Protein markers and seed size variation in common bean segregating populations

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Abstract

Selection and random genetic drift are the two main forces affecting allele frequencies in common bean breeding programs. Therefore, knowledge on allele frequency changes attributable to these forces is of fundamental importance for breeders. The changes in frequencies of alleles of biochemical markers were examined in F₂ to F₇ populations derived from crosses between cultivated Mesoamerican and Andean common bean accessions (*Phaseolus vulgaris* L.). Biochemical markers included the seed proteins phaseolin, lectin and other seed polypeptides, and six isozymes. The Schaffer's test detected a high significant linear trend of the 63% of the polymorphic loci studied, meaning that directional selection was acting on those loci. Associations between seed size traits, phaseolin seed-storage protein and isozyme markers were detected based on the comparisons of the progeny genotypic means. In the interracial populations the intermediate form PhaH/T, b6 and *Rbcs*⁹⁸ alleles had a positive effect on seed size. In the inter-gene pool populations, a higher transmission of Mesoamerican alleles in all loci was showed, although the Andean alleles Pha^T, *Skdh*¹⁰⁰, *Rbcs*⁹⁸ and *Diap*¹⁰⁰ showed positive effects on seed weight. Our results suggest that phaseolin and other seed proteins markers are linked to loci affecting seed size. These markers have good potential for improving the results of the selection and should be considered as a strategy for germplasm enhancement and to avoid the reduced performance of the inter-gene pool populations.

Keywords: *Phaseolus vulgaris* L., phaseolin, isozymes, seed size, selection, associations.

1 **Introduction**

2
3 Quantitative traits are often said to be controlled by many genes, each one with an individual
4 effect too small to be measured conveniently under usual experimental conditions (Miles and
5 Wayne, 2008). Studies designed to investigate Quantitative Trait Loci (QTL) have proceeded in
6 several ways to overcome this problem. Backcross or F₂ populations have been constructed to
7 maximize linkage disequilibrium between markers and QTL (Nodari et al. 1993; Mutlu et al.
8 2005). Alternatively, tracking marker frequencies in segregant populations may be used to
9 identify QTL (Edwards et al., 1987). This option has not been utilized as extensively as
10 backcross and F₂ methods, perhaps because of the lack of seed from cycles of populations under
11 selection, or lack of experimental populations selected for the same trait over many generations.
12 It is likely that genes affecting quantitative traits have a range of effects from large to small,
13 depending on the background in which they are expressed. Theoretically, alleles having positive
14 effects on the trait of interest should increase in frequency with selection and those having
15 negative effects should decrease. The frequency of alleles with large effects should also increase
16 or decrease faster than the frequency of alleles with relatively small effects. However, without
17 easily identifiable alleles, it is difficult to document changes in frequency.

18 Seed size in cultivated beans has been described as a polygenic trait (Sax 1923) and, from
19 crosses between cultivated and wild types, it has been estimated that there are at least 10 genes
20 or blocks of genes involved (Motto et al. 1978). Quantitative inheritance patterns of seed traits
21 such as seed length, width, and height have been reported by Vallejos and Chase (1991a). Nodari
22 (1993) found seven seed weight QTL on linkage groups (LGs) D1, D3, D4 and D7
23 corresponding to the linkage groups B1, B3, B4 and B7 by Freyre et al. (1998). Of these QTL,
24 those on B1 and B7 correspond to QTL for seed weight also found by Johnson et al. (1996). Park
25 et al. (2000) found five QTL for seed weight (B3, B4, B6, B7 and B8), four QTL for length
26 (three on B8 and one on B2) and three QTL (B4, B6, B11) for height. Guzman-Maldonado et al.
27 (2003) reported 5 QTL for seed weight in linkage groups B1, B2, B3, and B4. A total of 11 QTL
28 were identified across eight linkage groups by Blair et al. (2006) in an advanced backcross
29 population derived from a cultivated Andean x wild common bean cross. In short, 13 QTL have
30 been confirmed for seed size on the LGs B1, B2, B3, B4, B5, B6, B7, B8, B9 and B11.

31 Phaseolin and enzymatic markers offer a number of advantages for the analysis of genetic
32 variation which include: (a) lack of detectable effects on the morphology and physiology of the
33 plant, (b) codominant expression, permitting exact identification of genotypes, and (c) lack of
34 epistasis, allowing classification of any number of such markers segregating simultaneously.

1 Phaseolin and the members of the lectin family (phytohemagglutinin, arcelin and α -amylase
2 inhibitor) belong to vicilins that represent the major seed storage proteins in common bean. A
3 large fraction of these proteins is constituted by phaseolin (Pha) that accounts for 35 - 46% of
4 total seed proteins in common bean (Mennella et al., 2003). The electrophoretic variability of
5 phaseolin has contributed to the evidence for two gene pools in common bean (Gepts et al. 1986;
6 Singh et al. 1991a; Gepts 1998). The cultivars with "S" and "T" phaseolin patterns predominated
7 in Mesoamerica and in the Southern Andes, respectively. The "B" phaseolin type was presented
8 only in wild and cultivated common beans from Colombia. On the other hand, "C", "H" and "A"
9 phaseolin types were only found among landraces of Andes. Phytohemagglutinin, the major bean
10 seed lectin, accounts for 5-10% of mature seed total protein amount in common bean (Pusztai et
11 al. 1979). More than 10 electrophoretic variants of this protein were observed in wild genotypes
12 whereas only a limited heterogeneity was evidenced in domesticated material (Brown et al.
13 1982; Lioi 1991). Arcelin and α -amylase inhibitor, so far found only in Mesoamerican wild bean
14 accessions, showed seven and four different electrophoretic patterns respectively (Romero et al.
15 1986; Osborn et al. 1988).

16 Most studies have used isozymes to examine changes in allelic frequencies during selection.
17 Isozymes are useful tools to study the genetic structure of populations, making them good
18 subjects for detailed genetic study. Allelic frequencies at protein loci have been studied in
19 recurrent selection in common bean (Delaney and Bliss, 1991), and maize (Stuber et al. 1980;
20 Kahler 1983; Pollak et al. 1984; Butrón et al. 2005). These authors found significant associations
21 between protein frequencies and agronomic traits in selection programs. Alterations of protein
22 frequencies due to selection would cause a skew in the estimation of allele frequencies, as well
23 as a reduction of neutral polymorphism and expected heterozygosity (Kaplan et al. 1989;
24 Stephan et al. 1992). The number of selectively neutral polymorphic sites in a random sample of
25 genes can be reduced by selectively favored substitutions at linked loci. As other consequence,
26 genetic variation that is linked to any beneficial allele will survive the selective phase due to its
27 proximity to the selective target, and the reduction in diversity (measured e.g. by the number of
28 segregating sites or the average heterozygosity in a sample) would be less. These studies have
29 demonstrated presence of directional frequency changes in alleles associated with recurrent
30 selection for quantitative traits.

31 The assessment of the effects of genetic drift and selection is important for designing effective
32 and efficient selection breeding programs. Knowing which marker changes occur in populations
33 during selection might provide information on the genetic components underlying agronomic
34 responses to selection. Further, the use of markers might help to identify specific regions that

1 have been favored by selection. Labate et al. (1999) and De Koeyer et al. (2001) described
2 significant changes in the allele frequencies loci in a long-term recurrent selection programs
3 using RFLP (restriction fragment length polymorphism) markers. Johnson et al. (1996) found a
4 consistent association between the *Phs* locus (phaseolin locus) and seed weight and noted that
5 this association was conserved in other legume species. Weeden and Liang (1985) and Vallejos
6 and Chase (1991a) reported linkage between isozymes and seed components.

7 The present investigation on characterization of seed size traits and protein markers of several
8 populations developed under selection was undertaken with dual purpose. Firstly determine
9 changes in allele frequencies of protein alleles due to the effects of random genetic drift and
10 selection, and secondly to determine the proportion of the seed size variation that was explained
11 by each of the markers. Assessment of allele frequency changes in breeding programs can be
12 used to detect marker alleles linked to QTL regions under selection pressure.

28 **Material and methods**

30 Segregant populations

32 Thirty dry beans inbreeds displaying allelic differences at six enzyme loci, phaseolin and other
33 seed proteins, and difference in seed size were selected from the Misión Biológica de Galicia-

1 Spanish National Research Council (MBG-CSIC) breeding collection (Table 1) to produce F₁
2 progenies (Table 2).

3 Part of the seed of each F₁, together with their respective parents, was grown in the field to
4 ascertain hybridity origin and was harvested individually to create F₁-derived F₂ populations. F₃-
5 F₅ populations were planted as single rows and evaluated for seed quality traits (proportion of
6 seed coat, seed size and global quality seed) combined with seed yield. They were grown in
7 plant-to-progeny rows in the F₆ and F₇. F₂ to F₇ seed was stored at 4-5°C of temperature and 50%
8 of humidity. Seeds were carefully dissected in order to germinate and use in the seed storage
9 protein analysis. At the primary leaf stage, tissue samples for isozyme analysis were collected.

11 Morphological data

13 Four quantitative seed traits, which defined the seed commercial quality, were scored for each
14 individual: weight (g seed⁻¹), length (mm), defined as the longest distance across the seed
15 parallel to the hilum, height (mm), as the longest distance from the top to the bottom of the seed,
16 and width (mm), measured as the longest distance across the hilum seed.

17 Seed morphological characters studied reflect the F_{n-1} plant genotype since seed size and seed
18 coat color are maternal characteristics (Roach and Wulff 1987). Therefore, these traits were
19 determined from seeds of the following generation. For example, F₂ phenotypes were determined
20 from F_{2:3} seeds and F₇ phenotypes from F_{7:8} seeds.

22 Seed storage protein analysis

23 For storage protein analysis, a portion of the seed was manually removed and ground into a
24 fine dust. The flour sample was suspended at room temperature for at least 0.5 h in a mixture
25 consisting of equal volumes of a 0.5 M NaCl solution (adjusted to pH 2.4 with HCl) and
26 cracking buffer (0.625 M Tris-HCl pH 6.8, 2 mM EDTA; 2 % SDS, 40 % sucrose, 1 % 2-
27 mercaptoethanol and 0.01 % bromophenol blue marker dye). Five microliters of sample were
28 subjected to one-dimensional sodium dodecyl sulphate polyacrilamide gel electrophoresis (SDS-
29 PAGE) following the method of Brown et al. (1981) modified by Gepts et al. (1986).
30 Electrophoresis was carried out in 1-mm-thick, 15% (wt/vol) polyacrilamide slab gels (height, 16
31 cm; width, 18 cm) and carried out 25 mA at loading (approximately 1h) and thereafter to 30 mA
32 until the separation was completed. Proteins were stained with Coomassie brilliant blue R-250.

