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Assessment of Inter Simple Sequence Repeat Markers to Differentiate Sympatric Wild and Domesticated Populations of Common Bean

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ABSTRACT

Efficient assessment of genetic diversity is important for conservation and utilization of genetic resources. We sought to assess the ability of inter simple sequence repeats (ISSRs) as molecular markers to identify genetic diversity both within and among sympatric populations of common bean, *Phaseolus vulgaris* L. One wild and four domesticated populations originating from the Sierra Norte de Puebla, Mexico, were chosen because they were growing in the same region or field. The ISSR diversity was assessed using four primers that revealed a higher number of strong, polymorphic bands in preliminary analyses. Fifty of these bands could be mapped onto the core linkage map in population BAT93 × Jalo EEP558, whereas four were unlinked. The mapped bands were distributed over nine of the 11 linkage groups, suggesting that they were broadly distributed in the genome. On the basis of a sample of 50 intense bands in these five populations, ISSRs were able to clearly distinguish all populations. Most of the variation was distributed among populations rather than within populations, consistent with the predominantly selfing nature of the species. Differentiation among domesticated populations was much higher ($F_{ST} \approx$ 0.49–0.85) than between the wild and domesticated gene pools (F_{ST} = 0.05). Within each population, most loci had achieved near-fixation. Around 7% of individuals showed a lack of correlation between seed type and ISSR fingerprint. Furthermore, each population contained individuals with unusual markers but present in the other populations (frequency < 5 or 10%). Two nonmutually exclusive explanations were discussed—incomplete lineage sorting and introgression—to account for the presence of these unusual individuals. Overall, ISSR markers were very useful to differentiate closely related populations. Further research is necessary to quantify the actual level of outcrossing.

EFFICIENT MANAGEMENT of genetic resources, whether for conservation or for utilization in plant breeding programs, requires accurate and fast assessment of levels of genetic diversity and degrees of genetic relatedness. In the last decade, increasing use has been made of molecular markers based on DNA analyses (Bretting and Widrlechner, 1995; Gepts, 1995). An enduring concern with these markers is that they are fairly laborious and costly. Hence, there has been a trend to markers that, while highly polymorphic, are relatively easy and fast to analyze. In common bean, these include randomly amplified polymorphic DNA (RAPD) (e.g., Freyre et al., 1996) and amplified fragment length polymorphisms (AFLPs) (Tohme et al., 1996; Papa and Gepts, 2003). The ISSR marker technique involves polymerase chain reaction (PCR) amplification of DNA using a single primer composed of a microsatellite sequence, such as $(GACA)_4$, anchored at the 3′ or 5′ end by two to four arbitrary, often degenerate, nucleotides (Zietkiewicz et al., 1994). The ISSRs have proven to be a rapid, simple, and inexpensive way to assess genetic diversity (Kantety et al., 1995; Tsumura et al., 1996; Esselman et al., 1999; Gilbert et al., 1999; Sarla et al., 2003; Tanyolac, 2003; Jin et al., 2003), to identify closely related cultivars (Fang and Roose, 1997; Fang et al., 1997, 1998; Eiadthong et al., 1999; Prevost and Wilkinson, 1999; Martins et al., 2003), and to study evolutionary processes, such as reproductive systems (Liston et al., 2003) and gene flow (Wolfe et al., 1998). A recent paper by Galván et al. (2003) describes differences between 10 Argentinean and three French bean cultivars, in particular broad differences between the Andean and Mesoamerican gene pools.

In the current research, the level of genetic diversity and differentiation was determined among one wild and four domesticated sympatric populations of common bean (*Phaseolus vulgaris*; $2n = 2x = 22$) from the Sierra Norte de Puebla. Most studies of genetic diversity in common bean have been conducted in samples representing broad geographical ranges to identify major subdivisions in its germplasm, such as gene pools and races (Singh et al., 1991; Gepts, 1998). Fewer studies have been conducted to determine genetic relationships on smaller geographic levels (Cattan-Toupance et al., 1998; Papa and Gepts, 2003). These studies are useful because they provide information on evolutionary factors that can shape genetic diversity in common bean populations, such as gene flow. For example, Cattan-Toupance et al. (1998) showed the importance of a disease pressure in determining local population differentiation, in addition to genetic drift. They also considered partial outbreeding to be a potential factor affecting association of traits. Papa and Gepts (2003) showed that, in sympatry, gene flow from domesticated to wild types is three times more important than in the opposite direction. Furthermore, spatial autocorrelation studies showed that wild beans exhibited a stronger local population structure than domesticated beans.

