise, 0.5 g of root were ground and extracted with 0.5 ml of 0.1M Tris-malate buffer adjusted to pH 7.4. The extracts were stored at -80°C until use.

Material preparation and isoenzyme analyses was undertaken according to Hussain et al. (1986). The 10% (w/v) starch solution was prepared by dissolving electrophoresis-grade potato starch (Sigma Scientific) in 10% (v/v) 0.03M lithium-borate buffer adjusted to pH 8.1 with 0.05M Tris-citrate buffer pH 8.4. The final volume was adjusted with water, mixed and heated over an open flame until reaching a boiling, thick, translucent state, after which it was degassed for two minutes. The molten starch solution was poured into a precleaned, prelevelled plastic die-cast mold measuring 19 x

Table 1. Coomassie-G Blue stain for total proteins of *Phaseolus vulgaris* L.

A. Stock solution I

H_3PO_4 2%	2.35 ml
H_2O (distilled)	80.0 ml
$(NH_4)_2SO_4$	10.0 ml

B. Stock solution II (5% concentrated Coomassie stain solution)

Coomassie-G	1 g
H_2O (distilled)	20.0 ml

c. Stock solution III (Coomassie-G stain solution)

Stock solution I	80.0 ml
Stock solution II	1.6-3.0 ml
Methanol	20.0 ml

17 x 1.3 cm. Small bubbles were removed from the gel with Pasteur pipettes. The gel was left to solidify for approximately one hour. When thawed, the protein extracts were applied to the starch gel in the form of small, rectangular (0.5 x 1.5 cm) sample-impregnated paper wicks fashioned by strips of Whatman #3 filter paper. The gel was cut parallel to the width of the mold at an inside distance of 5 cm from one side. The wicks containing the sample extracts were fully inserted and placed upright into the slit with tweezers so that the upper portions of the wicks protruded perpendicularly from the gel. The gel mold, containing the cut gel and samples, was placed on top of the buffer chamber containing 0.03M lithium-borate buffer adjusted to pH 8.1. Gauze cloth was soaked in the buffer and applied, where it remained, on both of the gel extremities and buffer reservoirs to ensure conductivity. The chamber below the gel was packed with ice and the entire apparatus was placed in a refrigerator (4°C). The power supply was a Pharmacia ECPS 3000/150 unit (Pharmacia Fine Chemicals, Sweden) programmed to run at 300 volts, 25 watts, with an initial current of 50 milliamps. After 15 minutes, the wicks were removed from the gel and the current was increased to 60 milliamps to run for 6 hours at 4°C.

F. Gel slicing and staining

The gel was removed from the mold by inverting it onto a glass surface. The gel slab was cut horizontally into six slices of 0.3 cm thickness using nylon thread. Each of the gels was placed inside plastic trays containing enzyme-specific stains.

For root samples, the Diap-1, Diap-2, Me^{98}, Me^{96}, Mdh^{100}, Mdh^{98}, $Skdh^{100}$, and $Skdh^{98}$ alleles were visualised and studied for the following isoenzyme systems: diaphorase (DIAP), malic enzyme (ME), malate dehydrogenase (MDH), and shikimic dehydrogenase (SKDH), respectively.

For leaf samples, the Got^{100}, $Rbsc^{100}$, $Rbsc^{98}$, $Rbsc^{96}$, Acp^{100}, Acp^{98}, and Acp^{96} alleles were visualised and studied for the following isoenzyme systems: glutamate oxaloacetate transaminase (GOT), ribulose biphosphate carboxilase (rubisco or RBSC), and acid phosphatase (ACP), respectively.

The isoenzyme stains are listed in Table 2. To visualise the banding patterns, approximately 50-70 ml of stain were required. The gel slices were incubated at 37°C for one to two hours, after which the gels were washed free of the stain and were fixed in a solution of 50% ethanol containing 1% acetic

Table 2. Stains for isoenzyme systems analysed for root and leaf tissue of *Phaseolus vulgaris* L.

Root material

Diaphorase (DIAP) E.C.1.6.4.3

Buffer/stain:

Tris-HCl 1M, pH 8.5	5 ml
H_2O (distilled)	45 ml
NADH	40 mg
2.6 Dichloroindophenol (DCIP)	trace
MTT	10 mg

Malic enzyme (ME) E.C.1.1.1.40

Buffer/stain:

Tris-malate 0.1M, pH 7.2	45 ml
Magnesium chloride	5 ml
NADP	10 mg
MTT	6 mg
1-Malate	20 mg
Phenazine methosulphate (PMS)	trace

Malic dehydrogenase (MDH) E.C.1.1.1.37

Buffer/stain:

Tris-malate 0.1M, pH 7.2	50 ml
NAD	20 mg
MTT	8 mg
1-Malate	20 mg
PMS	trace

Shikimic dehydrogenase (SKDH) E.C.1.1.1.25

Buffer/stain:

Tris-HCl 1M, pH 8.5	5 ml
H_2O (distilled)	45 ml
Shikimic acid	30 ml
NADP	8 mg
MTT	6 mg
PMS	trace

Leaf material

Ribulose biphosphate carboxylase (Rubisco/RBSC) E.C.4.1.1.39

Buffer/stain:

Naphthol blue black	40 mg
Destaining solution:	40 ml
Methanol	50 ml
H_2O	50 ml
Acetic acid	10 ml

Glutamate oxaloacetate transaminase (GOT) E.C.2.6.1.1

Buffer/stain:

Tris-HCl 1M, pH 8.0	5 ml
H_2O (distilled)	45 ml
1-Aspartic acid	100 mg
∝-Ketoglutaric acid	50 mg
Pyridoxal-5-phosphate	4 mg
(mix before use and then add stain)	
Fast blue BB salt	50 mg

Acid phosphatase (ACP) E.C.3.1.3.2

Buffer/stain:

Sodium-acetate 0.1M, pH 5.0	50 ml
Sodium-naphthyl acid phosphate	50 mg
Fast Garnet GB salt	50 mg

acid. Those gels stained with reagents requiring the use of methyl thiazolyl tetrazolium (MTT) were soaked for 20 minutes. Those gels stained with reagents not including MTT were soaked overnight. After fixing, the gels were packed in clear plastic bags and stored at 4°C.

G. Correspondence analysis

The recombinant inbred lines were grouped according to the frequency distribution for each of the marker morphological traits, total protein patterns, and isoenzyme patterns. The means for agronomic traits were then compared pairwise for significant differences ($P < 0.05$) using a Student's t-test on SAS.

IV. RESULTS

A. General characteristics

Table 3 presents mean values for agronomic traits, including seed yield obtained from evaluations over two years at two locations, along with those for morphological markers, allozymes, and seed protein patterns for the eight parents used in intra- and interracial hybridization and development of the recombinant inbred lines. Significant differences ($P < 0.05$) were found for all agronomic traits among parents. ABA 58, followed by MAM 4 and Rio Tibagi, had the highest seed yield. A 486 had the lowest yield of all the parents. All large-seeded parents of Andean origin (e.g., Redkloud, Canadian Wonder, ICA L23, A 486, and ABA 58) had a high 100-seed weight. Two parents of race Mesoamerica, Brasil 2 and Rio Tibagi, had the lowest seed weight.

Canadian Wonder and A 486 did not differ for any of the agronomic traits, morphological markers, or proteins and allozymes, with the exception of bracteole type and the MDH isoenzyme. ICA L23 and Brasil 2 differed for bracteole type, leaf shape, phaseolin, and all other proteins. They also showed differences for isoenzymes ME, MDH, SKDH, RBSC, and ACP. Rio Tibagi and ABA 58 differed in terms of their growth

Table 3. Characteristics of common bean parents used in intra- and interracial hybridization and development of recombinant inbred lines.

Character	Canadian Wonder	A 486	ICA L23	Brasil 2	Rio Tibagi	ABA 58	Redkloud	MAM 4
Yield (kg/ha)[a]	1737	1427	1833	1623	2287	2749	1780	2685
100-seed weight (g)[a]	45.6	47.3	45.8	22.2	17.4	49.2	51.5	37.6
Biomass (kg/ha)[a]	3318	2800	3336	2628	4329	4887	3208	4469
Harvest index[a]	0.63	0.59	0.61	0.53	0.55	0.62	0.59	0.64
Pods/m^{2}[a]	165.3	120.6	134	263.5	278.8	266	159.2	253.2
Days to maturity[a]	71.7	74.5	84.2	81.8	84	79.9	73.8	75.2
Fifth internode length (cm)[a]	7.6	6.6	5.9	3.9	3.8	7.3	10.3	6.5
Growth habit	I	I	I	I	II	I	I	III
Flower colour[b]	L	L	W	W	P	W	W	W
Bracteole shape[c]	V	Lt	Lt	C	C	V	V	Lt
Leaf shape	R	R	R	C	C	C	C	R
Phaseolin	T	T	T	S	S	T	T	S
Lectin	1	1	2	1	2	1	1	2
Group 1 proteins	1	1	2	1	2	1	1	2
Group 2 proteins	1	1	2	1	2	1	1	2
DIAP1[d]	1	1	2	1	1	2	1	2
DIAP2[d]	1	1	2	2	2	2	2	2
ME	$\underline{Me}^{100}$	$\underline{Me}^{100}$	$\underline{Me}^{98}$	$\underline{Me}^{100}$	$\underline{Me}^{100}$	$\underline{Me}^{98}$	$\underline{Me}^{98}$	$\underline{Me}^{98}$
MDH	$\underline{Mdh}^{98}$	$\underline{Mdh}^{100}$	$\underline{Mdh}^{100}$	$\underline{Mdh}^{98}$	$\underline{Mdh}^{100}$	$\underline{Mdh}^{100}$	$\underline{Mdh}^{100}$	$\underline{Mdh}^{100}$
SKDH	$\underline{Skdh}^{100}$	$\underline{Skdh}^{100}$	$\underline{Skdh}^{98}$	$\underline{Skdh}^{100}$	$\underline{Skdh}^{100}$	$\underline{Skdh}^{98}$	$\underline{Skdh}^{98}$	$\underline{Skdh}^{98}$
RBSC	$\underline{Rbsc}^{100}$	$\underline{Rbsc}^{100}$	$\underline{Rbsc}^{100}$	$\underline{Rbsc}^{98}$	$\underline{Rbsc}^{98}$	$\underline{Rbsc}^{98}$	$\underline{Rbsc}^{98}$	$\underline{Rbsc}^{98}$
GOT	$\underline{Got}^{100}$	$\underline{Got}^{100}$	$\underline{Got}^{100}$	$\underline{Got}^{100}$	$\underline{Got}^{100}$	$\underline{Got}^{100}$	$\underline{Got}^{100}$	$\underline{Got}^{100}$
ACP	$\underline{Acp}^{100}$	$\underline{Acp}^{100}$	$\underline{Acp}^{100}$	$\underline{Acp}^{98}$	$\underline{Acp}^{100}$	$\underline{Acp}^{98}$	$\underline{Acp}^{98}$	$\underline{Acp}^{100}$

[a] mean over two years at two locations.
[b] L=lilac; W=white; P=purple
[c] V=variable; R=rhombohedric; C=cordate; Lt=lanceolate
[d] there are two banding patterns possible for both DIAP1 and DIAP2.

habit, flower colour, bracteole type, phaseolin, and all other proteins, as well as for isoenzymes ME, SKDH, and ACP. Redkloud had a type I determinate growth habit, whereas MAM 4 had a type III indeterminate growth habit. In addition, these two parents differed in bracteole type, leaf shape, phaseolin type, other various proteins, and demonstrated polymorphic differences for the ACP isoenzyme.