33 The storage-seed proteins studied were: S, T, C, B and H phaseolin patterns (43-53 kDa),
34 which were determined according to the genotypes of reference (Sanilac, Tendergreen,

Contender, Boyaca and Huevo de Huanchaco). We also present the results of the study of a subset of polypeptides: b4 and b5 (58-55 kDa), b6 and b7 (42-41 kDa), and b11, b12 and b13 polypeptides or lectins (33-31 kDa) (González et al., 2009).

Isozyme analysis

A combination of starch gel electrophoresis and enzyme activity staining was used to detect polymorphisms between parental lines for six enzymes: *Skdh* (E.C.1.1.1.25), *Me* (E.C.1.1.1.40), *Rbcs* (E.C.1.1.1.39), *Prx* (E.C.1.11.1.7), *Mdh-1* (E.C.1.1.1.37), and *Diap* (E.C.1.6.99). Both, *Mdh* and *Diap* enzyme systems had two independent loci.

The seeds used for seed protein analysis were sown, and plant and root tissues were analyzed at the first true-leaf stage, approximately 10 days after sowing. A crude tissue homogenate was produced by grinding the primary leaf or root apex (depending on the enzymes assayed) in a potassium phosphate grinding buffer (0.1 M pH 7.0 containing 20% sucrose, 5% PVP-40, 0.5%, Triton X-100 and 14 mM of 2-mercaptoethanol). A lithium borate/tris citrate discontinuous system was used. Electrophoresis was carried out at 25 mA for 20 min to load the proteins into the gel, and resumed at 30 mM. After electrophoresis, both anodal and cathodal sections of a gel slice 1.5 mm thick were placed in a tray along with the enzyme assayed, and stained for enzyme activity. Loci were labelled sequentially, with those migrating closest to the anodal end being designated as number 1 (Koenig and Gepts 1989a). In each gel, the cultivars ICA-Pijao and Dark Red Kidney were included as standards. ICA-Pijao has the following genotype at the polymorphic enzyme loci: *Skdh*¹⁰³, *Me*¹⁰⁰, *Rbcs*¹⁰⁰, *Prx*⁹⁸, *Diap-1*⁹⁵, *Diap-2*¹⁰⁵, *Mdh-1*¹⁰⁰ and *Mdh-2*¹⁰⁰. Dark Red Kidney exhibits the following genotype: *Skdh*¹⁰⁰, *Me*⁹⁸, *Rbcs*⁹⁸, *Prx*⁹⁸, *Diap-1*¹⁰⁰, *Diap-2*¹⁰⁰, *Mdh-1*¹⁰³ and *Mdh-2*¹⁰².

Data analysis

Comparisons of marker frequencies among cycles within populations were made to determine whether significant differences resulted from the selection process. A regression analysis (SAS 2002) described by Schaffer et al. (1977) and Wilson (1980) was used to determine if the changes in marker frequencies were due to genetic drift or selection. This test relies on the fact that if directional selection is acting on the locus studied, there should be an approximately linear directional trend in the gene frequency data that can be tested for significant deviations from the null hypothesis, i.e., genetic drift acting alone. The variance-covariance matrix for fluctuation in

gene frequency was derived for data subjected to the angular transformations $2 \sin^{-1}$ (marker frequency)^{1/2}, as described by Schaffer et al. (1977). The sum of squares of the deviation calculated to test the null hypothesis (changes due to random genetic drift) had a central χ^2 distribution with one degree of freedom less than the number of cycles evaluated. In addition, the sum of squares due to all departures from the model based solely on genetic drift is partitioned in two components, one of which has a central χ^2 distribution with one degree of freedom that accounts for any linear trend in the gene frequency. Therefore, the significance of this linear component determines whether the allelic frequency changes due to directional selection were important. The tabulated χ^2 value (2 df, $P \leq 0.01$) critical for declaring a significant deviation was 9.21, and the critical 2 value (1 df, $P \leq 0.01$) for declaring a significant linear trend was 6.63.

The proportion of the phenotypic variation that was explained by each one of the protein markers alleles was determined by calculating the coefficients of determination (R^2) due to regression of phenotypic values on allele frequencies in the populations (Edwards et al. 1987). A significant F-test indicated cosegregation of the marker locus genotypic classes with the quantitative trait phenotype. For the simplest case of digenic epistasis affecting a quantitative trait, a linear model in a two-way ANOVA (Edwards et al. 1987; Paterson et al. 1988; De Vicente and Tanksley 1993 and Damerval et al. 1994) was used. Two-factor analyse of variance was computed for each possible pair of loci to determine main effects of the two loci plus their interaction, and the model is as follows:

20

$$Y_{ijm} = \mu + \alpha_i + \alpha_j + \tau_{ij} + C_{ijm}, \text{ for } m = 1, 2, \dots, n_{ij},$$

22

where Y_{ijm} is the phenotype of the m^{th} individuals with the digenic genotype at loci i and j ; α_i and α_j are the main effects associated with the loci i and j , respectively; τ_{ij} are the effects arising from interactions between alleles at the loci i and j , and C_{ijm} is the residual effect including the genetic effect unexplained by the two loci in the model plus measurement of errors and other factors, which is assumed to be an independent random variable having a normal distribution.

“Partial R^2 ” parameters were estimated for each interaction effect and referred to the amount of variation explained by the interaction effect after accounting for the main effects of loci. This was calculated by dividing the Type III sums of squares for the interaction effect by the total sums of squares for the model which included both main effects and the interaction. A significant interaction factor suggests that the effect rendered by the interaction is not simply the sum of their independent effects (Tanksley 1993). In addition, the effect of allelic substitution

was estimated as the difference between mean values of the two genotypic classes. These values are relative to the phenotypic means used in the analyses.

Results

Seed traits data

Seed size characteristics exhibited quantitative inheritance patterns with a continuous distribution of phenotypic values in segregation populations. Combining acceptable seed size traits is crucial in the identification of useful genotypes in a breeding program. The selected lines from intra gene pool crosses exhibited higher seed size as compared to the parental lines. The means for seed weight, length, height and width of the parents and F₇ are shown in Table 2. On the one hand, PHA-257-01/323-02 had the highest mean weight, followed by PHA-257-06/306-

01, belonging both to interracial populations. In general, seed weight was excellent in most interracial populations. On the other hand, PHA-159-14/257-14 (inter-gene pool population) had the lowest mean, even lower than the intraracial PHA-20-07/159-11 (Mesoamerica x Mesoamerica).

The data did not fit a normal distribution in any population for seed weight, while seed width fitted in five of the 16 populations, seed length in four and seed height in two.

Changes in frequency of seed marker alleles

A fixation (frequency= 1) and an extinction (frequency= 0) of the parental alleles was observed at some of the marker loci. Of the 96 comparisons of marker frequency among generations, 67 significant marker frequency changes were detected in the test for selection (Tables 3 and 4).

The effect of selection on changing the frequency of the seed phaseolin alleles differed, depending on the allele and the genetic background. For all populations, the frequencies for the polymorphic markers from F₃ to the final selection cycles are shown in supplementary tables (ESM1, ESM2 and ESM3).

In intra-gene pool populations the phaseolin alleles contributed in a similar way to increase seed weight (Pha^B and Pha^S inside Mesoamerican pool and Pha^T, Pha^H and Pha^C inside Andean pool), and therefore the most changes in frequency of Pha appeared to be due to random drift.

The frequency of the Pha^T allele increased when it cosegregated with Pha^C allele in one interracial population, while a reduction or stability appeared when it cosegregated with Pha^H in the rest of interracial populations or with Pha^S in inter-gene pool populations. The Pha^T frequency changes exhibited significance in the test for selection only in two of the five crosses in which segregated with Pha^H (Table 3). Moreover, this allele showed non-linearity when it segregated with Pha^C. Thus the changes in frequency of Pha^T appeared to be due to random drift in the interracial populations, indicating similar contribution of Pha^T, Pha^H and Pha^C in seed weight. However, there was a highly linear trend in the frequencies of Pha^T allele in the inter-gene pool populations (Table 4).

Pha^S allele did not exhibit significant linear increases in frequency in the intraracial population PHA-20-07/PHA-159-11. In the inter-gene pool populations, Pha^S increased or kept stable throughout the generations. The Schaffer test detected that the allelic frequency changes of Pha^S were significantly greater than those expected from random genetic drift acting alone in the inter-gene pool crosses (Table 4). The χ^2 of the directional selection effect (χ^2 lineal) was highly

significant for this allele in all populations. Our finding suggests that selection was not effective in increasing seed size in inter-gene pool crosses in spite of differences in protein frequencies were observed. These changes were due to an increase of the S haplotype that exhibited dominance over the T haplotype and gave rise to lines with a smaller seed size than parentals.

Two of the isozyme loci examined (*Prx*⁹⁸ and *Diap-2*¹⁰⁵) did not revealed polymorphisms in any population studied. The alleles that encode for the remaining isoenzymes suffered a selective pressure in the crosses analyzed, increasing the frequencies of the alleles *Me*⁹⁸, *Rbcs*⁹⁸ and *Mdh1*¹⁰³ (Andean alleles) in the interracial populations, while in the inter-gene pool populations showed an increase of the Mesoamerican alleles *Skdh*¹⁰³, *Rbcs*¹⁰⁰ and *Diap1*⁹⁵.

The selection procedure resulted in a comparatively high selection response in interracial populations, which could be due to the accumulation of large-seeded alleles. Inter-gene pool populations showed an overrepresentation of the Mesoamerican alleles, many of which were fixed in the F₇ lines. The presence of the great percentage of Mesoamerican markers and the lowest mean in these populations revealed a strong association between seed size and the markers studied. These results revealed a great chance to find new favourable combinations of alleles and they could indicate a selective advantage on genetic level.

Proportion of variation explained by protein alleles

The genetic control and the location of phaseolin (Pha), lectins (*b11/b12/b13*), *Rbcs*, *Skdh*, *Me*, *Diap* and *Mdh* has been previously described (Vallejos and Chase 1991a; 1991b). These markers are distributed on seven linkage groups. The markers *b4/b5* and *b6/b7* have not been described previously in common bean.