In addition to studying the level of genetic diversity among sympatric populations of common bean, we sought to determine the usefulness of ISSR markers in common

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Abbreviations: AFLP, amplified fragment length polymorphism; ISSR, inter simple sequence repeat; PCR, polymerase chain reaction; RAPD, randomly amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; SAHN, sequential, agglomerative, hierarchical, and nested.

Table 1. Levels of polymorphism in wild and domesticated populations of common bean from the Sierra Norte de Puebla, Mexico.

		Number of polymorphic ISSR† fragments						
Population	No. of individuals	A (GACA)$_3$RT (12)‡	B YR(GACA)$_3$ (12)	C (GACAC)$_2$ (9)	D (ACTG)$_3$RG (17)	Total (50)	Nei's gene diversity	No. of haplotypes
Beige	51	6	4	1	4	15	0.02	14§
Negro Brillante	85	8	7	6	15	36	0.10	46¶
Negro Opaco	92	8	8	7	11	34	0.06	28¶
Rojo	24	9	5	5	10	29	0.10	10§
Domesticated#	252	12	9	7	15	43	0.19	95
Wild	77	10	8	6	16	40	0.20	75
All††	329	12	10	8	16	46	0.29	170

† ISSR, inter simple sequence repeat.
‡ ISSR primer (see Materials and Methods) and total number of fragments in parenthesis.
§ 1 haplotype shared between Beige and Rojo.
¶ 2 haplotypes shared between Negro Brillante and Negro Opaco.
# Beige + Negro Brillante + Negro Opaco + Rojo − 3 shared haplotypes.
†† Domesticated + wild.

bean with respect to their reproducibility, variation in DNA amplification among primers, relative linkage map location, and levels of polymorphism detected. The ISSRs were able to distinguish sympatric bean populations but also identify within-population polymorphism.

MATERIALS AND METHODS

Plant Materials

Seeds of a wild population of *P. vulgaris* were collected in early winter of 1995 by Francisco Basurto (FB1934) in the municipalities of Xochitlán de Romero Rubio and Huapalejcan in the Sierra Norte de Puebla (state of Puebla, Mexico). This wild population grows within bean fields, but surprisingly not outside fields. Seeds of four commonly grown landraces—labeled according to their seed color: Beige (B), Negro Brillante (NB, shiny black), Negro Opaco (NO, dull black), and Rojo (R, red)—were collected from local, indigenous farmers from the same area. As far as we can tell, these are not bred materials. All landraces were indeterminate strong climbers, classified as Type IV (Singh, 1982).