Effects of location, year, population, treatment (recombinant inbred lines), and interactions among them were significant for seed yield and yield components, harvest index, and days to maturity (Table 4). All main effects, except that of location, and the first order interactions were also significant for fifth internode length and biomass weight.

Mean, maximum, and minimum values for all agronomic traits for 79 recombinant inbred lines, along with parents for each of the four populations, are given in Table 5. Rio Tibagi x ABA 58 had the highest mean yield, followed by Redkloud x MAM 4. On the other hand, ICA L23 x Brasil 2 had the lowest mean yield, even lower than the intraracial Canadian Wonder x A 486 population. However, this was the only population for which the highest yielding line TY 5578-45 significantly outyielded the highest yielding parent. The lowest yielding

Table 4. Mean squares from pooled analysis of variance for recombinant inbred lines derived from intra- and interracial populations of common bean evaluated over two years at two locations in Colombia.

	df	Yield	100-seed weight	Biomass	Harvest index	Pods/m^2	Days to maturity	Fifth internode length
Location (L)	1	$2.2x10^{8**}$	2053.9^{**}	$9.9x10^{6}$	12.1^{**}	83542.8^{**}	22637.4^{**}	0.5
Year (Y)	1	$2.9x10^{8**}$	9393.3^{**}	$1.9x10^{8**}$	0.8^{**}	$1.9x10^{6**}$	19919.1^{**}	1193.7^{**}
Population (P)	3	$2.5x10^{7**}$	54741.4^{**}	$9.7x10^{7**}$	0.7^{**}	$1.6x10^{8**}$	12470.3^{**}	816^{**}
L x Y	1	$1.7x10^{8**}$	8577.2^{**}	$7.5x10^{8**}$	0.8^{**}	$5.4x10^{5**}$	17847.4^{**}	1334^{**}
L x P	3	$2.7x10^{7**}$	1140.6^{**}	$6.2x10^{7**}$	0.3^{**}	$2.3x10^{5**}$	725.6^{**}	164.6^{**}
Y x P	3	$8.8x10^{7**}$	3210.4^{**}	$2.9x10^{8**}$	0.3^{**}	$5.9x10^{5**}$	1017.6^{**}	311^{**}
L x Y x P	3	$3.3x10^{6}$	256.5^{**}	$2.7x10^{7*}$	$6.7x10^{-2}$	$1.1x10^{5**}$	759.9^{**}	51.8^{*}
Rep (L x Y x P)	16	$2x10^{6}$	22.3	$7.1x10^{6}$	$1.9x10^{-2}$	18606	21.5	11.1
Block/Rep L x Y x P	256	$5.9x10^{5}$	42.5	$1.7x10^{6}$	$7x10^{-3}$	5215.5	18.2	7.6
Inbred lines (T)[a]/P	320	$6.2x10^{5**}$	189.1^{**}	$1.9x10^{6**}$	$1.9x10^{-2**}$	9723.2^{**}	53.1^{**}	21.3^{**}
L x T/P	320	$4.5x10^{5**}$	27.5^{**}	$1.1x10^{6**}$	$9.1x10^{-3**}$	4503.6^{**}	21.4^{**}	5.8^{**}
Y x T/P	320	$3.8x10^{5**}$	16.6^{**}	$1.1x10^{6**}$	$8.1x10^{-3**}$	4736.1^{**}	13.4^{**}	3.3^{**}
L x Y x T/P	320	$3.8x10^{5**}$	13.5^{**}	$1x10^{6**}$	$7.5x10^{-3**}$	3922.3^{**}	14.5^{**}	2.8^{**}
Pooled error	1024	$1.3x10^{5}$	7.0	$4.6x10^{5}$	$3.7x10^{-3}$	1838.5	7.7	1.6

[a] T = treatment

*, ** P = 0.05, P = 0.01, respectively

Table 5. Mean, maximum, and minimum for yield and other agronomic traits for recombinant inbred lines from inter- and intraracial populations of common bean evaluated at two locations over two years in Colombia.

Population, parents, and recombinant inbred lines	Yield (kg/ha)	100-seed weight (g)	Biomass (kg/ha)	Harvest index	Pods/m^2	Days to maturity	Fifth internode length (cm)
Canadian Wonder x A 486							
Canadian Wonder	1737	45.6	3318	0.63	165.3	71.7	7.6
A 486	1427	47.4	2800	0.59	120.6	74.5	6.6
Recombinant inbred lines							
Mean	1571	45.3	2900	0.62	144.6	73.5	6.8
Maximum	2073	54.9	3846	0.68	204.0	76.7	8.1
Minimum	1073	37.3	1926	0.55	109.1	69.2	5.2
ICA L23 x Brasil 2							
ICA L23	1833	45.8	3336	0.61	134.0	84.2	5.9
Brasil 2	1623	22.2	2628	0.53	263.5	81.8	3.9
Recombinant inbred lines							
Mean	1434	26.9	2609	0.56	199.9	81.7	5.6
Maximum	2314	42.2	4175	0.69	310.1	86.4	10.4
Minimum	916	17.0	1726	0.44	123.3	77.2	2.2

Rio Tibagi x ABA 58							
Rio Tibagi	2287	17.4	4329	0.55	278.8	84.0	3.8
ABA 58	2749	49.2	4887	0.62	266.0	79.9	7.3
Recombinant inbred lines							
Mean	1879	24.6	3508	0.56	265.6	81.9	5.3
Maximum	2667	37.5	5023	0.65	397.0	87.3	10.6
Minimum	1269	17.5	2165	0.43	156.3	74.2	2.3
Redkloud x MAM 4							
Redkloud	1780	51.5	3208	0.59	159.2	73.8	10.3
MAM 4	2685	37.6	4469	0.64	253.2	75.2	6.5
Recombinant inbred lines							
Mean	1727	33.2	3104	0.59	206.2	75.1	7.7
Maximum	2537	49.1	4161	0.71	269.2	82.9	14.4
Minimum	1263	26.7	1858	0.45	144.4	66.0	4.7
LSD (0.05)[a]	352	2.6	661	0.06	42.0	2.7	1.2
LSD (0.05)[b]	155	0.5	301	0.01	15.5	0.5	0.4

[a] For comparison of values for parents and maximum and minimum for recombinant inbred lines within and among populations.
[b] For comparison of mean values of recombinant inbred lines among populations.

line yielded significantly less than the lowest yielding parent in all of the populations.

Population Rio Tibagi x ABA 58 significantly outyielded all other populations except population Redkloud x MAM 4 (Table 5). Rio Tibagi x ABA 58 outyielded intraracial population Canadian Wonder x A 486 by 308 kg/ha. The highest yielding recombinant inbred line (WA 7807-271, with yield of 2667 kg/ha) of the former population outyielded that of the latter population (TR 7562-186) by 594 kg/ha. The higher mean yield obtained by Rio Tibagi x ABA 58 over that of Canadian Wonder x A 486 possibly demonstrates the higher comparative yielding potential of interracial crosses as reported by Singh et al. (1992b). However, the highest yielding line of Rio Tibagi x ABA 58 did not outyield the highest yielding parent, ABA 58 (2749 kg/ha).

Interracial population Redkloud x MAM 4 significantly outyielded Canadian Wonder x A 486 by 156 kg/ha. The highest yielding recombinant inbred line from Redkloud x MAM 4 (TR 7618-210, 2537 kg/ha) outyielded the highest yielding recombinant inbred of Canadian Wonder x A 486 (a difference of 464 kg/ha) but did not outyield the highest yielding parent (MAM 4, 2685 kg/ha) for this population.

In population ICA L23 x Brasil 2, eight recombinant imbred lines (TY 5578-141, TY 5578-209, TY 5578-186, TY 5578-190, TY 5578-47, TY 5578-69, TY 5578-118, TY 5578-45) outyielded the highest yielding parent, ICA L23 (1833 kg/ha). The highest yielding recombinant inbred (TY 5578-45, 2314 kg/ha) also had a 100-seed weight of 41.5 g and outyielded ICA L23 by 481 kg/ha.

Twenty recombinant inbred lines of Canadian Wonder x A 486 outyielded the highest yielding parent, Canadian Wonder (1737 kg/ha). The highest yielding recombinant inbred line (TR 7562-186) outyielded Canadian Wonder by 336 kg/ha.

Rio Tibagi x ABA 58 had the largest biomass and highest number of pods/m^2 and days to maturity while possessing the smallest seed size and fifth internode length of all populations. Comparatively lower biomass yields were recorded for populations Canadian Wonder x A 486 and ICA L23 x Brasil 2, whereas the former population had the smallest number of pods/m^2. Canadian Wonder x A 486 and Redkloud x MAM 4 were found to be earlier maturing than the remaining populations. Canadian Wonder x A 486 had the highest value for harvest index (0.62). Mean harvest index for the three interracial populations ranged from 0.56 to 0.58.

B. Heritability and genetic gain

Of the seven agronomic traits studied, 100-seed weight had the highest heritability in all four populations (Table 6). This was followed by fifth internode length, number of days to maturity, and harvest index. Biomass yield was found to have the lowest overall heritability. Heritability for seed yield ranged from 0.19 $\pm$ 0.17 (Redkloud x MAM 4) to 0.50 $\pm$ 0.16 (Rio Tibagi x ABA 58).