The polypeptides of 56 kDa or 54 kDa (*b4/b5*) and 42 or 41 kDa (*b6/b7*) molecular weights segregated as products of different alleles of the same gene. In the classification of parental lines screened (Table 1), all lines with S-type phaseolin had the 54 kDa (*b5*) and 41 kDa (*b7*) polypeptides, and most lines with T, H or C type phaseolin had the 56 and 42 kDa (*b4* and *b6*) bands. Gepts et al. (1986) demonstrated that bean cultivars and landraces with S type phaseolin were domesticated in Mexico and Central America, and that beans with T- and C-type phaseolin were domesticated in the Andes. Although the number of lines screened is only a small sample, it suggests the possibility that the polypeptide patterns are also indicative of both centers of domestication.

In order to investigate possible linkage between seed size and protein and isozyme loci, F-test was used to compare the means of seed size parameters between homozygotes and heterozygotes

at all segregating marker loci. The means of seed weight, length, height and width were grouped according to class frequencies for each marker: protein band and isoenzyme. Those showing significant ($P \leq 0.05$) differences between the groups mean values for these traits are shown in Tables 5 and 6. Inspection of the mean seed weights of the three genotypes indicated that the mode of action of these genes are additive. The mean seed weight of heterozygotes was intermediate to the two homozygote classes.

Phaseolin explained from 5 to 26 % of the phenotypic variance of seed weight. Substitution of a lower-seed weight allele by a higher-seed weight allele resulted in a seed weight from 1 to 25 g 100 seed⁻¹, depending on the genetic background. For the *b6/b7* and *b4/b5* polypeptides, R^2 values ranged from 7 to 20 % and from 6 to 24 %, respectively. Thus, both markers, *b6/b7* and *b4/b5*, could be also useful for studying seed weight variability. Significant associations between isozymes markers and seed weight explained notable ranges of phenotypic variation: *Skdh* (4-14 %), *Rbcs* (5-12 %) and *Diap-1* (4-10 %).

Phaseolin explained from 7 to 48 % of the phenotypic variance of seed length, which was larger than the coefficient of determination (R^2) explained for seed weight. The polypeptides *b6/b7* and *b4/b5* explained from 0.8 to 16 % and from 6 to 8 % of the phenotypic variance, respectively.

The lectins explained from 4 to 16 % of the phenotypic variation of seed height. The interaction effect of loci of lectins and *Me* ranged from 4 to 7 % of the phenotypic variation after accounting for the main effects of the loci, while only one population showed interaction effect of loci of lectins with *Rbcs* (3 %).

Mdh-1 explained the largest percentage of phenotypic variation for seed width, from 6 to 12 %. The polypeptide *b6/b7* explained 4 to 10 % of the phenotypic variance. Both markers showed significant associations and explained from 3 to 6 % of the phenotypic variance.

Vallejos et al. (1991b) mapped five seed proteins, the *P* locus, and *Mdh-1* in the linkage group (B7) of the *Pha* locus. A significant interaction was observed between the polypeptides *b4/b5* and *b6/b7* and the isozyme *Mdh-1* in several crosses, indicating that these loci characterize close regions of the bean genome. These polypeptides must be positioned on the LG B7, closer than *Mdh-1* than *Pha*.

In the interracial populations the intermediate form *PhaH/T*, the homozygous *b6* and *Rbcs*⁹⁸ allele had a positive effect on seed size. The inter-gene pool populations increased the number of potential markers, showing an effect positive on seed size *PhaT*, *b4*, *b6*, *Skdh*¹⁰⁰, *Rbcs*⁹⁸ and *Diap*¹⁰⁰ alleles.

The observed allelic effects are shown in Table 7. An allelic effect of -0.5 for seed size traits indicates that the parental allele reduced seed size by half a percentage point in that cross. Although allelic effects varied depending on environmental conditions and genetic backgrounds, it has been noted that Andean alleles contributed a large seed size.

Discussion

The multigenic type of inheritance is in accord with the studies related to seed size in *Phaseolus* (Motto et al. 1978; Nienhuis and Singh, 1988; Vallejos and Chase 1991a; Park et al. 2000). The highest means and the widest variation for seed size traits belonged to interracial populations. This demonstrated the great usefulness of the interracial populations for improvement of large-seeded germplasm (Singh and Urrea 1994; Welsh et al. 1995; Singh et al. 2002). However, the seed size improvement within intraracial and inter-gene populations was very poor. One explanation for intraracial populations is the narrow genetic base that restricts the genetic gain. Possible causes for inter-gene populations include genetic factors affecting the transmission of the genes (González et al., 2009).

The data indicated that seed width showed a normal distribution in most populations. This could be due to the fact that width is a more stable trait, and it is not so important to identify common bean market classes, compared to seed weight, so it has gone more “unnoticed” to the selection process used in this study.

Selection increases the frequencies of favourable alleles while genetic drift is a random change in allele frequencies due to small population size. A loss of favourable alleles due to random genetic drift leads to a reduction in genetic variance and, thus, limits future selection response (Guzman and Lamkey 2000). Although the loss of genetic variation was generally consistent with that expected for a model in which random genetic drift acts alone on neutral alleles, the changes observed in the frequency of a large part of the markers were significantly greater from that which might be expected under such conditions. The selection resulted in the decrease of the genetic variation, thus random fluctuations were increased and caused that the genes of one generation were not a representative sample of the previous generation. As a result, a great part of the deviations were due to the action of any erratic factor, such as non-directional selection combined with random genetic drift and/or sampling error.

The Schaffer’s test detected a high significant linear trend of the 63% of the polymorphic loci studied, meaning that directional selection was acting on those loci (Schaffer et al. 1977). These results agreed with other works (Eagen and Goldman 1996; Heredia Diaz et al. 1996; Butrón et

al. 2005; Diaby and Casler 2005), which found significant changes in marker frequencies due to directional selection. The higher power of this study for detecting directional selection effects on allelic frequencies could be due to that in those studies the cycles evaluated were the result of selecting for different traits. Some of them are complex traits, as grain yield, and other traits could be negatively correlated, so the selection to increase one trait can result in the reduction of other trait, while in this study we selected for seed size, seed weight, length, height and width that are traits positively correlated (Vallejos and Chase 1991a; Park et al. 2000).

The difference in seed size between the progenitors suggests that either a large number of polygenes with similar effects or at least one major and a few others with minor effects would segregate in the progenies. With a limited number of markers, one can expect to detect minor genes with tight linkages or major genes with moderate linkage. The consistent results obtained with 16 independently obtained crosses strengthened the validity of the linkage between protein markers and the locus that contributes to seed size.

The statistically significant associations possibly infer a genetic linkage between the markers and the seed size traits in form of multilocus associations and/or pleiotropic effects of genes controlling these traits. Associations were found between T/H phaseolin protein and large seed size, and S phaseolin and small seed size. This association was consistent across the wide range of populations analyzed, and agreed with Johnson et al. (1996) and Murray et al. (2004) who also found a stable relationship between the locus *Phs* and seed weight in different environments. The locus *Phs* was significantly associated with seed weight in all populations in which it was polymorphic, except for the population PHA-257-01/PHA-323-02, in which the parents corresponded to T and C phaseolin patterns, respectively. Hartana (1983) reported that T and C phaseolin patterns were associated with large seed weight. This study showed that the alleles of both phaseolin patterns (T and C) contributed in a similar way to increase seed weight. The larger magnitude of the phenotypic variance explained by seed length than seed weight implies that the QTL for seed length could be mapped nearer locus *Phs* than the QTL for seed weight.

These populations represent the major variability of both gene pools in common bean, so the locus *Phs* provides an attractive model system for the molecular analysis of quantitative trait variation and the genetic expression of seed size. In addition, due to the large R^2 observed in this study, the locus *Phs* and some polypeptides, as b6/b7, could be useful for MAS (Marker Assisted Selection).

In common bean, associations between *Rbcs* and *Me* have been reported in different populations (Koenig and Gepts 1989b; Nodari et al. 1993). The interaction effect of the lectins

(*b11/b12/b13*), *Me* and *Rbcs* confirm the order *Rbcs*-*Lec*-*Me* reported by Koenig and Gepts (1989b). The lectins should be situated in LG B4, closer to *Me* than *Rbcs*.

The significant interaction observed between the polypeptides *b4/b5* and *b6/b7* and the isozyme *Mdh-1* indicates that these polypeptides should be situated in the same LG B7, near the locus *Phs*.

The isoenzymes are a useful tool, as the phaseolin, to characterize both gene pools, being characteristic the alleles *Skdh*¹⁰³, *Rbcs*¹⁰⁰, *MdhI*¹⁰⁰ and *DiapI*⁹⁵ from the Mesoamerican pool, while the alleles *Skdh*¹⁰⁰, *Rbcs*⁹⁸, *MdhI*¹⁰³ and *DiapI*¹⁰⁰ characterize Andean pool (Singh *et al.*, 1991b). Intra-gene pool populations showed an increase of isoenzymatic alleles: *Me*⁹⁸, *Rbcs*⁹⁸ and *MdhI*¹⁰³. The frequencies of the *b6* and *Rbcs*⁹⁸ alleles shifted in a manner consistent with its association with seed size. Both alleles, which have a positive effect on seed size, were increased markedly with each generation. Deviations for directional selection were significant, indicating that the changes were too large to be due to random drift alone.

The inter-gene pool populations showed a higher transmission of Mesoamerican alleles in all loci, although the Andean alleles, as *Pha*^T, *Skdh*¹⁰⁰, *Rbcs*⁹⁸ and *Diap*¹⁰⁰, showed positive effects on seed weight. The summary of the allelic effects on seed size within different genetic backgrounds showed a clear tendency for Andean alleles to increase seed size. Johnson *et al.* (1996) determinate that S haplotype was associated with lower seed weight and exhibited some degree of dominance over the T haplotype. Segregation distortion is a common feature of inter-gene pool crosses in common bean (Freyre *et al.* 1998; Johnson and Gepts 2002). It has been found that in crosses of Andean x Mesoamerican parents, those from the Mesoamerican gene pool usually possess negative general combining abilities for seed size (Singh *et al.* 1992). The negative phenotypic effect from Mesoamerican alleles was reflected in reduced performance of these populations. The bad performance of the progenies of Andean x Mesoamerican crosses has been reported by several authors (e.g. Santalla *et al.* 2005; Welsh *et al.* 1995).