DNA Extraction and ISSR Analysis

A total of 615 seeds (Table 1) were planted in a greenhouse at Davis. These seeds included 79, 156, 138, 34, and 208 individuals for the Beige, Negro Brillante, Negro Opaco, Rojo, and wild populations, respectively. DNA was extracted using the CTAB method as described by Doyle and Doyle (1987). Inter simple sequence repeats were analyzed using a total of 32 primers synthesized by the Protein Sequencing Laboratory of the University of California, Davis. From the 30 primers tested originally, four primers were selected that produced a high level of polymorphism among both landraces and wild populations (Table 1). Two of these primers, (GACA)$_3$ RT and (GACAC)$_2$, were run on the entire sample, whereas two additional primers, YR (GACA)$_3$ and (ACTG)$_3$ RG, were only run on a randomly chosen subset of 417 individuals, which included 61, 100, 117, 32, and 107 individuals of the Beige, Negro Brillante, Negro Opaco, Rojo, and wild populations, respectively. Each 20-μL amplification reaction consisted of 10 m*M* Tris-HCl (pH 9.0), 50 m*M* KCl, 0.1% Triton X-100, 2 m*M* $MgCl_2$, 200 μ*M* of each deoxyribonucleotide phosphate, 1 μ*M* of primer, 1 unit of *Taq* polymerase (Promega, Madison, WI), and 20 ng of template DNA. Each reaction mixture was overlaid with mineral oil. Amplification was performed in a 96-well Ericomp thermal cycler (Ericomp, Inc., San Diego, CA) under the following conditions: 4 min at 94°C for 1 cycle, followed by 2 min at 94°C, 1 min at 42°C, and 2 min at 70°C for 35 cycles, and 5 min at 72°C for final extension. Amplification products were separated on 320 by 380 by 0.4 mm of 6% nondenaturing 30:1 bis-acrylamide gels containing 3 *M* urea and 1 × tris-borate buffer (Zietkiewicz et al., 1994). A 123-bp molecular marker standard was included in each gel. Gels were run for 12 h at 300 V, and PCR products were detected by silver staining (Bassam et al., 1991) (Fig. 1). Each gel was loaded with PCR products of at least eight individuals of each accession to be able to compare the amplification pattern among accessions within the same gel. The PCR fragments generated by the ISSR primers were labeled with a code consisting of a description of the primer used for the reaction followed by the molecular size of the fragment in base pairs [i.e., (ACTG)$_3$ RG.434: fragment size of 434 bp].

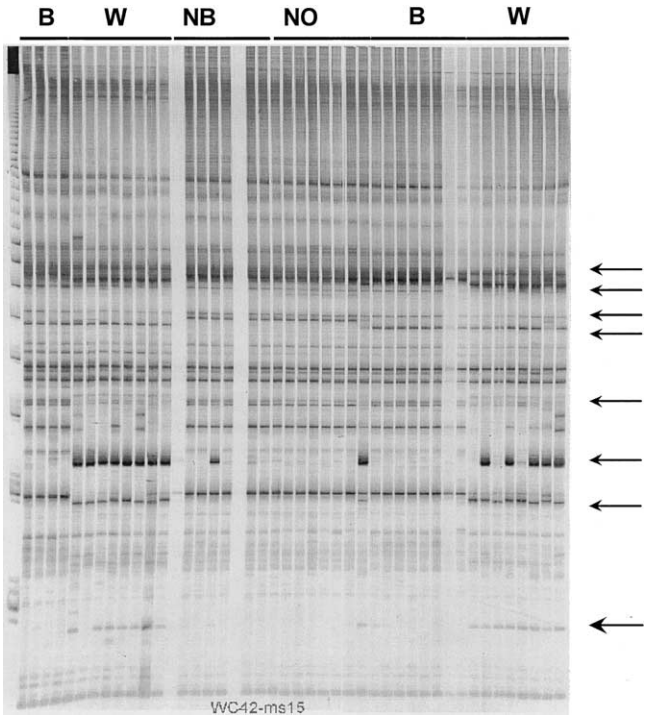

Fig. 1. Inter simple sequence repeat electrophoretic assessment of genetic diversity in common bean. A gel is shown in which several individuals showed one or more fragments (arrowheads on the right side of the gel) that were atypical for the populations (B, Beige; NB, Negro Brillante; NO, Negro Opaco; W, wild) after amplification using primer (GACA)$_3$RT.

Linkage Mapping

The same four ISSRs primers used to generate fingerprinting in the five populations (Table 1) were used to generate PCR-amplified DNA fragments in 75 lines of the BAT93 × Jalo EEP556 recombinant inbred population, which had reached the F_8 generation. This population has been used previously to develop a RFLP genetic linkage map (Nodari et al., 1993) and to develop a core linkage map for the common bean genome (Freyre et al., 1998). Segregation was scored for presence or absence of the ISSRs bands. Segregation of polymorphic fragments was analyzed by a chi-square test for goodness-of-fit to a 1:1 ratio. Linkage analysis of the polymorphic set of markers was done using Mapmaker 3.0b (Lander et al., 1987) as described in Menéndez et al. (1997) (Fig. 2). Briefly, markers already previously established in the common bean map (Freyre et al., 1998) were used to anchor linkage groups. The *Assign* command was then used to allocate ISSRs markers into linkage groups. To establish the most likely order of markers within each linkage group, the command *Order* was applied under the following conditions: (i) LOD score above or equal to 3.00, and (ii) a maximum distance of 30 cM. Markers not placed under this limit were placed using lower LOD scores (between 3 and 2).