Genetic gain for seed yield ranged from 3.9% for Redkloud x MAM 4 to 11.4% for Rio Tibagi x ABA 58. It was found that fifth internode length had the highest percentage genetic gain (51.5%), for populations Rio Tibagi x ABA 58 and ICA L23 x Brasil 2 (45.6%). Days to maturity had the lowest overall percentage genetic gain, with a range of 2.5% (ICA L23 x Brasil 2) to 5.0% (Redkloud x MAM 4).

C. Correlation coefficients among agronomic traits

Table 7 presents correlation coefficients among yield, its components, and other agronomic traits, using mean values across locations and years for recombinant inbred lines from all four populations. Days to maturity and pods/m^2 shared significant correlations ($P < 0.05$) with all of the other

Table 6. Heritability (h^2) and percentage genetic gain from selection[a] (GS) in recombinant inbred lines from intra- and interracial populations of common bean evaluated over two years at two locations in Colombia.

Character	Canadian Wonder x A 486		ICA L23 x Brasil 2		Rio Tibagi x ABA 58		Redkloud x MAM 4	
	h^2 SE	GS	h^2 SE	GS	h^2 SE	GS	h^2 SE	GS
Yield	0.40 ± 0.16	7.8	0.31 ± 0.17	9.4	0.50 ± 0.16	11.4	0.19 ± 0.17	3.9
100-seed weight	0.80 ± 0.15	11.1	0.91 ± 0.15	26.6	0.94 ± 0.15	25.1	0.91 ± 0.15	20.6
Biomass	0.26 ± 0.17	4.6	0.37 ± 0.17	10.0	0.55 ± 0.16	12.5	0.36 ± 0.17	6.8
Harvest index	0.51 ± 0.15	3.0	0.51 ± 0.16	6.3	0.72 ± 0.21	7.7	0.69 ± 0.16	9.6
Pods/m^2	0.38 ± 0.16	6.3	0.61 ± 0.16	17.7	0.61 ± 0.16	15.1	0.38 ± 0.17	6.8
Days to maturity	0.72 ± 0.16	2.6	0.48 ± 0.16	2.5	0.63 ± 0.16	3.5	0.89 ± 0.16	5.0
Fifth internode length	0.30 ± 0.16	4.4	0.88 ± 0.16	45.6	0.91 ± 0.15	51.5	0.79 ± 0.15	29.6

[a] Calculated at 20% selection pressure and expressed as the % of the mean values of all recombinant inbred lines within each population.

Table 7. Pearson correlation coefficients among some agronomic traits obtained from F_2-derived F_6 recombinant inbred lines from intra- and interracial populations of common bean evaluated at two locations over two years in Colombia.

Character	Yield	100-seed weight	Biomass	Harvest index	Pods/m^2	Days to maturity
100-seed weight	-0.03					
Biomass	0.89**	-0.01				
Harvest index	0.03	0.34**	-0.18**			
Pods/m^2	0.55*	-0.70**	0.54**	-0.25**		
Days to maturity	0.17**	-0.60**	0.28**	-0.46**	0.47**	
Fifth internode length	0	0.37**	-0.06	0.03	-0.27**	-0.47**

**, * P = 0.01 and P = 0.05, respectively.

agronomic traits. The relationship of days to maturity and pods/m^2 with 100-seed weight, harvest index, and fifth internode length was negative. Biomass and yield shared highly significant positive associations ($P < 0.01$). Biomass had a negative association with harvest index. Fifth internode length did not share any correlation with yield, but demonstrated a highly significant ($P < 0.01$) positive association with 100-seed weight and negative associations with pods/m^2 and days to maturity.

D. Marker traits

Occurrence and frequencies of morphological traits, seed protein patterns, and allozymes are presented in Table 8. The three interracial populations demonstrated a predominance of small- and medium-sized heart-shaped bracteoles, whereas population Canadian Wonder x A 486 demonstrated a mixture of lanceolate and other shaped bracteoles of a mostly medium size.

Population Canadian Wonder x A 486 displayed ovate-shaped leaves. The interracial populations also showed some recombinant inbred lines with oval leaves, but a greater percentage of cordate-shaped leaves was evident.

Both Canadian Wonder and A 486 had lilac flower colour. The majority (82.7%) of recombinant inbred lines from this population had lilac flower colour, but white flower colour (16%) was also found. The parents of population Rio Tibagi x ABA 58 possessed purple and white flower colours, respectively, whereas the recombinant inbred lines displayed white, lilac, and purple colours. Redkloud and MAM 4 each had white flower colour. The majority (51.9%) of the recombinant inbred lines also had white flower colour, but lilac and purple flowers were found in 4.9% and 37.0% of the lines, respectively.

E. Total proteins

Table 8 presents the frequencies for patterns of different protein fractions. The electrophoregrams (i.e., total protein banding patterns) constituted four groups of proteins, including phaseolins and lectins. In addition, a group of proteins between the phaseolins and lectins, and another group between the lectins and albumins, were faintly but consistently detected. These were termed "Group 1" and "Group 2" proteins, respectively. The recombinant inbred lines of population Canadian Wonder x A 486 did not differ markedly from one another nor from either of the parents. In contrast, interracial populations demonstrated varying degrees of

Table 8. Class frequencies (%) for marker morphological traits and seed protein and allozyme patterns in recombinant lines from intra- and interracial populations of common bean.

	Canadian Wonder x A 486	ICA L23 x Brasil 2	Rio Tibagi x ABA 58	Redkloud x MAM 4
Bracteole type				
Cordate	0	80.2	75.3	45.7
Lanceolate	49.4	7.4	11.1	19.8
Variable	50.6	12.3	13.6	34.6
Bracteole size				
Medium	87.7	46.9	70.4	44.4
Small	4.9	40.7	19.8	48.1
Large	7.4	12.3	9.9	7.4
Leaf type				
Cordate	7.4	23.5	38.3	30.9
Lanceolate	0	6.2	33.3	1.2
Rhombohedric	76.5	34.6	28.4	55.6
Variable	16.0	35.8	0	12.3
Flower colour				
White	16.0	93.8	22.2	51.9
Lilac	82.7	6.2	40.7	4.9
Purple	0	0	37.0	37.0
Variable	1.2	0	0	6.2
Seed proteins				
Phaseolin				
S	0	39.5	74.1	56.8
T	100.0	37.0	25.9	29.6
Variable	0	23.5	0	13.6
Lectin				
Maternal-type	0*	44.4	58.0	37.0
Paternal-type	0*	46.9	38.3	59.3
Variable	0*	8.6	3.7	3.7

Group 1 proteins				
Maternal-type	0[a]	0[a]	44.4	25.9
Paternal-type	0[a]	0[a]	53.1	74.1
Variable	0[a]	0[a]	0	0
Group 2 proteins				
Maternal-type	0[a]	84.0	58.0	34.2
Paternal-type	0[a]	16.0	33.3	56.2
Variable	0[a]	0	8.6	9.6
Allozymes				
Diap 1				
Pattern 1	100.0	61.0	33.0	51.0
Pattern 2	0	39.0	67.0	49.0
Diap 2				
Pattern 1	100.0	0	0	0
Pattern 2	0	100.0	100.0	100.0
Mdh^{98}	58.0	39.0	0	0
Mdh^{100}	42.0	61.0	100.0	100.0
Me^{96}	0	5.0	0	0
Me^{98}	0	42.0	5.0	100.0
Me^{100}	100.0	53.0	95.0	0
$Skdh^{98}$	0	52.0	37.0	51.0
$Skdh^{100}$	100.0	48.0	63.0	49.0
Got^{100}	100.0	100.0	100.0	100.0
Acp^{96}	100.0	5.0	0	0
Acp^{98}	0	44.0	33.0	25.0
Acp^{100}	100.0	51.0	67.0	75.0
$Rbsc^{96}$	100.0	10.0	10.0	15.0
$Rbsc^{98}$	100.0	48.0	33.0	41.0
$Rbsc^{100}$	100.0	42.0	57.0	44.0

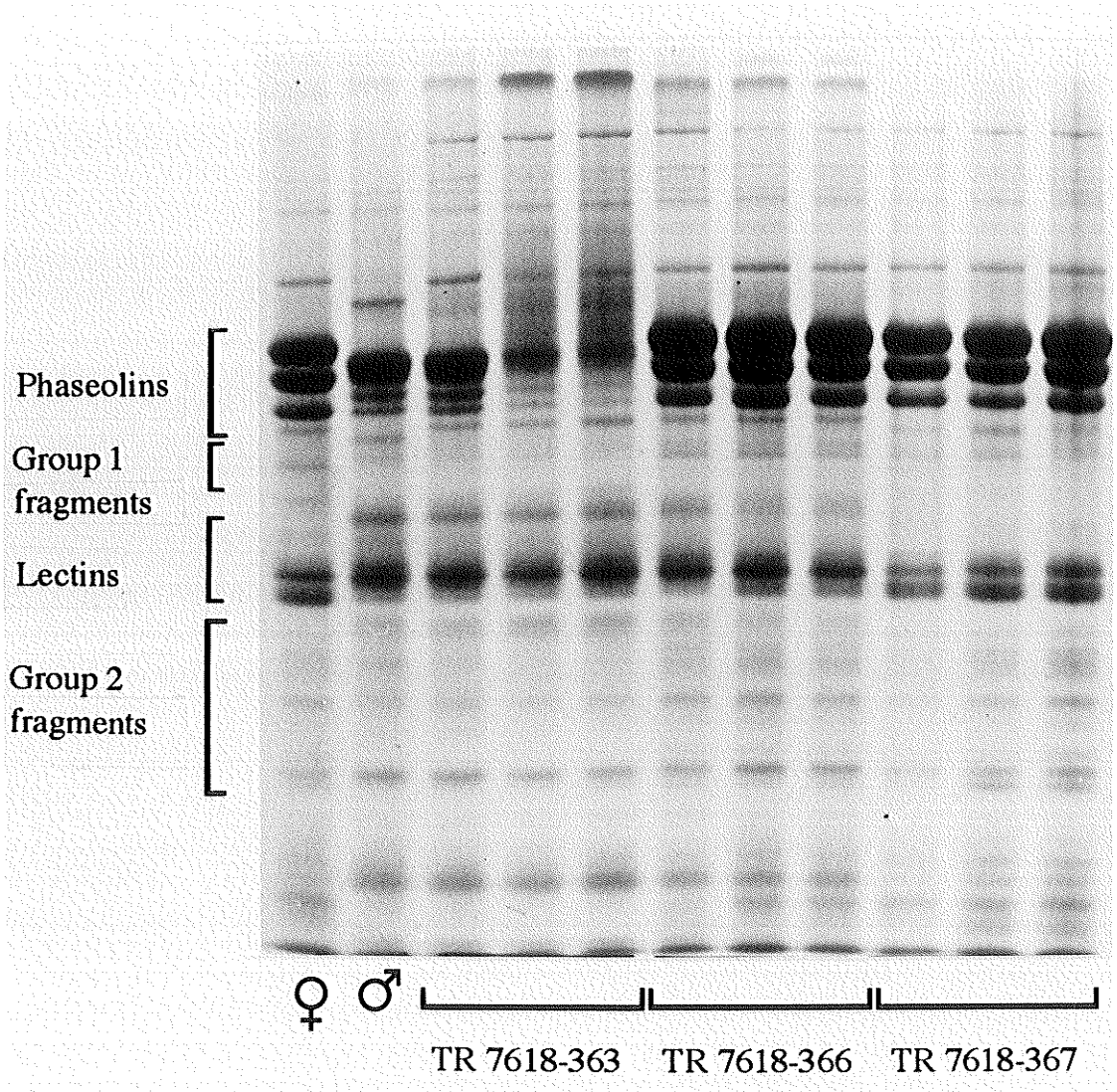
[a] No differences (i.e., no polymorphism) between parents.