Our results suggest that Phaseolin and other seed proteins are linked to favorable alleles at loci affecting seed size. These markers have good potential for improving the results of the selection and should be considered by breeders as a strategy for germplasm enhancement and to avoid the reduced performance of the inter-gene pool populations by means of a marker-assisted selection to identify Andean alleles in the progenies that posses a positive effect on seed size.

The availability of tags for genes that control seed size can be useful in studies of bean evolution. In addition to phaseolin type, seed size is one of the major differentiating characters between Mesoamerican and Andean *Phaseolus vulgaris* beans (Gepts 1988). The availability of

genetic markers for genes involved in seed size control in beans will certainly aid physiological and evolutionary studies centered on this important agronomic character.

There are several implications of our results to breeding programs. Based on molecular markers, crosses involving Mesoamerican x Andean genotypes were found to be the most divergent and may generate more significant progress from selection. The transfer of polygenic traits such as seed size potential of Andean types to Mesoamerican types may, however, be a problem, because distorted segregations make it more difficult to recover certain recombinants. This may explain in part why it has been difficult to transfer quantitative traits between cultivars of Mesoamerican and Andean origin.

We conclude that selection for seed size may lead to molecular changes in protein frequencies in the selected populations. Results from this work are relevant to ongoing common bean breeding programs that are aimed at developing beans with a good seed commercial quality, in which protein markers can be used as tools to select.

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Table 1 Characteristics of common bean genotypes used in intra-and interracial hybridization

Line	Race ^a	Seed		Growth habit ^b	Pha	Protein ^c			Isoenzym		
		Color	Weight (g100 seed ⁻¹)			b4/5	b6/7	b11/12/13	<i>Skdh</i>	<i>Me</i>	<i>Rbcs</i>
PHA-020-07	M	white	35	III	S	b5	b7	b13	103	102	98
PHA-119-01	PE	beige	25	II	H	b4	b7	b12	100	100	100
PHA-159-08	M	white	27	II	S	b5	b7	b13	103	102	98
PHA-159-09	M	white	24	II	S	b5	b7	b13	103	102	98
PHA-159-10	M	white	30	II	S	b5	b7	b13	103	102	98
PHA-159-11	M	white	25	II	B	b5	b7	b11	103	100	98
PHA-159-12	M	white	29	II	B	b5	b7	b11	103	100	100
PHA-159-13	M	white	21	II	S	b5	b7	b13	103	102	100
PHA-159-14	M	white	27	II	S	b5	b7	b13	103	100	100
PHA-257-01	NG	white	70	I	T	b4	b6	b11	100	98	98
PHA-257-04	NG	white	54	I	T	b4	b6	b11	100	98	98
PHA-257-06	NG	white	67	I	T	b4	b6	b11	100	98	98
PHA-257-08	NG	white	58	I	T	b4	b6	b11	100	98	100
PHA-257-10	NG	white	58	I	H	b4	b7	b11	100	98	98
PHA-257-14	NG	white	36	I	T	b4	b6	b12	100	98	98
PHA-257-23	NG	white	63	I	T	b4	b6	b12	100	98	98
PHA-267-15	NG	white	62	I	T	b4	b6	b12	100	100	98
PHA-267-18	NG	white	63	I	T	b4	b6	b11	100	100	98
PHA-267-20	NG	white	51	I	T	b4	b6	b12	100	100	100
PHA-269-12	NG	white	51	I	T	b4	b6	b12	100	98	100
PHA-269-13	NG	white	58	I	T	b4	b6	b12	100	98	100
PHA-272-01	PE	purple	45	IV	H	b4	b7	b12	100	98	100
PHA-272-02	PE	purple	43	IV	H	b4	b7	b12	100	98	100
PHA-306-01	PE	beige	55	I	H	b4	b7	b12	100	100	100
PHA-306-11	PE	beige	65	I	H	b4	b7	b12	103	100	100
PHA-306-21	PE	beige	56	I	H	b4	b7	b12	100	100	98
PHA-323-02	CH	beige	54	IV	C	b4	b6	b13	100	98	100
PHA-338-19	PE	beige	56	I	H	b4	b7	b12	100	100	98
PHA-338-27	PE	beige	60	I	H	b4	b7	b12	100	98	100
PHA-452-01	NG	white	74	IV	T	b4	b6	b12	100	98	98

^a M: Mesoamerica; PE: Peru; NG: Nueva Granada; CH: Chile

^b I: determinate, erect; II: indeterminate, erect; III: indeterminate, non climber or semi-climbing, prostrate, and IV: indeterminate, climbing

^c Pha: phaseolin; b4/b5: legumin; b6/b7: polypeptides; b11/b12/b13: lectin

^d *Skdh*: Shikimic acid dehydrogenase; *Me*: Malic enzyme; *Rbc*: Ribulose bisphosphate carboxylase; *Diap*:- Diaphorase; *Mdh*:- Malate dehydrogenase

1 **Table 2** Parental and F₇ means for seed traits and the probability of fitting a normal distribution
2 by using the Smirnov–Kolmogorov (K) test in the 16 intraracial, interracial and inter-gene pool
3 common bean populations studied

Population		Seed															
P ₁ / P ₂	N ^a	Weight				Length				Width				Height			
		P ₁	P ₂	F ₇	K	P ₁	P ₂	F ₇	K	P ₁	P ₂	F ₇	K	P ₁	P ₂	F ₇	K
Intraracial populations																	
PHA-20-07/159-11	300	0.25	0.35	0.30	**	12.0	10.1	10.7		8.0	7.4	7.3	**	5.9	5.1	5.3	**
PHA-267-20/257-23	714	0.51	0.63	0.61	**	15.1	16.3	15.5	**	7.5	8.0	8.1	**	6.1	6.7	6.4	**
PHA-338-19/306-21	320	0.56	0.56	0.57	*	14.3	14.0	14.2	**	8.4	8.7	8.4	**	7.2	7.1	7.0	**
Interracial populations																	
PHA-452-01/119-01	50	0.75	0.25	0.62	**	19.7	10.1	16.0		8.7	7.4	7.7		6.4	5.1	6.4	**
PHA-257-01/323-02	125	0.70	0.54	0.80	**	17.2	14.3	17.4	*	8.3	8.8	9.1	**	7.1	7.6	7.4	**
PHA-257-06/306-01	589	0.58	0.65	0.69	**	13.3	13.1	15.4	**	5.7	6.7	8.2	**	4.3	5.1	6.5	**
PHA-257-10/306-11	459	0.67	0.55	0.65	**	16.6	14.6	16.5	**	8.2	8.2	8.3	**	6.8	7.3	6.8	**
PHA-267-18/338-27	515	0.46	0.60	0.66	**	15.3	14.7	16.0		7.5	8.5	8.6	**	6.0	7.1	7.1	**
PHA-272-01/257-01	69	0.45	0.64	0.61	**	12.4	16.5	15.0		8.0	8.1	8.2		6.6	6.5	6.7	
PHA-272-02/257-04	45	0.43	0.54	0.37	**	12.1	15.7	11.9	**	8.2	7.6	8.0		6.4	6.1	5.5	**
Inter-gene pool populations																	
PHA-159-09/257-08	140	0.24	0.58	0.36	**	10.2	15.6	11.2	**	6.9	7.8	7.4		4.5	6.4	5.7	**
PHA-159-12/267-15	148	0.63	0.21	0.41	**	16.1	9.7	13.3	**	7.7	6.6	7.6		6.7	4.5	5.8	**
PHA-159-13/267-18	250	0.27	0.36	0.28	**	10.1	15.3	13.4	**	7.1	7.5	8.2	**	5.3	6.0	6.0	**
PHA-159-14/257-14	150	0.29	0.62	0.27	**	11.8	17.9	11.2	**	7.1	8.4	6.7	*	5.9	6.4	4.9	**
PHA-159-10/269-12	30	0.30	0.51	0.34	**	12.0	15.0	10.2	**	7.2	8.0	7.0	**	5.9	5.9	6.1	
PHA-159-08/269-13	60	0.27	0.58	0.37	**	11.7	15.9	11.3	**	7.1	8.4	8.2	**	5.2	7.0	6.6	**

4
5 ^a Number of individuals analyzed in F₇
6 *, ** Significant at P ≤ 0.05 or 0.01, respectively
7

Table 3 Chi square values for deviations from genetic drift and for directional selection of significant allelic frequency changes at protein markers in intra-gene pool crosses.

Marker	AFCC ^a	χ^2_{drift}	$\chi^2_{\text{selection}}$
Intraracial crosses			
PHA-267-20/PHA-257-23 ^b			
<i>Rbc</i> ¹⁰⁰ / <i>Rbc</i> ⁹⁸	0.09	160.9	***
<i>MdhI</i> ¹⁰³ / <i>MdhI</i> ¹⁰⁰	0.12	70.38	***
PHA-338-19/PHA-306-21 ^b			
<i>Mdh2</i> ¹⁰⁰ / <i>Mdh2</i> ¹⁰²	0.07	167.81	***
Interracial crosses			
PHA-452-01/PHA-119-01 ^b			
Phaseolin H/T	0.04	7.28	16.49
<i>b6/b7</i>	0.17	37.49	***
PHA-257-01/PHA-323-02 ^b			
<i>b11/b13</i>	0.38	378.8	***
<i>Rbc</i> ¹⁰⁰ / <i>Rbc</i> ⁹⁸	0.10	1.18	42.96
<i>MdhI</i> ¹⁰³ / <i>MdhI</i> ¹⁰⁰	0.10	505.05	***
PHA-257-06/PHA-306-01 ^b			
Phaseolin H/T	0.04	128.82	***
<i>b6/b7</i>	0.09	697.74	***
<i>b11/b12</i>	0.07	61.15	***
<i>Me</i> ¹⁰⁰ / <i>Me</i> ⁹⁸	0.19	86.39	***
<i>Rbc</i> ¹⁰⁰ / <i>Rbc</i> ⁹⁸	0.09	12.67	8.86
<i>MdhI</i> ¹⁰³ / <i>MdhI</i> ¹⁰⁰	0.19	71.21	***
PHA-257-10/PHA-306-11 ^b			
<i>Skdh</i> ¹⁰³ / <i>Skdh</i> ¹⁰⁰	0.08	34.74	***
<i>Me</i> ¹⁰⁰ / <i>Me</i> ⁹⁸	0.14	40.45	***
<i>Rbc</i> ¹⁰⁰ / <i>Rbc</i> ⁹⁸	0.24	49.46	***
<i>MdhI</i> ¹⁰³ / <i>MdhI</i> ¹⁰⁰	0.14	30.02	***
PHA-267-18/PHA-338-27 ^b			
<i>b11/b12</i>	0.11	52.34	***
<i>Me</i> ¹⁰⁰ / <i>Me</i> ⁹⁸	0.06	7.87	5.15
<i>Rbc</i> ¹⁰⁰ / <i>Rbc</i> ⁹⁸	0.06	199.86	***
<i>MdhI</i> ¹⁰³ / <i>MdhI</i> ¹⁰⁰	0.11	41.52	***
PHA-272-01/PHA-257-01 ^b			
<i>b6/b7</i>	0.15	7.85	7.72
<i>Rbc</i> ¹⁰⁰ / <i>Rbc</i> ⁹⁸	0.06	3.75	3.32
PHA-272-02/PHA-257-04 ^b			
<i>b6/b7</i>	0.17	13.76	***
<i>Rbc</i> ¹⁰⁰ / <i>Rbc</i> ⁹⁸	0.12	10.36	7.70