Analyses of Genetic Diversity

The PCR products amplified using the ISSR primers were scored as present (1) or absent (0) for the five populations. Analyses were initially conducted on individuals with results from four or two primers. Because results were similar between the two groups, only the results based on individuals with complete data sets (four primers) are presented here. Of a total of 20 850 data points (417 individuals × 50 markers), 1079 (5%) were considered as missing data. Individuals with such missing data were excluded without effect on the results (data not shown). Thus, our final sample contained 329 individuals, including 51, 85, 92, 24, and 77 individuals from the Beige, Negro Brillante, Negro Opaco, Rojo, and wild populations, respectively. When considering all 50 fragments together, 170 multilocus combinations (haplotypes) could be identified. Unless otherwise mentioned, standard indices of genetic diversity were calculated using the Arlequin (Schneider et al., 2000) or the POPGENE (Yeh et al., 1997) softwares. Indices of genetic similarity were calculated using Dice's coefficient (Dice, 1945) to estimate the relationship between accessions and to observe the placement of putative outcrossed individuals. From the similarity matrix, a sequential, agglomerative, hierarchical, and nested (SAHN) cluster analysis was performed using the unweighted pair group method with arithmetic means (UPGMA) algorithm computed using NTSYS-pc (Rohlf, 1997). A dendrogram was generated using NTSYS-pc (Rohlf, 1997) to show the genetic relationships and distances of each accession (Fig. 3).

RESULTS

Development and Mapping of ISSR Markers in Common Bean

The pattern of DNA amplification using ISSR primers was very reproducible based on results from at least 20 different DNA extractions and PCR reactions from each landrace, which produced reproducible banding patterns (Fig. 1). All primers tested amplified some bands in common bean except primers $(AT)_8YG$ and $(AT)_8RG$, with R = G, A and Y = C, T (data not shown). The four ISSR primers selected amplified 50 bands, which were strongly amplified and well-stained across gels. The bands ranged in size from 250 to 1300 bp. To examine the distribution of these ISSR markers in the bean genome, a mapping study was conducted in the BAT93 × Jalo EEP558 recombinant inbred population, the core mapping population in common bean (Freyre et al., 1998). The core map established in this population includes some 560 markers, including 120 RFLPs and 430 RAPDs. The four ISSR primers used in the gene flow studies amplified 54 polymorphic fragments in this population. The segregation data of these fragments were added to those of existing framework markers to determine their location on the map. The Chi-square test (1:1 expectation, $P = 0.99$) indicated that nine markers (16.6%) showed a segregation distortion that was significantly different from the expected 1:1 ratio at the 1% level. Fifty of the amplified bands mapped onto nine of the 11 linkage groups of the common bean linkage map, indicating a broad coverage of the genome by the ISSRs (Fig. 2). None of the ISSRs markers scored mapped on linkage groups 4 or 9 (Fig. 2) and four bands were unlinked. The mapped ISSRs markers were distributed mainly on linkage group 7 (11 markers), 10 (9 markers), and 11 (9 markers). Further inspection of Fig. 2 suggests some degree of clustering of these markers on individual linkage groups.

Polymorphisms in *Phaseolus vulgaris* of the Sierra Norte de Puebla

Ninety-two (46/50) percent of the analyzed markers displayed variation in this study (Tables 1 and 2). Furthermore, the five populations analyzed in this study showed contrasting levels of polymorphism. The wild population was the most diverse, whether measured by the number of polymorphic fragments, Nei's (1973) gene diversity, or the number of haplotypes (Table 1). As a whole, the domesticated group ($n = 252$) had higher or similar levels of polymorphism compared with the sample of the wild population ($n = 77$) (Table 1). None of the regressions between population size (independent variable) and measures of genetic diversity as dependent variables (number of polymorphic bands, Nei's gene diversity, and number of haplotypes) were statistically significant at the $P = 0.05$. Thus, differences in genetic diversity among populations were not due to differences in population sizes.