recombination. A typical example appears in Figure 1. Parents Redkloud (Andean maternal material - T phaseolin type) and MAM 4 (Middle American paternal material - S phaseolin type) were the first two banding columns from left to right. Recombinant inbred lines from this population (TR 7618-363, TR 7618-366, and TR 7618-367) were run in triplicate. Recombinant inbreds TR 7618-363 and TR 7618-367 displayed electrophoregrams similar to parents MAM 4 and Redkloud, respectively. The second recombinant inbred, TR 7618-366, however, displayed the phaseolin banding pattern of Redkloud and the lectin profile of MAM 4. There were no detectable differences amongst the Group 1 nor Group 2 proteins in Figure 1.

The recombinant inbred lines from Rio Tibagi x ABA 58 demonstrated a skewed segregation ratio for phaseolin protein patterns. Apparently, there was an excess of lines with S phaseolin (Table 8). Similarly, skewed ratios were found for the lectin and Group 1 proteins for populations Rio Tibagi x ABA 58 and Redkloud x MAM 4. All three interracial populations demonstrated varying degrees of distorted frequencies for Group 2 proteins.

In this study, polymorphism for six isoenzyme systems (MDH, ME, SKDH, ACP, RBSC, GOT) originated at a single locus for each. Two independent loci had polymorphism for DIAP1 and

Figure 1. SDS-PAGE of Redkloud (♀) x MAM 4 (♂) and three F_2-derived F_6 recombinant inbred line progeny.


Phaseolins
Group 1 fragments
Lectins
Group 2 fragments
♀
♂
TR 7618-363
TR 7618-366
TR 7618-367

DIAP2. All isoenzymes, except for GOT and DIAP2, displayed polymorphism for all populations and are summarized in Table 8. It was found that DIAP1 displayed a high degree of polymorphism for the three interracial populations, whereas DIAP2 showed minimal polymorphic behavior. The results from the analysis of malate dehydrogenase (MDH) and malic enzyme (ME) systems seemed to serve as a basis for distinguishing among populations Redkloud x MAM4, Rio Tibagi x ABA 58, and ICA L23 x Brasil 2. For example, of the three populations, only ICA L23 x Brasil 2 demonstrated polymorphism for the two alleles involved in the expression of MDH (i.e., $\underline{Mdh}^{100}$ and $\underline{Mdh}^{98}$). Population Redkloud x MAM 4 did not display the common $\underline{Me}^{100}$ but instead expressed allele $\underline{Me}^{98}$. In contrast, population Rio Tibagi x ABA 58, while not demonstrating a high level of polymorphism, was found to express only the $\underline{Me}^{100}$ allele in 95% of the recombinant inbred lines. The remaining 5% of the lines in this population expressed the $\underline{Me}^{98}$ allele. All three alleles of ME (i.e., $\underline{Me}^{100}$, $\underline{Me}^{98}$, $\underline{Me}^{96}$) were expressed in population Rio Tibagi x ABA 58.

All interracial populations (ICA L23 x Brasil 2, Rio Tibagi x ABA 58, and Redkloud x MAM 4) displayed polymorphism between the two alleles of SKDH ($\underline{Skdh}^{100}$ and $\underline{Skdh}^{98}$) and for the three alleles of RBSC ($\underline{Rbsc}^{100}$, $\underline{Rbsc}^{98}$, and $\underline{Rbsc}^{96}$).

Distorted isoenzyme frequencies were detected for MDH and ACP in ICA L23 x Brasil 2. Rio Tibagi x ABA 58 showed distorted frequencies for DIAP1, SKDH, ACP, and RBSC. Redkloud x MAM 4 demonstrated similar ratios for SKDH, ACP, and RBSC.

A number of skewed segregation ratios were found for the combined morphological-allozyme/protein marker traits for the three interracial populations. Canadian Wonder x A 486 displayed somewhat distorted ratios for bracteole type, bracteole size, and flower colour, and $\underline{Mdh}^{98}/\underline{Mdh}^{100}$ (the only allozymes that demonstrated polymorphism in this population).

In the remaining populations, distorted ratios favouring cordate bracteole were found for all isoenzymes and proteins. Distorted ratios were observed for DIAP1, MDH, ME, RBSC, lectins, and Group 2 fragments (ICA L23 x Brasil 2); DIAP1, ME, SKDH, ACP, RBSC, phaseolin, lectins, and Group 2 fragments (Rio Tibagi x ABA 58); and ACP, RBSC, phaseolin, Group 1 fragments, lectins, and Group 2 fragments (Redkloud x MAM 4). Distorted ratios for both bracteole size and isoenzyme markers were found between medium- and large-sized bracteole for DIAP1, ME, ACP, RBSC, and Group 2 fragments (ICA L23 x Brasil 2); DIAP1, ME, SKDH, ACP, RBSC, phaseolin, lectins, and Group 2 fragments (Rio Tibagi x ABA 58); and ACP, RBSC, phaseolin, Group 1 and 2 fragments, and lectins (Redkloud x MAM 4). Leaf

shape and isoenzyme/protein markers also demonstrated skewed ratios. Flower colour, as with the other morphological marker traits, showed abnormal ratios.

F. Relationship between agronomic traits and morphological, seed protein, and allozyme marker traits

The recombinant inbred lines from each population were grouped according to class frequencies for each of the morphological, protein, and allozyme marker traits. Those showing significant ($P < 0.05$) differences between the group mean values for major agronomic traits are presented in Table 9. For 100-seed weight, recombinant lines with white flower colour, *Diap1*^{1}, and S phaseolin had smaller sized seeds; and those with lilac flower, *Diap1*^{2}, and T phaseolin had higher seed weight. For biomass, lines with indeterminate growth habit, lanceolate leaf, white flower colour, *Acp*^{96}, and T phaseolin were found to yield more dry matter than their counterparts. Similarly, recombinant lines with large bracteole, *Skdh*^{100}, *Me*^{100}, and S phaseolin had a significantly larger number of pods/m^{2} than their counterparts. Mean values for fifth internode length were higher for groups of recombinant inbreds possessing determinate growth habit, cordate bracteole, *Rbsc*^{100}, *Acp*^{100}, and T phaseolin than for their counterparts. Lines with indeterminate growth habit,

Table 9. Association among agronomic and marker morphological traits and seed protein and allozyme patterns in recombinant inbred lines of common bean.

Marker trait	Agronomic trait	Population
	Seed yield (kg/ha)	
Growth habit (determinate)	1642**	Redkloud x MAM 4
Growth habit (indeterminate)	1835**	
	100-seed weight (g)	
White flower	26.6*	ICA L23 x Brasil 2
Lilac flower	34.1*	
*Diap*1[1]	26.0*	ICA L23 x Brasil 2
*Diap*1[2]	28.7*	
S phaseolin	24.4**	ICA L23 x Brasil 2
T phaseolin	30.1**	
S phaseolin	24.0*	Rio Tibagi x ABA 58
T phaseolin	27.0*	
S phaseolin	32.0**	Redkloud x MAM 4
T phaseolin	36.9**	
	Biomass (kg/ha)	
Growth habit (determinate)	2853**	Redkloud x MAM 4
Growth habit (indeterminate)	3384**	
Growth habit (determinate)	3368**	Rio Tibagi x ABA 58
Growth habit (indeterminate)	3691**	
Rhombohedric leaf	2512*	ICA L23 x Brasil 2
Lanceolate leaf	2814*	
Lilac flower	3400**	Rio Tibagi x ABA 58
White flower	3989**	

Acp^{98}	2593*	ICA L23 x Brasil 2
Acp^{96}	2866*	
Acp^{100}	2616*	
S phaseolin	3445*	Rio Tibagi x ABA 58
T phaseolin	3792*	
	Harvest index	
Growth habit (determinate)	0.56**	Redkloud x MAM 4
Growth habit (indeterminate)	0.51**	
Growth habit (determinate)	0.53*	Rio Tibagi x ABA 58
Growth habit (indeterminate)	0.50*	
Mdh^{98}	0.59*	Canadian Wonder x A 486
Mdh^{100}	0.56*	
Rbsc^{98}	0.55*	Redkloud x MAM 4
Rbsc^{100}	0.52*	
S phaseolin	0.52*	Rio Tibagi x ABA 58
T phaseolin	0.49*	
S phaseolin	0.55*	Redkloud x MAM 4
T phaseolin	0.51*	
	Pods/m^2	
Medium bracteole	198.0*	Redkloud x MAM 4
Large bracteole	211.1*	
Skdh^{98}	184.5**	ICA L23 x Brasil 2
Skdh^{100}	216.4**	
Me^{98}	189.1*	ICA L23 x Brasil 2
Me^{100}	209.9*	
S phaseolin	210.4**	ICA L23 x Brasil 2
T phaseolin	180.8**	

	Days to maturity	
Growth habit (determinate)	73**	Redkloud x MAM 4
Growth habit (indeterminate)	77**	
Growth habit (determinate)	80**	Rio Tibagi x ABA 58
Growth habit (indeterminate)	84**	
White flower	72.4*	Canadian Wonder x A 486
Lilac flower	73.7*	
White flower	75.1*	Redkloud x MAM 4
Purple flower	74.2*	
*Diap*1[1]	81.3*	ICA L23 x Brasil 2
*Diap*1[2]	82.3*	
S phaseolin	80.7**	ICA L23 x Brasil 2
T phaseolin	82.3**	
	Fifth internode length (cm)	
Growth habit (determinate)	5.8**	Rio Tibagi x ABA 58
Growth habit (indeterminate)	4.2**	
Lanceolate bracteole	6.1*	Redkloud x MAM 4
Cordate bracteole	7.4*	
Rbsc[96]	3.4*	Rio Tibagi x ABA 58
Rbsc[98]	5.1*	
Rbsc[100]	5.3*	
Acp[98]	5.2*	Redkloud x MAM 4
Acp[100]	6.8*	
S phaseolin	4.7*	ICA L23 x Brasil 2
T phaseolin	6.5*	

'*,**' Significantly different at P = 0.05 and P = 0.01, respectively.

lilac flower in one population and white in the other, *Diap1*^{2}, and T phaseolin were relatively late maturing. Similarly, lines with determinate growth habit, *Mdh*^{98}, and S phaseolin had a relatively higher harvest index.