^a Average frequency change per cycle

^b Race Nueva Granada= PHA-267-20, PHA-257-23, PHA-452-01, PHA-257-01, PHA-257-04, PHA-257-06, PHA-257-10 and PHA-267-18. Race Peru= PHA-338-19, PHA-306-21, PHA-

1 306-01, PHA-306-11, PHA-338-27, PHA-272-01, PHA-272-02, PHA-119-01. Race Chile=
2 PHA-323-02

3 **, *** Significant at $P \leq 0.01$ or 0.005 , respectively

4

Table 4 Chi square values for deviations from genetic drift and for directional selection of significant allelic frequency changes at protein markers in the inter-gene pool crosses

Marker	AFCC ^a	χ^2_{drift}	$\chi^2_{\text{selection}}$		
PHA-159-09/PHA-257-08 ^b					
Phaseolin S/T	0.025	0.99		20.71	***
<i>b6/b7</i>	0.182	6.27		10.58	***
<i>b11/b13</i>	0.483	66.29	***	23.88	***
<i>Skdh</i> ¹⁰³ / <i>Skdh</i> ¹⁰⁰	0.095	7.05		32.22	***
<i>Me</i> ¹⁰² / <i>Me</i> ⁹⁸	0.102	26.89	***	25.89	***
<i>Rbc</i> ¹⁰⁰ / <i>Rbc</i> ⁹⁸	0.244	104.66	***	19.53	***
<i>DiapI</i> ¹⁰⁰ / <i>DiapI</i> ⁹⁵	0.030	347.86	***	175.28	***
<i>MdhI</i> ¹⁰³ / <i>MdhI</i> ¹⁰⁰	0.090	16.61	***	14.92	***
PHA-159-12/PHA-267-15 ^b					
Phaseolin B/T	0.245	11.80	***	34.60	***
<i>b6/b7</i>	0.245	26.02	***	26.00	***
<i>Skdh</i> ¹⁰³ / <i>Skdh</i> ¹⁰⁰	0.249	52.81	***	25.60	***
<i>Rbc</i> ¹⁰⁰ / <i>Rbc</i> ⁹⁸	0.189	66.80	***	62.72	***
<i>DiapI</i> ¹⁰⁰ / <i>DiapI</i> ⁹⁵	0.089		***		***
<i>MdhI</i> ¹⁰³ / <i>MdhI</i> ¹⁰⁰	0.325	333.40	***	212.52	***
PHA-159-13/PHA-267-18 ^b					
Phaseolin S/T	0.157	11.18	***	61.15	***
<i>b4/b5</i>	0.153	57.91	***	33.30	***
<i>b6/b7</i>	0.136	81.72	***	39.60	***
<i>b11/b13</i>	0.169	51.09	***	23.62	***
<i>Skdh</i> ¹⁰³ / <i>Skdh</i> ¹⁰⁰	0.145	54.60	***	21.41	***
<i>Rbc</i> ¹⁰⁰ / <i>Rbc</i> ⁹⁸	0.059	395.96	***	199.01	***
<i>DiapI</i> ¹⁰⁰ / <i>DiapI</i> ⁹⁵	0.080	122.95	***	42.11	***
<i>MdhI</i> ¹⁰³ / <i>MdhI</i> ¹⁰⁰	0.093	936.27	***	342.97	***
PHA-159-14/PHA-257-14 ^b					
Phaseolin S/T	0.045	12.52	***	22.32	***
<i>b12/b13</i>	0.282	201.96	***	75.16	***
<i>Skdh</i> ¹⁰³ / <i>Skdh</i> ¹⁰⁰	0.278	245.26	***	77.07	***
<i>Me</i> ¹⁰⁰ / <i>Me</i> ⁹⁸	0.191	81.57	***	32.53	***
<i>MdhI</i> ¹⁰³ / <i>MdhI</i> ¹⁰⁰	0.200	233.84	***	155.36	***
PHA-159-10/PHA-269-12 ^b					
Phaseolin S/T	0.167	2.34		8.23	***
<i>b12/b13</i>	0.095	91.85	***	57.20	***
<i>Skdh</i> ¹⁰³ / <i>Skdh</i> ¹⁰⁰	0.258	39.29	***	25.64	***
<i>Me</i> ¹⁰⁰ / <i>Me</i> ⁹⁸	0.130	54.35	***	19.89	***
<i>DiapI</i> ¹⁰⁰ / <i>DiapI</i> ⁹⁵	0.206	125.06	***	71.28	***

Table 4 Continued

Marker	AFCC ^a	χ^2 drift	χ^2 selection		
PHA-159-08/PHA-269-13 ^b					
Phaseolin S/T	0.109	5.09		85.67	***
<i>b4/b5</i>	0.398	26.18	***	9.39	***
<i>b6/b7</i>	0.0220	35.70	***	16.28	***
<i>b12/b13</i>	0.112	113.04	***	73.87	***
<i>Skdh</i> ¹⁰³ / <i>Skdh</i> ¹⁰⁰	0.136	29.10	***	15.82	***
<i>Me</i> ¹⁰² / <i>Me</i> ⁹⁸	0.121	714.85	***	603.24	***
<i>Rbc</i> ¹⁰⁰ / <i>Rbc</i> ⁹⁸	0.243	23.62	***	8.31	***
<i>Diap1</i> ¹⁰⁰ / <i>Diap1</i> ⁹⁵	0.231	106.33	***	72.86	***
<i>Mdh1</i> ¹⁰³ / <i>Mdh1</i> ¹⁰⁰	0.400		***		***

^a Average frequency change per cycle

^b Race Mesoamerica= PHA-159-08, PHA-159-09, PHA-159-10, PHA-159-12, PHA-159-13 and PHA-159-14. Race Nueva Granada= PHA-257-08, PHA-267-15, PHA-267-18, PHA-257-14, PHA-269-12 and PHA-269-13

, * Significant at $P \leq 0.01$ or 0.005, respectively

Table 5 Coefficient of determination (R^2), significant means of marker classes and partial R^2 for seed weight and seed length of the 16 intraracial, interracial and inter-gene pool common bean populations

Marker/interaction	Seed weight (g 100 seed ⁻¹)						Seed length (mm)			
	LG ^a	R ^{2b}	MM ^c	MS	SS		R ²	MM	MS	SS
Intraracial crosses										
PHA-20-07/PHA-159-11										
Pha B/S	B7	5.1	0.29	0.31	0.33	***	9.1	10.7	10.2	11.0 ***
<i>Me</i> ^{102/100}	B4	3.5	0.32	0.34	0.31	***				
<i>b11/b13*Me</i> ^{102/100}		3.1				***	3.6			**
PHA-267-20/PHA-257-23										
<i>Me</i> ^{100/98}	B4	1.1	0.53	0.67	0.56	*				
<i>Rbcs</i> ^{100/98}	B4	8.6	0.48	0.64	0.58	***				
Interracial crosses										
PHA-452-01/PHA-119-01										
Pha H/T	B7	6.1	0.59	0.73	0.70	**	11.4	14.2	17.6	17.2 **
<i>b6/b7</i>		7.2	0.72	0.57	0.49	**	6.3	17.4	15.1	15.4 **
<i>b6/b7*Mdh-1</i> ^{103/100}		5.1				**				
PHA-257-01/PHA-323-02										
Pha T/C	B7						7.0	16.8	15.5	15.7 **
<i>Rbcs</i> ^{100/98}	B4	8.8	0.60	0.70	0.75	***				
<i>b11/b13*Rbcs</i> ^{100/98}		3.1								
PHA-257-06/PHA-306-01										
Pha H/T	B7	19.1	0.71	0.96	0.83	***	9.0	16.0	17.5	16.7 **
<i>b6/b7</i>		11.8	0.90	0.83	0.67	***	4.4	18.6	17.5	16.7 **
<i>Me</i> ^{100/98}	B4	4.2	0.63	0.82	0.67	***				
<i>Rbcs</i> ^{100/98}	B4	4.6	0.65	0.68	0.74	**				
Pha H/T* <i>Mdh-1</i> ^{103/100}		2.0				*				
<i>b6/b7*Mdh-1</i> ^{103/100}		4.3				***				
PHA-257-10/PHA-306-11										
<i>Skdh</i> ^{103/100}	B3	4.2	0.42	0.55	0.53	**				
<i>Rbcs</i> ^{100/98}	B4	11.7	0.41	0.83	0.63	**				
PHA-267-18/PHA-338-27										
Pha H/T	B7	12.9	0.68	0.59	0.66	***	12.0	15.0	16.2	15.2 *
<i>b6/b7</i>		10.4	0.69	0.65	0.52	***				
<i>Rbcs</i> ^{100/98}	B4	4.9	0.60	0.64	0.69	*				
<i>Diap-1</i> ^{100/95}	B5	4.3	0.65	0.62	0.59	***				
<i>Me</i> ^{100/98} * <i>b11/b12</i>	B4	6.7				***				
PHA-272-01/PHA-257-01										
Pha H/T	B7	13.5	0.54	0.69	0.64	***	16.3	14.5	16.0	15.3 **
<i>b6/b7</i>		16.1	0.70	0.65	0.57	***	15.2	15.8	15.1	14.5 **
Pha H/T* <i>b6/b7</i>		6.1				***				
PHA-272-02/PHA-257-04										
Pha H/T	B7	21.1	0.41	0.61	0.58	***	18.8	12.6	15.6	14.3 **
<i>b6/b7</i>		19.6	0.80	0.74	0.51	***	15.4	14.7	14.5	12.6 **
<i>b11/b12</i>	B4	8.2	0.72	0.83	0.78	***				
Pha H/T* <i>b6/b7</i>		6.0				***				