Genetic Distances and Differentiation

The ISSR markers clearly distinguished the wild from the domesticated populations included in this study (Fig. 3). Among the 50 markers used for the final analysis, 22% showed highly contrasting frequencies in the wild and domesticated gene pools: A12, A13, A40, B23, B26, B27, C6, C18, D9, D28, and D33. Frequencies of these markers in one gene pool were below 20% and above 80% in the other gene pool (Table 2). Fourteen percent were monomorphic (frequencies ≥ 0.95) among these two groups (i.e., markers A24 [$(GACA)_3$RG.703], A29 [$(GACA)_3$RG.616], B17 [YR$(GACA)_3$], C8 [$(GACAC)_2$.887], C10 [$(GACAC)_2$.821], C12 [$(GACAC)_2$.727], and

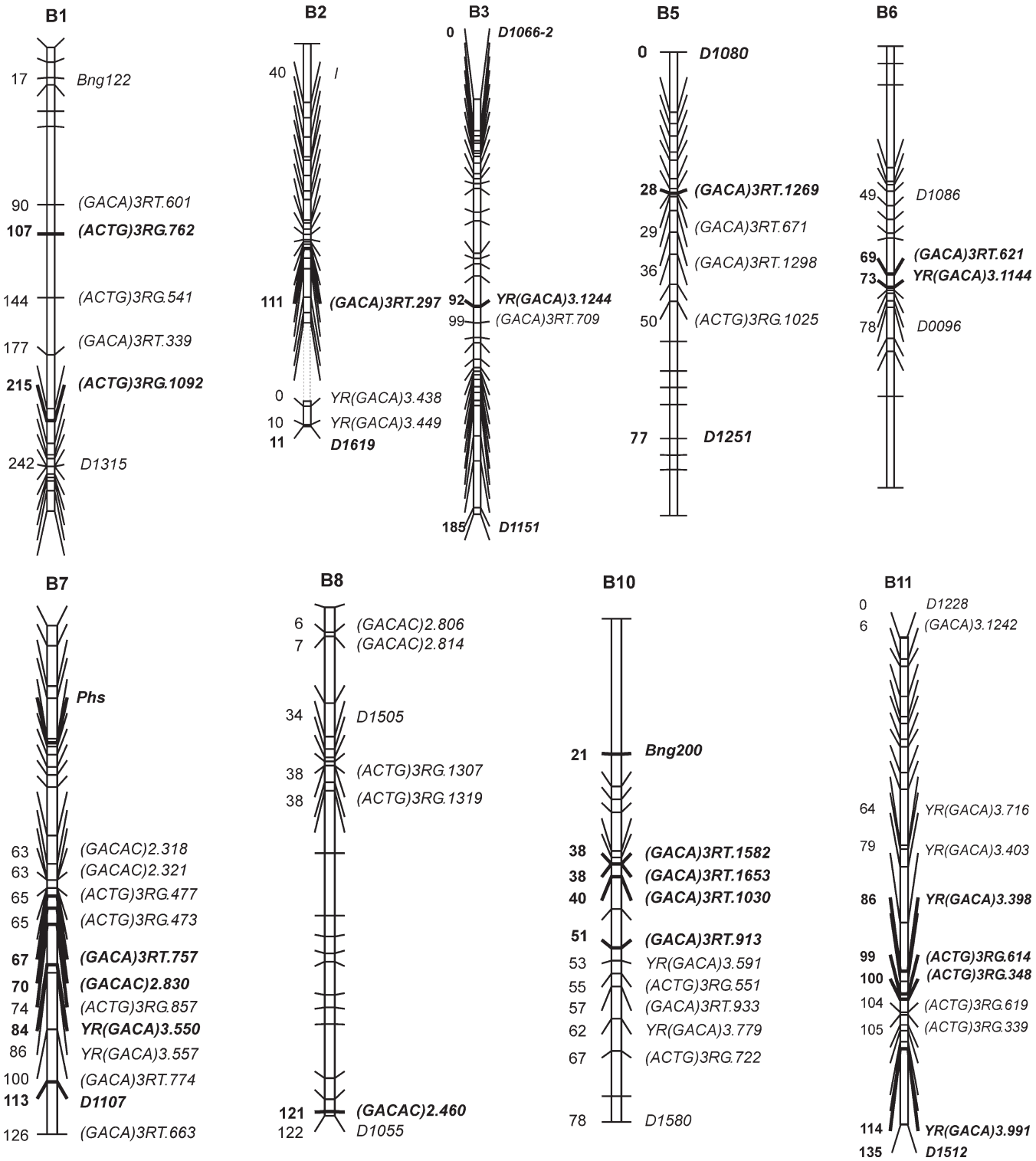

Fig. 2. Distribution of inter simple sequence repeat (ISSR) markers among linkage groups of the recombinant inbred population BAT93 × Jalo EEP558. No ISSRs mapped to linkage groups B4 and B9. Only ISSR loci are shown, in addition to two previously mapped markers (Freyre et al., 1998) on each linkage group to orient them with respect to other common bean maps. The ISSR locus names include the primer sequence followed after the period by the size of the scored band in bp. Markers in bold were mapped with a log-likelihood ratio above 3, those in normal type with a log-likelihood ratio below 3. Distances to the left of each linkage group are expressed in Kosambi cM. Linkage groups were not drawn to scale.

D5 [$(ACTG)_3$RG.1013] in Table 2). The remaining percentage consisted of bands with various frequency patterns in the wild and domesticated components (Table 2). In addition, ISSR markers were also able to clearly distinguish the four domesticated populations, including the Negro Opaco and Negro Brillante populations, in spite