Indeterminate growth habit was positively associated with yield for Redkloud x MAM 4, but inversely associated with biomass for this population. Indeterminate growth habit was also associated with reduced harvest index, a longer maturing period, and shorter internode lengths.

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V. DISCUSSION

A. Analysis of variance

Location and year effects and their interactions on most agronomic traits were significant, demonstrating the occurrence of contrasting growing conditions at Palmira and Popayán over two years (Table 4). Higher yields were found in Popayán (see Appendix i), possibly reflecting better growing conditions for parents and recombinant inbred lines from the four populations. Large-seeded common beans from the Andes are usually better adapted to relatively cooler sites such as Popayán. Because all populations involved at least one large-seeded parent, this may have helped improve their performance at Popayán compared with Palmira. Significant differences for all agronomic traits between the two years could be due to differences in precipitation and other factors. More precipitation was received at both locations in 1991 than in 1990 (Appendix ii).

Population and treatment (recombinant inbred line entries) differences were found for all traits. This indicated genetic differences among populations and among recombinant lines within the populations for each of the traits studied.

B. Agronomic traits

The lack of recombinant inbred lines yielding significantly higher than the highest yielding parents in populations Rio Tibagi x ABA 58 and Redkloud x MAM 4 is worth discussing. Although high genetic variation was created for morphological markers, allozymes, and proteins, no useful genetic variation for seed yield was found in these interracial populations. One reason could be zero or negative combining abilities for yield of Rio Tibagi and Redkloud (Nienhuis and Singh, 1986 and 1988a). Canadian Wonder and A 486 both had positive combining ability for yield (Singh et al., 1992a; Nienhuis and Singh, 1988b), which may explain higher yields for 20 recombinant inbred lines than their highest parent in this population. Moreover, it is possible that a threshold for genetic diversity among parents exists in order to create recombinants with potentially increased yields. For example, in population ICA L23 x Brasil 2, one recombinant inbred (TY 5578-45) outyielded the highest yielding parent and had a large seed size. The two parents of this population differ principally for their seed size and evolutionary origins. In contrast, the parents of more divergent Rio Tibagi x ABA 58 and Redkloud x MAM 4 populations differed for evolutionary origin, growth habit, and seed size and did not show any recombinant inbred lines with increased

yield. This could be a consequence of excessive genetic distance between the parents and an accumulation of deleterious genes whose effects could not be seen until brought together, thus resulting in depressed yields for the recombinant inbred lines. Also, because yield is a quantitative trait controlled by several genes with relatively small effects, it is very likely that a random sample of 79 recombinant inbred lines was not large enough to contain genotypes with all favourable genes from either parent. Which of these alternative hypotheses are tenable needs to be validated.

The three interracial populations demonstrated mean values depicting small seed size. Except for line TY 5578-45, an inverse relationship between seed size and seed yield was detected for the recombinant inbred lines, as has been demonstrated (Nienhuis and Singh, 1986; White and González, 1990). It was found that in crosses of Andean x Middle American parents, those from the Middle American genepools usually possessed negative general combining abilities for seed size (Singh et al., 1992a).

The highest yielding population, Rio Tibagi x ABA 58, was found to produce the most dry matter, or biomass (although not significantly). However, this population obtained a

significantly lower harvest index than that of Canadian Wonder x A 486. A similar finding for ICA L23 x Brasil 2 suggests that increased biomass acts as a sink in the developmental processes of the plant, resulting in diminished plant efficiency. Thus, independent selection for increased biomass (due to its positive association with yield) should be avoided.

While Andean and Middle American germplasm may be differentiated according to seed size, it was also found that seed size possessed a positive correlation with internode length (Nienhuis and Singh, 1985) (Table 7). Therefore, internode length could serve both as an indirect selection criterion for seed size and a distinguishing trait for genepool identification.

Late maturity has been associated with higher yields (White and Singh, 1991). The relatively highest yielding recombinant lines from Rio Tibagi x ABA 58 matured significantly later than those from Canadian Wonder x A 486 and Redkloud x MAM 4, supporting White and Singh (1991). Redkloud x MAM 4 matured earlier than the other interracial populations. The mean seed weight for recombinant inbred lines of this population had an intermediate value, but significantly larger than that for ICA L23 x Brasil 2 and Rio

Tibagi x ABA 58. This agrees with White and Laing (1989), who reported the early maturity tendency for larger seeded varieties.

C. Heritability and percentage genetic gain

Heritability of a character is a measure of the relative importance of the ratio of additive to non-additive genetic variance among test materials. Heritability is important to breeders as characters with higher values can be improved more rapidly with less intensive evaluation than those characters with lower heritabilities. Therefore, when heritability is high, a breeder can rely on selection methods requiring fewer evaluations. In contrast, when heritability is low, more effort must be spent in progeny evaluations in contrasting environments over a period of time for reliable selection.

The heritability values for 100-seed weight were the highest among all traits (Table 6). This supports the results of Motto et al. (1978), Nienhuis and Singh (1988b), and Singh et al. (1991a). Thus, selection for larger or smaller seed size should be very effective among and within populations varying for the trait. Among the four populations, Rio Tibagi x ABA 58 had the highest heritability values for yield, 100-seed weight, biomass, harvest index, and fifth internode

length. This suggests a high level of additive genetic variance for each of these traits in this population. The moderate to high heritability in the genotypically fixed recombinant inbred lines suggests that non-additive genetic and environmental variances were low, thus allowing a breeder to reliably use these lines for selection for these traits.

Relatively low heritability for harvest index for intraracial population Canadian Wonder x A 486 and interracial population ICA L23 x Brazil 2 implies reduced additive genetic variation among the recombinant inbred lines and a relatively larger environmental impact on its expression. The inherent weakness of harvest index, coupled with low heritability, as is the case with these two populations, render this trait of minimal use to a breeder. However, higher heritabilities for harvest index for two interracial populations would allow more reliable selection among their recombinant inbreds.

Percentage genetic gain under 20% selection pressure appears in Table 6. The highest expected gains for yield are found for the recombinant inbred lines of Rio Tibagi x ABA 58. Percentage genetic gain for 100-seed weight for this and the other large-seeded x small-seeded population (ICA L23 x Brazil 2) was found to be extremely high.

The overall percentage genetic gains and narrow-sense heritabilities for the interracial populations are found to be higher than those for Canadian Wonder x A 486. This means the presence of greater additive genetic variance in the interracial population for those traits with higher heritabilities and genetic gains. Nonetheless, it is important to note that the heritability of yield for population ICA L23 x Brasil 2 and Redkloud x MAM 4 was low to moderate. The expected gain for yield in the latter population is markedly low (Table 6).

The fact that advanced generation recombinant inbreds are genetically fixed means that the traits will continue to maintain their heritabilities, thus allowing reliable evaluation and selection among lines in contrasting environments.

D. Correlation coefficients

The inverse relationship of seed weight to seed yield and biomass (Table 7) supports the seed size effect as reported by White and González (1990). Dry biomass yield was closely related to seed yield, suggesting closely related development patterns. Both of these traits were significantly related to pods/m^2. The significant negative relationship of yield,

biomass, and therefore pods/m^2 to seed weight supports the yield component compensation effect (Adams, 1967). Pods/m^2 and biomass are significantly and negatively correlated with harvest index, which implies that greater non-economic yield results from diminished reproductive efficiency. An increased number of days to maturity allows more dry matter accumulation, explaining the positive and negative relationships of yield and harvest index to days to maturity, respectively. This finding supports that of White and Singh (1991), who found that later maturing germplasm had higher yields than its earlier maturing counterparts.

The seed size effect argument is further supported by considering the effect of increased days to maturity on yield, fifth internode length, and seed size. As the number of days to maturity increases, so does yield. However, seed size is diminished. This further supports White and González (1990) and White and Singh (1991), who reported that a longer period to maturity favours small-seeded, higher yielding Middle American germplasm. This also implies that large-seeded determinate Andean materials are comparatively early in their maturity and therefore possess relatively lower yields. This further illustrates the dilemma faced by breeders attempting to improve early-maturing cultivars.

E. Morphological, seed protein, and allozyme marker traits

The use of morphological markers (bracteole size and shape, leaf shape, etc.), proteins, and allozymes as indirect selection criteria is largely unknown in common bean. Breeder preference towards intragenepool and intraracial populations has resulted in diminished genetic variation and, hence, marker availability. Increased genetic variation among recombinant inbred lines from intergenepool and interracial populations increases not only the number of potential markers available as indirect selection criteria but should also increase the number of favorable genes for seed yield and resistance to both biotic and abiotic stress factors.

Variation for leaf and bracteole shapes found among parents used in this study corresponded to the findings of Urrea and Singh (1991). Some variability in morphological traits and protein and isoenzyme patterns was found within some recombinant inbred lines, possibly because of ongoing segregation.