Table 5 Continued

Marker/interaction	Seed weight (g 100 seed ⁻¹)					Seed length(mm)				
	LG ^a	R ² ^b	MM ^c	MS	SS	R ²	MM	MS	SS	
Inter-gene pool crosses										
PHA-159-09/PHA-257-08										
<i>b4/b5</i>		5.9	0.46	0.43	0.36	***				
Pha S/T	B7	10.9	0.31	0.39	0.65	***	22.0	12.1	12.8	18.1 ***
<i>Skdh</i> ^{103/100}	B3	5.6	0.36	0.40	0.44	***				
<i>Me</i> ^{102/98}	B4	2.2	0.36	0.43	0.41	*				
<i>Rbcs</i> ^{100/98}	B4	9.6	0.33	0.41	0.46	***				
<i>Diap-I</i> ^{100/95}	B5	10.2	0.43	0.37	0.36	***				
<i>b4/b5</i> * <i>Mdh-I</i> ^{103/100}		3.8				**				
<i>b6/b7</i> * <i>Mdh-I</i> ^{103/100}		5.2				***				
PHA-159-12/PHA-267-15										
<i>b4/b5</i>		24.0	0.45	0.29	0.32	***				
Pha B/T	B7	25.6	0.31	0.42	0.47	***	48.2	10.9	13.6	14.3 **
<i>b6/b7</i>		15.8	0.47	0.31	0.34	***	15.1	14.3	10.5	11.5 **
<i>Rbcs</i> ^{100/98}		11.6	0.32	0.28	0.44	***	9.6	11.2	10.1	13.7 **
PHA-159-13/PHA-267-18										
<i>b4/b5</i>		10.5	0.48	0.45	0.36	***	4.0	13.1	13.5	12.6 ***
Pha S/T	B7	13.0	0.30	0.39	0.44	***				
<i>b6/b7</i>		15.9	0.49	0.40	0.34	***	0.8	13.7	13.1	13.0 *
<i>Skdh</i> ^{103/100}	B3	5.8	0.40	0.45	0.48	***				
<i>Rbcs</i> ^{100/98}	B4	6.3	0.40	0.42	0.47	***				
<i>Diap-I</i> ^{100/95}	B5	8.5	0.50	0.47	0.40	***				
<i>b4/b5</i> * <i>b6/b7</i>							7.4			***
<i>b4/b5</i> * <i>Mdh-I</i> ^{103/100}		1.8				*				
Pha S/T * <i>Mdh-I</i> ^{103/100}		1.6				*				
<i>b6/b7</i> * <i>Mdh-I</i> ^{103/100}		2.6				**				
PHA-159-14/PHA-257-14										
Pha S/T	B7	15.2	0.25	0.30	0.57	***	24.6	10.6	11.7	15.5 **
<i>b6/b7</i>		10.2	0.35	0.31	0.27	***	2.2	11.8	11.3	11.8 **
<i>Skdh</i> ^{103/100}	B3	12.3	0.27	0.34	0.40	***				
<i>Diap-I</i> ^{100/95}	B5	4.7	0.41	0.32	0.24	***				
PHA-159-10/PHA-269-12										
<i>b4/b5</i>		12.5	0.40	0.35	0.31	***	8.4	12.8	11.5	10.2 **
Pha S/T	B7	23.9	0.78	0.80	0.83	***	22.2	10.6	11.1	13.3 **
<i>b6/b7</i>		14.3	0.41	0.36	0.31	***	5.9	12.6	12.1	10.8 **
<i>Skdh</i> ^{103/100}	B3	14.3	0.31	0.27	0.37	***				
PHA-159-08/PHA-269-13										
<i>b4/b5</i>		10.9	0.47	0.37	0.37	***	3.1	12.8	12.4	12.2 **
Pha S/T	B7	12.0	0.32	0.42	0.57	**	17.8	11.3	12.6	15.9 **
<i>b6/b7</i>		14.4	0.54	0.48	0.41	***	5.9	14.2	13.6	12.5 **
<i>Skdh</i> ^{103/100}	B3	5.6	0.39	0.35	0.44	**				
<i>b4/b5</i> * <i>b6/b7</i>							6.1			**
<i>b4/b5</i> * <i>Mdh</i> ^{103/100}		3.8				**				
Pha S/T * <i>Mdh</i> ^{103/100}		2.2				*				
<i>b6/b7</i> * <i>Mdh</i> ^{103/100}		5.7				***				

^a Linkage group

^b Coefficient of determination: phenotypic variation explained by the marker

^c MM and SS are homozygous for parental alleles; MS is heterozygous

*, **, *** Significant at $P \leq 0.05$, 0.01 or 0.001, respectively

1 **Table 6** The coefficient of determination (R^2), significant means of marker classes and partial R^2
2 and for seed height and width of the 16 intraracial, interracial and inter-gene pool common bean
3 populations
4

Marker/interaction	Seed height (mm)					Seed width (mm)					
	LG ^a	R ^{2b}	MM ^c	MS	SS	R ²	MM	MS	SS		
Intraracial populations											
PHA-20-07/PHA-159-11											
<i>b11/b13</i>	B4	4.1	7.4	7.7	6.8	**					
<i>Me</i> ^{102/100}	B4	0.9	7.5	7.6	7.4	*					
<i>b11/b13 * Me</i> ^{102/100}		4.4				***					
PHA-267-20/PHA-257-23											
<i>Me</i> ^{100/98}	B4	1.2	7.8	8.6	7.8	***					
<i>Rbcs</i> ^{100/98}	B4	6.7	7.5	8.4	7.9	***					
PHA-338-19/PHA-306-21											
<i>Mdh-1</i> ^{103/100}	B7					9.0	7.1	6.8	***		
Interracial populations											
PHA-257-01/PHA-323-02											
<i>b11/b13</i>	B4	9.1	8.5	8.0	9.9	***	2.1	7.0	6.4	7.3	**
<i>Rbcs</i> ^{100/98}	B4	3.2	8.9	9.1	9.8	***					
<i>Mdh-1</i> ^{103/100}	B7					6.9	7.8	7.6	6.1	*	
<i>b11/b13 * Rbc</i> ^{100/98}		3.1				**					
PHA-257-06/PHA-306-01											
<i>Me</i> ^{100/98}	B4	12.4	8.3	8.3	9.3	***					
<i>Mdh-1</i> ^{103/100}	B7					8.6	6.4	7.3	7.0	***	
<i>b11/b12 * Me</i> ^{100/98}		6.5				**					
PHA-257-10/PHA-306-11											
<i>b11/b12</i>	B4					1.8	6.0	6.2	6.3	***	
<i>Mdh-1</i> ^{103/100}	B7					8.9	6.3	5.9	5.8	***	
PHA-267-18/PHA-338-27											
<i>b6/b7</i>						5.1	6.9	7.2	7.3	***	
<i>b11/b12</i>	B4	15.9	7.1	8.8	8.9	***					
<i>Mdh-1</i> ^{103/100}	B7					8.5	7.2		6.4	***	
<i>b6/b7 * Mdh-1</i> ^{103/100}						6.4				***	
PHA-272-01/PHA-257-01											
<i>b11/b12</i>	B4	14.2	8.1	8.5	8.9	***					
PHA-272-02/PHA-257-04											
<i>b6/b7</i>						9.9	6.4	7.0	5.7	***	
<i>Mdh-1</i> ^{103/100}	B7					8.3	6.9	6.1	5.8	***	
<i>b6/b7 * Mdh-1</i> ^{103/100}						6.2				*	
Inter-gene pool populations											
PHA-159-09/PHA-257-08											
<i>b6/b7</i>						4.1	6.9	6.4	6.2	**	
<i>b11/b13</i>	B4	8.0	7.6	7.9	7.9	*	1.7	6.0	6.1	6.3	*
PHA-159-12/PHA-267-15											
<i>b6/b7</i>						4.4	6.0	5.0	5.6	***	
<i>b11/b13</i>	B4	15.5	7.4	6.9	7.8	***					
<i>Rbcs</i> ^{100/98}	B4	9.0	7.2	7.3	8.5	***					
<i>Mdh-1</i> ^{103/100}	B7					12.	6.3	5.6	5.1	***	
PHA-159-13/PHA-267-18											
<i>b6/b7</i>						6.8	5.3	5.5	5.1	*	
<i>b11/b13</i>	B4	14.2	8.0	7.7	8.2	***					
<i>Mdh-1</i> ^{103/100}	B7					12.	5.9	5.7	5.5	**	
<i>b6/b7 * Mdh-1</i> ^{103/100}						2.5				**	

Table 6 Continued

Marker/interaction	Seed height (mm)						Seed width (mm)				
	LG ^a	R ^{2b}	MM ^c	MS	SS		R ²	MM	MS	SS	
PHA-159-14/PHA-257-14											
Pha S/T	B7	4.0	6.9	7.1	7.8	***	4.3	5.1	5.3	4.9	**
<i>b6/b7</i>											
<i>b12/b13</i>	B4	12.1	7.2	7.3	6.6	***					
<i>Me</i> ^{100/98}	B4	15.6	6.8	7.1	7.8	**	6.1	5.2	5.0	4.7	**
<i>Mdh-1</i> ^{103/100}	B7										
<i>Me</i> ^{100/98} * <i>b12/b13</i>		7.1				***					
PHA-159-10/PHA-269-12											
<i>b6/b7</i>							3.4	5.9	5.7	5.6	*
<i>b12/b13</i>	B4	10.0	7.7	8.0	7.2	***					
<i>Me</i> ^{100/98}	B4	14.8	7.1	7.4	8.0	***					
PHA-159-08/PHA-269-13											
<i>Me</i> ^{100/98}	B4	10.5	8.2	8.4	8.8	***	2.4	6.7	5.2	6.0	**
<i>b12/b13</i>	B4	11.3	9.0	8.5	8.3	*					
<i>Mdh-1</i> ^{103/100}	B7										
							8.5	5.7	6.5	6.2	**