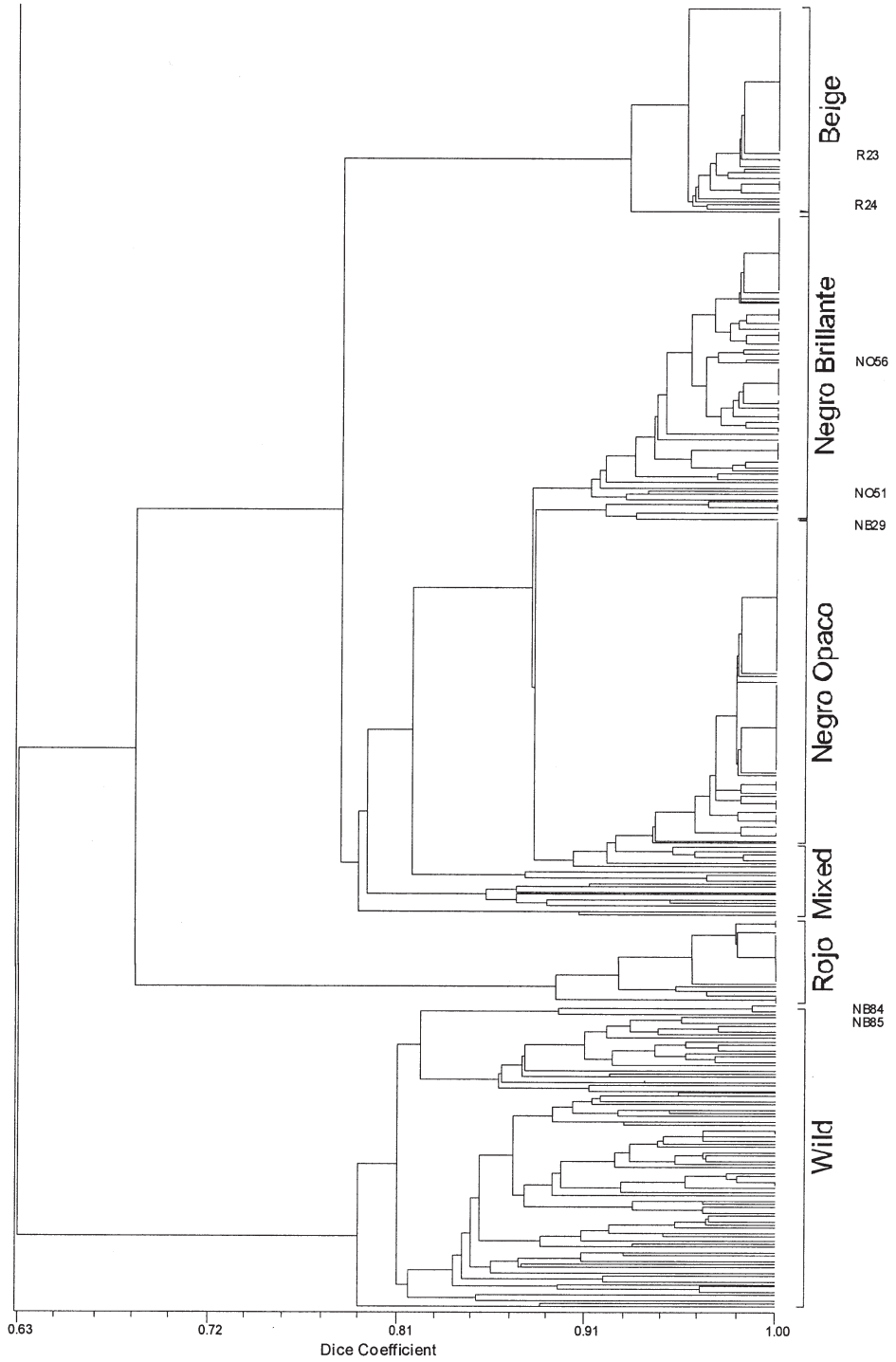

Fig. 3. Dendrogram generated by unweighted pair group method with arithmetic means based on Dice (1945) coefficients. The clusters correspond to the populations, indicated at the right of the dendrogram. The mixed cluster includes individuals from the Negro Brillante (NB), Negro Opaco (NO), and wild (W) populations (see text for composition). Individuals listed at the right constitute exceptions to the clustering into the Beige, Negro Brillante, Negro Opaca, Rojo, and wild populations. All 170 haplotypes are shown in this figure, although some haplotypes were observed in more than one individual.

of the phenotypic similarities of the latter (Fig. 3). Although the main difference between the two black-seeded populations—the shininess of the seed—is controlled by a single gene (*Asp*; Beninger et al., 2000), there were four markers that were able to differentiate these two populations: D12 [$(ACTG)_3$RG.652], D25 [$(ACTG)_3$RG.485], D27 [$(ACTG)_3$RG.434], and D29 [$(ACTG)_3$RG.415]. A more heterogeneous fingerprinting pattern was observed in the wild accessions, but nevertheless, some