Population Canadian Wonder x A 486 did not display any skewed ratios for any of the morphological markers, because of the close similarity of the parents. However, although both

parents had lilac flower colour, some recombinant inbreds were observed to have white flower colour. This was due to the presence of different genes in both parents, which permitted the expression of recessive gene coding for white flower colour in some recombinant inbred lines. Interestingly, whereas Redkloud and MAM 4 both had white flower colour, lilac and purple flower colours were also detected for several lines (4.9% and 37.0%, respectively). This could have been because of the complementary action of genes controlling flower colour. Thus, the two parents possessed different genes that together produced a different flower colour not found in either parent.

The close similarity of Canadian Wonder and A 486 resulted in no differences for most traits being recorded between the parents or for the recombinant inbred lines. Similar results were found for isoenzyme polymorphism, except for the $\underline{Mdh}^{98}$ and $\underline{Mdh}^{100}$ allozymes. Similarly, the absence of polymorphism for proteins in this population was expected because of the common evolutionary origin of the parents, and represents minimal genetic variation.

Recombination amongst the proteins of the recombinant inbred lines in the interracial populations was readily visualised in SDS-PAGE systems. This was because the parents

used for each interracial population had different phaseolin types and, in certain cases, different lectins, as well as different smaller fractions of proteins. Recombination occurred amongst the total proteins as four distinct groups in order of descending relative molecular mass: the phaseolins, a group of proteins located between the phaseolins and lectins referred to as "Group 1" proteins, the lectins, and a group of proteins located below the lectins referred to as "Group 2" proteins. No effort was made to characterise the "Group 1" and "Group 2" proteins. They are possibly protein fragment artifacts from the dissociation stage used in preparing the samples for electrophoresis. Conversely, they could be associated with the prolamine or alkaline-soluble protein groups as described by Ma and Bliss (1978).

The lectin protein was found to participate actively in recombination between the genepools, and was tightly linked to the albumin set of proteins as both entities behaved as a single unit in recombination. Although common bean lectin has been extensively characterised (Brown et al., 1982b and 1982c; Osborn et al., 1983, 1984, and 1985), it was not characterised in this study.

As Table 8 shows, there were several instances of distorted ratios for morphological, seed protein, and allozyme

traits. Koenig and Gepts (1989b) state that the distorted frequencies for specific alleles suggest the action of some form of female/male-specific mechanism that affects gene exchange between parental germplasm. They argue that the lack of reciprocity in the exchange results from nuclear-cytoplasm interactions and point out that genetic background and environment are commonly known to influence the level of recombination frequency. However, reversing the maternal/paternal roles in the populations used in this study could possibly confirm whether or not the distorted segregation ratios are attributed to female/male exchange mechanisms. If not, then it could be that the distorted segregation ratios are caused by interactions between the embryo and endosperm of the maternal parent as argued by Shii et al. (1982).

Gene exchange between parents from the same genepool or race should proceed without metabolical, hormonal, or physiological hindrance because of evolutionary similarity. In contrast, greater degrees of biochemical adaptation and modification would be necessary to accomodate gene exchange and adaptation among distantly related parents. The greater genetic distances between two materials that have evolved in separate regions in response to different selection pressures would predicate greater degrees of biochemical/hormonal

adaptation in order to allow successful crossing between the two genepools. Specifically, such biochemical adaptation would (depending upon genetic distance of the parents) necessitate potentially large-scale modifications of both major and minor metabolic and anabolic pathways in the viable progeny. This is probably affected by changes in genome expression, resulting in distorted segregation ratios amongst the progeny.

F. Association between agronomic and marker traits

Pairwise t-tests of the associated means for each of the morphological, protein, and allozyme marker traits are presented in Table 9. Thirty-three significant associations were detected. Among them, associations were found between the T phaseolin protein and large seed size and S phaseolin and small seed size. This association has been widely documented (Brown et al., 1981a; Bliss and Brown, 1983; Gepts et al., 1986).

Only two associations (flower colour with maturity and polymorphism for MDH with harvest index) were established for Canadian Wonder x A 486, further illustrating the numerical lack of molecular markers in intragenepool or intraracial populations relative to intergenepool and interracial populations.

The statistically significant associations possibly infer a genetic linkage between the markers listed and the agronomic traits in the form of multiloci associations and/or pleiotropic effects of genes controlling these traits.

VI. SUMMARY

A greater range of allozymes and other markers was expressed in the interracial populations in contrast to the intraracial population. This primarily testifies to the presence of greater genetic diversity in the former.

Skewed frequencies for several morphological and molecular markers were found in interracial populations, but not in intraracial populations. In order to study the distorted frequency/segregation ratios, the proteins (including phaseolin) and isozymes should be analysed in reciprocal crosses.

While no markers except growth habit were found to be associated with yield, associations were detected with other traits such as seed weight, biomass yield, pods/m^2, days to maturity, harvest index, and fifth internode length (Table 9). The associations outlined in Table 9 can eventually be integrated, after mapping, into the existing genetic linkage map for *Phaseolus vulgaris* L. Future studies should embark on determining the usefulness of the genetic variation generated in these populations with respect to resistance to diseases, drought, and other factors. Moreover, potential usefulness of molecular markers with more resolving power such as RAPD and

RFLP for indirect selection criteria for seed yield needs to be explored.

The mean yield of an intraracial recombinant inbred population was lower than that of two interracial recombinant inbred populations. The highest yielding lines from the latter significantly outyielded their counterparts from the former. However, none of the recombinant inbreds outyielded the highest yielding parent of two out of the three interracial populations. The recombinant inbreds outyielded the highest yielding parent in the intraracial and low-yielding interracial population.

The highest heritability values were recorded for 100-seed weight followed by fifth internode length and days to maturity. Values were low to moderately high for other traits including seed yield. The highest values for gains from selection were found for fifth internode length followed by 100-seed weight. Values for seed yield ranged from 3.9% to 11.4%. Days to maturity and harvest index tended to have the lowest values. In general, for most traits, gains tended to be comparatively larger in interracial populations compared with the intraracial one.

Seed yield was positively associated with biomass, pods/m^2, and days to maturity. However, pods/m^2 and days to maturity, although positively associated with each other, both had negative correlations with 100-seed weight and harvest index.

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VIII. APPENDIX

Appendix i. Yield performances (kg/ha) for F_2-derived F_6 recombinant inbred lines and parents evaluated over two years at two locations in Colombia.

Part A. Population Canadian Wonder x A 486

Entry number	Line code	Palmira			Popayan			Overall line mean
		1990	1991	Mean	1990	1991	Mean	
1	TR 7562-012	1158	1935	1547	1665	1776	1720	1633
2	TR 7562-022	498	2553	1526	1897	1895	1896	1711
3	TR 7562-027	907	1893	1400	1947	1698	1822	1611
4	TR 7562-031	693	1905	1299	2322	1268	1795	1547
5	TR 7562-033	1062	2369	1716	1936	1290	1613	1664
6	TR 7562-038	1030	2140	1585	2197	1892	2045	1815
7	TR 7562-039	1146	1716	1431	2084	1437	1761	1596
8	TR 7562-041	1355	1772	1564	2259	1229	1744	1654
9	TR 7562-042	926	1971	1449	1704	1419	1562	1505
10	TR 7562-043	990	1513	1252	1339	1377	1358	1305
11	TR 7562-046	761	1682	1222	1877	1840	1858	1540
12	TR 7562-055	474	1624	1049	2166	1976	2071	1560
13	TR 7562-056	1023	1429	1226	1184	657	920	1073
14	TR 7562-058	792	1582	1187	1148	1738	1443	1315
15	TR 7562-070	768	1835	1302	2444	1808	2126	1714
16	TR 7562-071	1171	2019	1595	2124	1877	2001	1798
17	TR 7562-079	612	2292	1452	1466	1520	1493	1473
18	TR 7562-082	609	1733	1171	1051	961	1006	1088
19	TR 7562-094	774	2036	1405	1620	1134	1377	1391
20	TR 7562-106	943	2120	1532	1587	1548	1568	1549
21	TR 7562-109	894	1949	1422	1385	1269	1327	1374
22	TR 7562-111	1531	1962	1747	607	1088	848	1297
23	TR 7562-112	1068	2185	1627	2259	2574	2417	2022
24	TR 7562-113	902	2172	1537	1584	1711	1648	1592
25	TR 7562-122	669	1650	1160	1834	1335	1585	1372
26	TR 7562-127	1138	1507	1323	1343	1503	1423	1373

27	TR 7562-132	842	1920	1381	3039	1960	2500	1940
28	TR 7562-135	836	1440	1138	1291	1438	1365	1251
29	TR 7562-146	774	1647	1211	2392	1256	1824	1517
30	TR 7562-156	1189	2099	1644	2313	1628	1971	1807
31	TR 7562-164	848	1734	1291	1168	1413	1291	1291
32	TR 7562-166	814	2196	1505	1758	2037	1897	1701
33	TR 7562-169	802	1929	1366	1368	1703	1536	1451
34	TR 7562-173	764	2041	1403	692	1341	1016	1209
35	TR 7562-185	1041	2090	1566	2254	1984	2119	1843
36	TR 7562-186	825	2349	1587	2829	2287	2558	2073
37	TR 7562-187	993	2341	1667	975	1335	1155	1411
38	TR 7562-188	1215	1878	1547	1393	1673	1533	1540
39	TR 7562-190	721	1706	1214	2628	1919	2274	1744
40	TR 7562-193	1096	1452	1274	2371	1272	1822	1547
41	TR 7562-197	827	2119	1473	1699	2368	2033	1753
42	TR 7562-227	828	2036	1432	1643	1818	1731	1581
43	TR 7562-232	533	1741	1137	1399	1694	1547	1342
44	TR 7562-233	719	1548	1134	1263	864	1064	1098
45	TR 7562-234	562	1808	1185	2518	1428	1973	1579
46	TR 7562-237	866	1605	1236	1522	1106	1314	1275
47	TR 7562-239	1024	2014	1519	1781	1989	1885	1702
48	TR 7562-240	929	2146	1538	1503	2044	1773	1655
49	TR 7562-242	675	1416	1046	1797	1304	1550	1298
50	TR 7562-243	895	1641	1268	1861	996	1428	1348
51	TR 7562-257	1142	1836	1489	1435	1408	1422	1455
52	TR 7562-258	747	1732	1240	1302	1613	1458	1348
53	TR 7562-266	907	1882	1395	2503	1909	2206	1800
54	TR 7562-268	1675	2243	1959	758	1452	1105	1532
55	TR 7562-271	1192	2178	1685	1351	1497	1424	1554
56	TR 7562-276	1176	2298	1737	2111	1635	1873	1805
57	TR 7562-277	865	1887	1376	2246	1632	1939	1657
58	TR 7562-278	859	2016	1438	1324	1185	1255	1346
59	TR 7562-287	626	2064	1345	866	1788	1327	1336
60	TR 7562-293	735	2127	1431	2420	2402	2411	1921
61	TR 7562-318	972	1785	1379	2611	1340	1076	1677