^a Coefficient of determination: phenotypic variation explained by the marker

^b MM and SS are homozygous for parental alleles; MS is heterozygous

*, **, *** Significant at $P \leq 0.05$, 0.01 or 0.001, respectively

1 **Table 7** Allelic effects for seed size traits of the intraracial, interracial and inter-gene pool
2 common bean populations
3

Allelic effect ^a				
Marker	% weight	% length	% height	% width
Intraracial crosses				
PHA-20-07/PHA-159-11				
Pha B/S	-0.04	-0.3		
<i>b11/b13</i>			0.6	
<i>Me</i> ^{102/100}	0.01		0.1	
PHA-267-20/PHA-257-23				
<i>Me</i> ^{100/98}	-0.03			
<i>Rbcs</i> ^{100/98}	-0.10		-0.4	
Interracial crosses				
PHA-452-01/PHA-119-01				
Pha H/T	-0.11	-3		
<i>b6/b7</i>	0.23	2		
PHA-257-01/PHA-323-02				
Pha T/C		1.1		
<i>b11/b13</i>			-1.4	-0.3
<i>Rbcs</i> ^{100/98}	-0.15		-0.9	
<i>Mdh-1</i> ^{103/100}				1.7
PHA-257-06/PHA-306-01				
Pha H/T	-0.12	-0.7		
<i>b6/b7</i>	0.23	1.9		
<i>Me</i> ^{100/98}	-0.04		-1	
<i>Rbcs</i> ^{100/98}	-0.09			
<i>Mdh-1</i> ^{103/100}				-0.6
PHA-257-10/PHA-306-11				
<i>b11/b12</i>				-0.3
<i>Skdh</i> ^{103/100}	-0.11			
<i>Rbcs</i> ^{100/98}	-0.22			
<i>Mdh-1</i> ^{103/100}				0.5
PHA-267-18/PHA-338-27				
Pha H/T	0.02	-0.2		
<i>b6/b7</i>	0.17			-0.4
<i>b11/b12</i>			-1.8	
<i>Rbcs</i> ^{100/98}	-0.09			
<i>Diap-1</i> ^{100/95}	0.06			
<i>Mdh-1</i> ^{103/100}				0.8
PHA-272-01/PHA-257-01				
Pha H/T	-0.10	-0.8		
<i>b6/b7</i>	0.13	1.3		
<i>b11/b12</i>			-0.8	
PHA-272-02/PHA-257-04				
Pha H/T	-0.17	-1.7		
<i>b6/b7</i>	0.29	2.1		0.7
<i>b11/b12</i>	-0.06			
<i>Mdh-1</i> ^{103/100}				1.1

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Table 7 Continued

Marker	Allelic effect ^a			
	% weight	% length	% height	% width
Inter-gene pool crosses				
PHA-159-09/PHA-257-08				
<i>b4/b5</i>	0.10			
<i>b6/b7</i>				0.7
<i>b11/b13</i>			-0.3	-0.3
Pha S/T	-0.34	-6		
<i>Skdh</i> ^{103/100}	-0.08			
<i>Me</i> ^{102/98}	-0.05			
<i>Rbcs</i> ^{100/98}	-0.13			
<i>Diap-1</i> ^{100/95}	0.07			
PHA-159-12/PHA-267-15				
<i>b4/b5</i>	0.13			
Pha B/T	-0.16	-3.4		
<i>b6/b7</i>	0.13	2.8		0.4
<i>b11/b13</i>			-0.4	
<i>Rbcs</i> ^{100/98}	-0.12	-2.5	-1.3	
<i>Mdh-1</i> ^{103/100}				1.2
PHA-159-13/PHA-267-18				
<i>b4/b5</i>	0.12	0.5		
Pha S/T	-0.14			
<i>b6/b7</i>	0.15	0.7		0.2
<i>b11/b13</i>			-0.2	
<i>Skdh</i> ^{103/100}	-0.08			
<i>Rbcs</i> ^{100/98}	-0.07			
<i>Diap-1</i> ^{100/95}	-0.10			
<i>Mdh-1</i> ^{103/100}				0.4
PHA-159-14/PHA-257-14				
Pha S/T	-0.32	-4.9	-0.9	
<i>b6/b7</i>	0.08	0		0.2
<i>b12/b13</i>			0.6	
<i>Skdh</i> ^{103/100}	-0.13			
<i>Me</i> ^{100/98}			-1	
<i>Diap-1</i> ^{100/95}	0.17			
<i>Mdh-1</i> ^{103/100}				0.8
PHA-159-10/PHA-269-12				
<i>b4/b5</i>	0.09	2.6		
Pha S/T	-0.05	-2.7		
<i>b6/b7</i>	0.10	1.8		0.3
<i>b12/b13</i>			0.5	
<i>Skdh</i> ^{103/100}	-0.06			
<i>Me</i> ^{100/98}			-0.9	
PHA-159-08/PHA-269-13				
<i>b4/b5</i>	0.10	0.6		
Pha S/T	-0.25	-4.6		
<i>b6/b7</i>	0.13	1.7		
<i>b12/b13</i>			0.7	
<i>Skdh</i> ^{103/100}	-0.05			
<i>Me</i> ^{100/98}			-0.6	
<i>Mdh-1</i> ^{103/100}				-0.5

^a The allelic effect correspond to the first allele of the segregating pair in the population

1 **ESM1** Frequencies for the polymorphic markers in intraracial populations

2

PHA-20-07/PHA-159-11						
	Pha		<i>b11/b13</i>		<i>Me</i>	
	B	S	<i>b11</i>	<i>b13</i>	<i>102</i>	<i>100</i>
F ₃	0.37	0.63	0.37	0.63	0.51	0.49
F ₄	0.34	0.66	0.54	0.46	0.57	0.43
F ₅	0.30	0.70	0.35	0.65	0.64	0.36
F ₆	0.41	0.59	0.51	0.49	0.85	0.15
F ₇	0.67	0.33	0.67	0.33	0.61	0.39
PHA-267-20/PHA-257-23						
	<i>Me</i>		<i>Rbc</i>		<i>Mdh1</i>	
	<i>100</i>	<i>98</i>	<i>100</i>	<i>98</i>	<i>103</i>	<i>100</i>
F ₃	0.23	0.77	0.44	0.56	0.88	0.12
F ₄	0.36	0.64	0.32	0.68	1	0
F ₅	0.32	0.68	0.27	0.73		
F ₆	0.52	0.48	0.17	0.83		
F ₇	0.31	0.69	0.12	0.88		
PHA-338-19/PHA-306-21						
	<i>Mdh1</i>		<i>Mdh2</i>			
	<i>103</i>	<i>100</i>	<i>102</i>	<i>100</i>		
F ₃	0.29	0.71	0.50	0.50		
F ₅	0.20	0.80	0.45	0.55		
F ₆	0.17	0.83	0.44	0.56		
F ₇	0.17	0.83	0.30	0.70		

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4

1 **ESM2** Frequencies for the polymorphic markers in interracial populations

2

PHA-452-01/PHA-119-01												
	Pha		<i>b6/b7</i>		<i>Me</i>		<i>Rbc</i>		<i>Diap</i>		<i>Mdh1</i>	
	H	T	<i>b6</i>	<i>b7</i>	<i>100</i>	<i>98</i>	<i>100</i>	<i>98</i>	<i>100</i>	<i>95</i>	<i>103</i>	<i>100</i>
F ₄	0.3	0.66	0.91	0.09	0.41	0.59	0.33	0.67	0.33	0.67	0.29	0.71
F ₅	0.2	0.71	0.99	0.01	0.30	0.70	0.37	0.63	0.48	0.52	0	1
F ₇	0	1	1	0	0.16	0.84	0.33	0.67	0.23	0.77		
PHA-257-01/PHA-323-02												
	Pha		<i>b11/b13</i>		<i>Rbc</i>		<i>Mdh1</i>					
	T	C	<i>b11</i>	<i>b13</i>	<i>100</i>	<i>98</i>	<i>103</i>	<i>100</i>				
F ₃	0.9	0.04	0.60	0.40	0.43	0.57	0.86	0.14				
F ₄	0.6	0.39	0.75	0.25	0.15	0.85	0.85	0.15				
F ₅	1	0	1	0	0.05	0.95	0.82	0.18				
F ₆					0.04	0.96	0.79	0.21				
F ₇					0.06	0.94	0.78	0.22				
PHA-257-06/PHA-306-01												
	Pha		<i>b6/b7</i>		<i>b11/b12</i>		<i>Me</i>		<i>Rbc</i>		<i>Mdh1</i>	
	H	T	<i>b6</i>	<i>b7</i>	<i>b11</i>	<i>b12</i>	<i>100</i>	<i>98</i>	<i>100</i>	<i>98</i>	<i>103</i>	<i>100</i>
F ₃	0.4	0.60	0.66	0.34	0.74	0.26	0.85	0.15	0.17	0.83	0.59	0.41
F ₄	0.5	0.47	0.91	0.09	0.82	0.18	0.52	0.48	0.32	0.68	0.58	0.42
F ₅	0.5	0.50	0.99	0.01	0.88	0.12	0.17	0.83	0.39	0.61	0.65	0.35
F ₆	0.5	0.50	0.98	0.02	0.98	0.02	0.15	0.85	0.31	0.69	0.79	0.21
F ₇	0.4	0.51	0.96	0.04	1	0	0.22	0.88	0.37	0.63	1	0
PHA-257-10/PHA-306-11												
	<i>b11/b12</i>		<i>Skdh</i>		<i>Me</i>		<i>Rbc</i>		<i>Mdh1</i>			
	<i>b11</i>	<i>b12</i>	<i>103</i>	<i>100</i>	<i>100</i>	<i>98</i>	<i>100</i>	<i>98</i>	<i>103</i>	<i>100</i>		
F ₃	0.7	0.24	0.35	0.65	0.47	0.53	0.46	0.54	0.38	0.62		
F ₄	0.7	0.30	0.23	0.77	0.36	0.64	0.57	0.43	0.48	0.52		
F ₅	0.6	0.36	0.20	0.80	0.53	0.47	0.40	0.60	0.67	0.33		
F ₆	0.5	0.43	0.13	0.87	0.40	0.60	0.36	0.64	0.61	0.39		
F ₇	0.4	0.54	0.03	0.97	0.23	0.78	0.16	0.84	0.84	0.16		
PHA-267-18/PHA-338-27												
	Pha		<i>b6/b7</i>		<i>b11/b12</i>		<i>Me</i>		<i>Rbc</i>		<i>Mdh1</i>	
	H	T	<i>b6</i>	<i>b7</i>	<i>b11</i>	<i>b12</i>	<i>100</i>	<i>98</i>	<i>100</i>	<i>98</i>	<i>103</i>	<i>100</i>
F ₃	0.67	0.33	0.78	0.22	0.83	0.17	0.40	0.60	0.34	0.66	0.68	0.32
F ₄	0.70	0.30	0.87	0.13	0.83	0.17	0.25	0.75	0.30	0.70	0.85	0.15
F ₆	0.77	0.23	0.61	0.39	0.95	0.05	0.23	0.77	0.27	0.73	0.70	0.30
F ₇	0.82	0.18	0.72	0.72	0.74	0.26	0.22	0.88	0.18	0.82	0.72	0.28
PHA-272-01/PHA-257-01												
	Pha		<i>b6/b7</i>		<i>b11/b12</i>		<i>Rbc</i>					
	H	T	<i>b6</i>	<i>b7</i>	<i>b11</i>	<i>b12</i>	<i>100</i>	<i>98</i>				
F ₃	0.5	0.42	0.40	0.60	0.33	0.67	0.37	0.63				
F ₄	0.6	0.37	0.57	0.43	0.30	0.70	0.50	0.50				
F ₇	1	0	0.70	0.30	0	1	0.45	0.55				
PHA-272-02/PHA-257-04												
	Pha		<i>b6/b7</i>		<i>b11/b12</i>		<i>Rbc</i>					
	H	T	<i>b6</i>	<i>b7</i>	<i>b11</i>	<i>b12</i>	<i>100</i>	<i>98</i>				
F ₃	0.73	0.27	0.66	0.34	0.37	0.65	0.50	0.50				
F ₄	0.92	0.08	0.85	0.15	0.11	0.89	0.37	0.63				
F ₅	1	0	1	0	0	1	0.35	0.65				
F ₆							0.40	0.60				
F ₇							0.31	0.69				