62	TR 7562-319	847	2203	1525	1629	1388	1508	1517
63	TR 7562-325	641	1900	1271	2917	1435	2176	1723
64	TR 7562-328	921	1869	1395	2088	2126	2107	1751
65	TR 7562-335	760	1866	1313	2204	1536	1870	1592
66	TR 7562-337	902	2224	1563	2265	2247	2256	1910
67	TR 7562-350	1231	2015	1623	1671	1795	1733	1678
68	TR 7562-353	1136	2238	1687	2776	1755	2266	1976
69	TR 7562-354	953	1994	1474	1522	1725	1624	1549
70	TR 7562-367	978	1959	1469	2194	2110	2152	1810
71	TR 7562-369	1699	1972	1836	1804	2270	2037	1936
72	TR 7562-372	378	2072	1225	1418	1828	1623	1424
73	TR 7562-373	1467	1539	1503	1607	1567	1587	1545
74	TR 7562-376	1276	1353	1315	1973	2353	2163	1739
75	TR 7562-378	1591	1902	1747	1829	1892	1861	1804
76	TR 7562-379	1151	2488	1820	1917	1518	1718	1769
77	TR 7562-382	871	1241	1056	2130	1536	1833	1444
78	TR 7562-384	717	1657	1187	1390	1604	1497	1342
79	TR 7562-387	1206	2092	1649	1412	1183	1298	1473
80	Canadian Wonder	1136	1354	1245	2392	2067	2230	1737
81	A486	939	1692	1316	1553	1524	1538	1427

Part B. Population Redkloud x MAM 4

Entry number	Line code	Palmira			Popayan			Overall line mean
		1990	1991	Mean	1990	1991	Mean	
82	TR 7618-001	972	722	847	2743	1409	2076	1461
83	TR 7618-008	1865	1096	1481	1641	1792	1716	1598
84	TR 7618-013	1261	935	1098	2747	1228	1987	1543
85	TR 7618-015	995	1427	1211	2799	1644	2221	1716
86	TR 7618-016	1679	1339	1509	2041	985	1513	1511
87	TR 7618-018	1696	795	1246	2764	1298	2031	1638
88	TR 7618-021	1399	1483	1441	3352	990	2171	1806
89	TR 7618-036	914	967	941	4154	1559	2857	1899
90	TR 7618-041	1710	1226	1468	1863	1242	1552	1510
91	TR 7618-042	2221	1137	1679	4313	1762	3038	2358
92	TR 7618-047	1475	1138	1307	3044	1475	2260	1783
93	TR 7618-049	819	765	792	2381	1326	1853	1323
94	TR 7618-050	1397	1691	1544	2773	1010	1892	1718
95	TR 7618-063	1327	375	851	2773	1684	2229	1540
96	TR 7618-080	1529	1695	1612	3538	1948	2743	2178
97	TR 7618-081	1393	991	1192	2862	840	1851	1522
98	TR 7618-088	1774	1104	1439	3239	1216	2227	1833
99	TR 7618-093	1504	1081	1293	1726	1416	1571	1432
100	TR 7618-097	1593	722	1158	3456	2051	2753	1955
101	TR 7618-111	1412	1138	1275	3302	1665	2484	1879
102	TR 7618-112	1816	1383	1600	2177	1461	1819	1709
103	TR 7618-115	1583	631	1107	3349	1824	2587	1847
104	TR 7618-116	1222	409	816	3499	1032	2266	1541
105	TR 7618-119	1328	1197	1263	2407	1093	1750	1506
106	TR 7618-128	1505	1161	1333	2536	1682	2109	1721
107	TR 7618-131	1800	950	1375	4532	1107	2819	2097
108	TR 7618-138	1581	630	1106	3282	2255	2768	1937
109	TR 7618-150	861	577	719	2104	1568	1836	1277
110	TR 7618-155	2362	925	1644	4294	1519	2907	2275
111	TR 7618-157	1859	1447	1653	2409	1394	1901	1777

112	TR 7618-159	1464	1323	1394	4289	1556	2923	2158
113	TR 7618-161	1497	1386	1442	1436	1288	1362	1402
114	TR 7618-164	1366	1307	1337	2252	1432	1842	1589
115	TR 7618-166	2103	934	1519	2484	2433	2459	1989
116	TR 7618-167	1555	957	1256	3304	1437	2370	1813
117	TR 7618-170	1457	904	1181	725	1966	1346	1263
118	TR 7618-172	1811	1140	1476	3446	1620	2533	2004
119	TR 7618-178	1166	1049	1108	2816	880	1848	1478
120	TR 7618-180	1617	1293	1455	2896	1357	2126	1791
121	TR 7618-182	761	1192	977	2700	1502	2101	1539
122	TR 7618-183	1767	869	1318	2593	1717	2155	1736
123	TR 7618-202	1224	1060	1142	2498	1666	2082	1612
124	TR 7618-205	1702	743	1223	3187	1653	2420	1821
125	TR 7618-207	1863	827	1345	3182	1406	2294	1820
126	TR 7618-209	1374	923	1149	3452	2018	2735	1942
127	TR 7618-210	2393	1688	2041	3976	2092	3034	2537
128	TR 7618-216	1293	1463	1378	3911	1687	2799	2088
129	TR 7618-230	1511	683	1097	3520	1372	2446	1771
130	TR 7618-235	1972	1468	1720	2929	1448	2188	1954
131	TR 7618-240	1412	1297	1355	2389	2288	2339	1847
132	TR 7618-254	724	1171	948	3024	1450	2237	1592
133	TR 7618-256	2169	821	1495	2305	1497	1901	1698
134	TR 7618-263	1772	1149	1461	1389	1593	1491	1476
135	TR 7618-268	1813	867	1340	3178	1885	2532	1936
136	TR 7618-275	978	611	795	4241	1052	2647	1721
137	TR 7618-281	1484	723	1104	1669	1432	1551	1327
138	TR 7618-282	1501	959	1230	3629	904	2266	1748
139	TR 7618-287	2179	1339	1759	1780	1478	1629	1694
140	TR 7618-288	1182	820	1001	3200	1867	2534	1767
141	TR 7618-291	1076	886	981	2739	1767	2253	1617
142	TR 7618-302	1680	883	1282	3062	1271	2167	1724
143	TR 7618-314	1548	1389	1469	2945	1011	1978	1723
144	TR 7618-316	1154	1006	1080	4151	2135	3143	2112
145	TR 7618-319	1672	855	1264	2374	1312	1843	1553
146	TR 7618-326	1474	834	1154	2766	1169	1957	1561

147	TR 7618-329	1572	1104	1338	3229	1736	2483	1910
148	TR 7618-344	1003	839	921	3973	1572	2763	1847
149	TR 7618-347	1438	1143	1291	3733	1558	2645	1968
150	TR 7618-351	2192	817	1505	2954	1277	2115	1810
151	TR 7618-352	1342	689	1016	2857	1190	2023	1520
152	TR 7618-356	1117	737	927	3587	1694	2640	1784
153	TR 7618-361	1539	1568	1554	2031	1360	1696	1625
154	TR 7618-363	1792	1378	1585	2427	1309	1868	1726
155	TR 7618-366	1852	1078	1465	1994	1324	1659	1562
156	TR 7618-367	1403	583	993	2482	1130	1806	1399
157	TR 7618-368	1377	924	1151	2479	1683	2081	1616
158	TR 7618-005	1740	309	1025	2207	1014	1610	1317
159	TR 7618-019	1506	1097	1302	2073	1265	1669	1485
160	TR 7618-147	1164	954	1059	3194	1072	2133	1596
161	Redkloud	1879	922	1401	2542	1775	1570	1780
162	MAM 4	2162	1241	1702	5073	2265	3669	2685

Part C. Population Rio Tibagi x ABA 58

Entry number	Line code	Palmira			Popayan			Overall line mean
		1990	1991	Mean	1990	1991	Mean	
163	WA 7807-002	2521	1011	1766	3371	1762	2566	2166
164	WA 7807-004	1641	1089	1365	3267	1484	2375	1870
165	WA 7807-005	1734	1324	1529	3287	1424	2355	1942
166	WA 7807-010	1268	1444	1356	3276	1267	2272	1814
167	WA 7807-014	2117	1058	1588	3647	1311	2479	2033
168	WA 7807-020	1878	1124	1501	4765	1995	3380	2441
169	WA 7807-024	1794	807	1301	2874	1912	2393	1847
170	WA 7807-025	1279	999	1139	3528	1380	2454	1796
171	WA 7807-028	2195	1178	1687	3010	1116	2063	1875
172	WA 7807-033	1665	965	1315	4791	859	2825	2070
173	WA 7807-036	2049	1363	1706	4142	1477	2810	2258
174	WA 7807-038	881	993	937	3199	1253	2226	1582
175	WA 7807-039	1484	412	948	3668	1471	2569	1759
176	WA 7807-040	781	627	704	2634	1707	2171	1437
177	WA 7807-049	1384	923	1154	2688	1048	1868	1511
178	WA 7807-054	1389	1501	1445	4079	1191	2635	2040
179	WA 7807-055	979	1197	1088	3144	1007	2076	1582
180	WA 7807-058	1689	535	1112	1830	1371	1601	1356
181	WA 7807-060	1889	1183	1536	5279	1022	3151	2343
182	WA 7807-061	1724	1075	1400	3311	2170	2740	2070
183	WA 7807-073	1296	1068	1182	3529	1212	2371	1776
184	WA 7807-078	2365	2390	2382	3373	1800	2586	2482
185	WA 7807-079	2721	1544	2133	2961	1116	2039	2086
186	WA 7807-084	2193	1323	1758	4318	1555	2937	2347
187	WA 7807-091	1782	929	1356	3165	935	2050	1703
188	WA 7807-092	2754	1287	2021	2041	1539	1790	1905
189	WA 7807-094	2173	791	1482	3232	1350	2290	1886
190	WA 7807-095	1930	864	1397	2634	1385	2010	1703
191	WA 7807-098	1208	1025	1117	3292	1136	2214	1665
192	WA 7807-099	1479	632	1056	4454	2249	3351	2203
193	WA 7807-101	2382	1090	1736	2893	1749	2321	2028