ESM3 Frequencies for the polymorphic markers in the inter-gene pool populations

PHA-159-09/PHA-257-08																					
Pha		<i>b4/b5</i>		<i>b6/b7</i>		<i>b11/b13</i>		<i>Skdh</i>		<i>Me</i>		<i>Rbc</i>		<i>Diap1</i>		<i>Mdh1</i>		<i>Mdh2</i>			
S	T	<i>b4</i>	<i>b5</i>	<i>b6</i>	<i>b7</i>	<i>b11</i>	<i>b13</i>	<i>103</i>	<i>100</i>	<i>102</i>	<i>98</i>	<i>100</i>	<i>98</i>	<i>100</i>	<i>95</i>	<i>103</i>	<i>100</i>	<i>102</i>	<i>100</i>		
F ₃	0.54	0.46	0.32	0.68	0.11	0.89	0.35	0.65	0.67	0.33	0.31	0.69	0.36	0.64	0.52	0.48	0.88	0.11	0.36	0.64	
F ₄	0.49	0.51	0.31	0.69	0.40	0.60	0.55	0.45	0.76	0.24	0.13	0.87	0.68	0.32	0.46	0.54	0.78	0.22	0.24	0.76	
F ₅	0.50	0.50	0.73	0.27	0.56	0.44	0.85	0.15	0.81	0.19	0.07	0.93	0.43	0.57	0.46	0.54	0.74	0.26	0.43	0.57	
F ₇	0.50	0.50	1	0	0.64	0.36	1	0	0.78	0.22	0	1	0.60	0.40	0.44	0.56	0.61	0.39	0	1	
PHA-159-12/PHA-267-15																					
Pha		<i>b4/b5</i>		<i>b6/b7</i>		<i>b11/b12</i>		<i>Skdh</i>		<i>Rbc</i>		<i>Diap1</i>		<i>Mdh1</i>							
B	T	<i>b4</i>	<i>b5</i>	<i>b6</i>	<i>b7</i>	<i>b11</i>	<i>b12</i>	<i>103</i>	<i>100</i>	<i>100</i>	<i>98</i>	<i>100</i>	<i>95</i>	<i>103</i>	<i>100</i>						
F ₃	0.89	0.11	0.20	0.80	0.20	0.80	0.98	0.02	0.88	0.12	0.79	0.21	0.23	0.77	0.75	0.25					
F ₄	0.76	0.24	0.40	0.60	0.32	0.68	0.78	0.22	0.67	0.33	0.56	0.44	0.22	0.78	1	0					
F ₇	0.50	0.50	0.67	0.33	0.31	0.69	0.33	0.67	0.38	0.62	0.51	0.49	0.20	0.80							
PHA-159-13/PHA-267-18																					
Pha		<i>b4/b5</i>		<i>b6/b7</i>		<i>b11/b13</i>		<i>Skdh</i>		<i>Me</i>		<i>Rbc</i>		<i>Diap1</i>		<i>Mdh1</i>					
S	T	<i>b4</i>	<i>b5</i>	<i>b6</i>	<i>b7</i>	<i>b11</i>	<i>b13</i>	<i>103</i>	<i>100</i>	<i>102</i>	<i>100</i>	<i>100</i>	<i>98</i>	<i>100</i>	<i>95</i>	<i>103</i>	<i>100</i>				
F ₂	0.54	0.45	0.23	0.77	0.27	0.73	0.36	0.64	0.60	0.40	0.65	0.35	0.55	0.45	0.41	0.59	0.55	0.45			
F ₃	0.55	0.45	0.39	0.61	0.50	0.50	0.53	0.47	0.58	0.42	0.46	0.54	0.60	0.40	0.39	0.61	0.61	0.39			
F ₄	0.66	0.34	0.41	0.59	0.58	0.42	0.57	0.43	0.77	0.23	0.62	0.38	0.68	0.32	0.29	0.71	0.38	0.62			
F ₅	0.50	0.50	0.61	0.38	0.45	0.55	0.41	0.59	0.83	0.17	0.88	0.12	0.65	0.35	0.30	0.70	0.32	0.68			
F ₆	0.45	0.55	0.89	0.11	0.23	0.77	0.64	0.36	0.54	0.46	0.46	0.54	0.88	0.12	0.20	0.80	0.32	0.68			
F ₇	0.50	0.50	0.79	0.21	0.20	0.80	0.39	0.69	0.61	0.39	0.66	0.34	0.90	0.10	0.11	0.89	0.24	0.76			

ESM3 Continued

PHA-159-14/PHA-257-14																		
	Pha		<i>b4/b5</i>		<i>b6/b7</i>		<i>b12/b13</i>		<i>Skdh</i>		<i>Me</i>		<i>Rbc</i>		<i>Diap1</i>		<i>Mdh1</i>	
	S	T	<i>b4</i>	<i>b5</i>	<i>b6</i>	<i>b7</i>	<i>b12</i>	<i>b13</i>	<i>103</i>	<i>100</i>	<i>100</i>	<i>98</i>	<i>100</i>	<i>98</i>	<i>100</i>	<i>95</i>	<i>103</i>	<i>100</i>
F ₃	0.51	0.49	0.01	0.99	0.44	0.56	0.49	0.51	0.48	0.52	0.39	0.61	0.708	0.29	0.26	0.73	0.75	0.25
F ₄	0.51	0.49	0	1	0.51	0.59	0.92	0.08	0.67	0.33	0.67	0.33	0.44	0.56	0.11	0.89	0.89	0.11
F ₅	0.50	0.50			0.72	0.28	0.74	0.26	0.76	0.24	0.44	0.56	0.44	0.56	0.03	0.97	0.97	0.03
F ₆	0.50	0.50			0.67	0.33	1	0	1	0	0.30	0.70	1	0	0	1	1	0
F ₇	0.67	0.33			0.42	0.58					0.21	0.79						
PHA-159-10/PHA-269-12																		
	Pha		<i>b4/b5</i>		<i>b6/b7</i>		<i>b12/b13</i>		<i>Skdh</i>		<i>Me</i>		<i>Rbc</i>		<i>Diap1</i>			
	S	T	<i>b4</i>	<i>b5</i>	<i>b6</i>	<i>b7</i>	<i>b12</i>	<i>b13</i>	<i>103</i>	<i>100</i>	<i>102</i>	<i>98</i>	<i>100</i>	<i>98</i>	<i>100</i>	<i>95</i>		
F ₃	0.50	0.50	0.20	0.80	0.10	0.90	0.90	0.10	0.40	0.60	0.70	0.30	0.70	0.30	0.21	0.79		
F ₄	0.55	0.45	0.39	0.61	0.35	0.65	0.78	0.22	0.57	0.43	0.88	0.22	0.51	0.49	0.09	0.91		
F ₅	0.73	0.27	0.05	0.95	0.05	0.95	0.94	0.06	0.54	0.46	0.81	0.27	0.68	0.42	0	1		
F ₇	1	0	0	1	0	1	1	0	0.77	0.23	1	0	0.89	0.11				
PHA-159-08/PHA-269-13																		
	Pha		<i>b4/b5</i>		<i>b6/b7</i>		<i>b12/b13</i>		<i>Skdh</i>		<i>Me</i>		<i>Rbc</i>		<i>Diap1</i>		<i>Mdh1</i>	
	S	T	<i>b4</i>	<i>b5</i>	<i>b6</i>	<i>b7</i>	<i>b12</i>	<i>b13</i>	<i>103</i>	<i>100</i>	<i>102</i>	<i>98</i>	<i>100</i>	<i>98</i>	<i>100</i>	<i>95</i>	<i>103</i>	<i>100</i>
F ₅	0.55	0.45	0.55	0.45	0.05	0.95	0.05	0.95	0.65	0.35	0.46	0.54	0.50	0.50	0.69	0.31	0.85	0.15
F ₃	0.60	0.40	0.71	0.29	0.06	0.94	0.06	0.94	0.46	0.53	0.25	0.75	0.48	0.52	0.60	0.40	0.34	0.66
F ₄	0.69	0.31	0.34	0.66	0.05	0.95	0.16	0.84	0.48	0.52	0.42	0.58	0.66	0.34	0.23	0.77	0.16	0.84
F ₇	0.50	0.50	1	0	0	1	0.08	0.82	0.29	0.71	0.18	0.82	1	0	0	1	0	1