194	WA 7807-103	1397	1071	1234	4530	1440	2985	2110
195	WA 7807-105	1243	1548	1396	2827	1292	2060	1728
196	WA 7807-109	1604	1403	1504	3770	1101	2436	1969
197	WA 7807-121	1233	976	1105	2824	1639	2231	1668
198	WA 7807-122	1866	1339	1603	3189	1423	2306	1954
199	WA 7807-129	2519	1024	1772	3443	1122	2283	2027
200	WA 7807-131	1617	803	1210	2623	1306	1965	1587
201	WA 7807-132	2085	1076	1581	2992	1815	2404	1992
202	WA 7807-133	1621	1345	1483	2957	1391	2174	1828
203	WA 7807-136	1794	1343	1569	2563	1662	2112	1840
204	WA 7807-142	869	941	905	2367	969	1668	1287
205	WA 7807-148	1863	1257	1560	2635	1438	2037	1798
206	WA 7807-150	1741	1118	1430	3478	1231	2355	1892
207	WA 7807-151	1829	1269	1549	3536	1198	2367	1958
208	WA 7807-157	895	1137	1016	2681	1398	2039	1528
209	WA 7807-158	1178	1218	1198	2664	1250	1957	1578
210	WA 7807-163	1793	792	1293	3607	1415	2511	1902
211	WA 7807-165	1282	829	1056	1991	1106	1549	1302
212	WA 7807-166	2672	1527	2100	4404	1650	3027	2563
213	WA 7807-170	1866	1753	1810	4392	1663	3027	2419
214	WA 7807-172	1782	1377	1580	2722	1036	1879	1729
215	WA 7807-175	1033	1108	1071	1811	1240	1525	1298
216	WA 7807-178	1745	903	1324	3889	778	2333	1829
217	WA 7807-182	1148	944	1046	2991	1344	2168	1607
218	WA 7807-187	1236	944	1090	4058	1196	2627	1859
219	WA 7807-204	1553	754	1154	2941	1446	2194	1674
220	WA 7807-206	1549	1181	1365	3787	828	2308	1836
221	WA 7807-207	1989	899	1444	2863	1211	2037	1741
222	WA 7807-208	1506	1440	1473	2897	1617	2257	1865
223	WA 7807-213	1820	896	1358	2897	1536	2217	1788
224	WA 7807-223	1721	986	1354	2618	1497	2058	1705
225	WA 7807-225	1920	1035	1478	3874	1421	2648	2062
226	WA 7807-239	1677	1314	1496	2194	706	1450	1473
227	WA 7807-243	792	1103	948	2178	1072	1625	1286
228	WA 7807-248	1941	1477	1709	3795	1440	2617	2163

229	WA 7807-251	1232	921	1077	2073	850	1462	1269
230	WA 7807-257	2192	1421	1807	4079	1570	2825	2316
231	WA 7807-258	1002	973	988	3445	1060	2252	1620
232	WA 7807-262	1941	1381	1661	4249	1200	2725	2193
233	WA 7807-267	2602	1106	1854	4557	1418	2988	2421
234	WA 7807-269	2247	694	1471	2164	1246	1705	1588
235	WA 7807-271	2010	1686	1848	5362	1611	3486	2667
236	WA 7807-279	2255	1469	1862	3406	1574	2490	2176
237	WA 7807-294	2004	524	1264	3712	853	2282	1773
238	WA 7807-297	1506	1445	1476	3965	1317	2641	2058
239	WA 7807-298	1637	830	1234	4269	1416	2842	2038
240	WA 7807-303	1268	1294	1281	3838	1176	2507	1894
241	WA 7807-305	1512	1406	1459	3558	1624	2591	2025
242	Rio Tibagi	2598	1521	2060	3816	1212	2514	2287
243	ABA 58	2905	1496	2201	3874	2719	3297	2749

Part D. Population ICA L23 x Brasil 2

Number entry	Line code	Palmira			Popayan			Overal l line mean
		1990	1991	Mean	1990	1991	Mean	
244	TY 5578-11	1461	1097	1279	1787	1064	1425	1352
245	TY 5578-15	1850	1561	1706	2571	718	1644	1675
246	TY 5578-23	1373	1444	1409	2311	901	1606	1507
247	TY 5578-28	2495	1172	1834	1484	933	1208	1521
248	TY 5578-35	1701	1037	1369	3298	863	2081	1725
249	TY 5578-45	2357	1434	1896	4236	1230	2733	2314
250	TY 5578-47	1416	1606	1511	3659	1303	2481	1996
251	TY 5578-50	1068	982	1025	2283	708	1496	1260
252	TY 5578-51	1553	862	1208	1636	892	1264	1236
253	TY 5578-56	1682	1077	1380	2324	949	1637	1508
254	TY 5578-61	1762	586	1174	1546	545	1045	1110
255	TY 5578-66	1738	1362	1550	1078	840	959	1255
256	TY 5578-69	2198	1363	1781	3096	1499	2298	2039
257	TY 5578-75	1829	1313	1571	2258	514	1386	1479
258	TY 5578-79	1526	1173	1350	1032	725	878	1114
259	TY 5578-81	1948	1663	1806	1278	478	878	1341
260	TY 5578-82	2147	972	1560	1654	1017	1335	1448
261	TY 5578-85	1441	1456	1449	3158	1012	2085	1767
262	TY 5578-88	1717	1009	1363	778	742	760	1061
263	TY 5578-112	2242	1031	1637	2487	892	1689	1663
264	TY 5578-114	1106	1067	1087	1826	642	1243	1160
265	TY 5578-117	1817	1158	1488	1354	846	1100	1294
266	TY 5578-118	1434	1504	1469	3904	1423	2664	2066
267	TY 5578-120	1633	1126	1380	481	574	527	953
268	TY 5578-129	1312	1424	1368	2413	833	1623	1496
269	TY 5578-135	870	1070	970	1292	432	862	916
270	TY 5578-137	1418	624	1021	3558	850	2204	1612
271	TY 5578-138	1388	1017	1203	2863	850	1856	1529
272	TY 5578-141	1679	919	1299	3877	935	2406	1853
273	TY 5578-153	1919	968	1444	1962	1065	1514	1479
274	TY 5578-155	2289	1124	1707	2802	895	1849	1778

275	TY 5578-156	1557	1575	1566	2304	932	1618	1592
276	TY 5578-161	1394	854	1124	2499	975	1737	1431
277	TY 5578-162	1651	628	1140	2259	706	1482	1311
278	TY 5578-165	1474	1244	1359	987	1104	1046	1202
279	TY 5578-170	1491	1563	1527	1565	916	1240	1384
280	TY 5578-182	1476	879	1178	2854	607	1730	1454
281	TY 5578-183	1385	733	1059	1712	910	1311	1185
282	TY 5578-186	1225	1431	1328	3810	1216	2513	1921
283	TY 5578-187	1598	775	1187	1894	658	1276	1231
284	TY 5578-189	1352	1071	1212	2269	961	1615	1414
285	TY 5578-190	1988	1009	1499	3544	1174	2359	1929
286	TY 5578-191	2101	1375	1738	1325	1050	1188	1463
287	TY 5578-192	1122	921	1022	2485	1382	1933	1478
288	TY 5578-193	1279	824	1052	2593	804	1699	1375
289	TY 5578-201	1159	1084	1122	1572	1102	1337	1229
290	TY 5578-202	1151	1115	1133	3491	1140	2315	1724
291	TY 5578-206	1433	748	1091	1877	892	1385	1237
292	TY 5578-209	1948	1547	1748	2843	1230	2037	1892
293	TY 5578-211	1168	1002	1085	1370	1344	1357	1221
294	TY 5578-216	2317	922	1620	1649	995	1322	1471
295	TY 5578-218	1577	762	1170	2541	1033	1787	1478
296	TY 5578-219	1278	1063	1171	1980	594	1287	1229
297	TY 5578-220	1599	1083	1341	2237	588	1413	1377
298	TY 5578-221	1175	949	1062	876	854	865	963
299	TY 5578-226	1448	833	1141	1111	617	864	1002
300	TY 5578-228	1582	1127	1355	3001	763	1882	1618
301	TY 5578-230	2522	1192	1857	2701	696	1698	1778
302	TY 5578-238	1374	1261	1318	2462	986	1724	1521
303	TY 5578-258	1706	801	1254	1198	827	1013	1133
304	TY 5578-260	1403	980	1192	2011	959	1485	1338
305	TY 5578-262	1719	750	1235	685	975	830	1032
306	TY 5578-263	1028	1098	1063	1035	875	955	1009
307	TY 5578-276	1377	963	1170	1222	835	1029	1099
308	TY 5578-283	1797	1443	1620	2191	1649	1920	1770
309	TY 5578-284	1599	1139	1369	3178	1053	2116	1742

310	TY 5578-289	1512	952	1232	3474	859	2166	1699
311	TY 5578-292	1372	1650	1511	1973	969	1471	1491
312	TY 5578-293	1415	1996	1706	1143	989	1066	1386
313	TY 5578-297	1809	1439	1624	1251	748	1000	1312
314	TY 5578-299	1628	1402	1515	2114	1028	1571	1543
315	TY 5578-302	1273	795	1034	1296	909	1103	1068
316	TY 5578-304	1466	1676	1571	982	628	805	1188
317	TY 5578-305	1171	1127	1149	2002	851	1426	1288
318	TY 5578-306	1615	633	1124	1739	374	1057	1090
319	TY 5578-310	1791	872	1332	1378	1023	1201	1266
320	TY 5578-311	2271	695	1483	1630	824	1227	1355
321	TY 5578-315	1631	694	1163	1391	1011	1201	1182
322	TY 5578-361	2009	1186	1598	2759	1342	2051	1824
323	ICA L23	1319	1661	1490	3326	1027	2176	1833
324	Brasil 2	2343	741	1542	2440	966	1703	1623

Appendix ii. Total precipitation received in Palmira and Popayán in 1990 and 1991.

	Precipitation (mm)	Total annual precipitation (mm)
Palmira 1990	0.9	70.1
Palmira 1991	3.1	310.1
Popayán 1990	1.9	197.5
Popayán 1991	11.7	1135.0