bands were found that had a high frequency in one group of accessions (either wild or domesticated) and were present at a much lower frequency in the other group (Table 2).

All ISSRs markers were present at some frequency in the wild and the two black-seeded landraces (Table 2).

Table 2. Frequency of inter simple sequence repeat markers within the different populations.†

Fragment no.	Marker	B	NB	NO	R	W
A11	$(GACA)_3$ RG.1000‡	0.98	1.00	1.00	0.18	0.95
A12	$(GACA)_3$ RG.957	0.98	0.96	0.98	0.00	0.13
A13	$(GACA)_3$ RG.936	0.02	0.05	0.06	1.00	0.98
A16	$(GACA)_3$ RG.896	1.00	0.92	0.96	1.00	0.96
A18	$(GACA)_3$ RG.820	1.00	1.00	1.00	0.00	1.00
A19	$(GACA)_3$ RG.802	0.03	1.00	0.96	1.00	0.15
A21	$(GACA)_3$ RG.785	1.00	0.08	0.12	0.86	0.93
A24	$(GACA)_3$ RG.703	1.00	1.00	1.00	1.00	1.00
A26	$(GACA)_3$ RG.658	1.00	1.00	1.00	0.14	1.00
A29	$(GACA)_3$ RG.616	1.00	1.00	1.00	1.00	1.00
A37	$(GACA)_3$ RG.500	1.00	0.92	1.00	1.00	0.28
A40	$(GACA)_3$ RG.429	0.03	0.11	0.06	0.11	0.90
A43	$(GACA)_3$ RG.359	0.02	0.21	0.33	0.18	0.73
A44	$(GACA)_3$ RG.355	0.00	0.05	0.09	0.04	0.34
B15	YR $(GACA)_3$.873	0.88	0.09	0.04	0.27	0.49
B16	YR $(GACA)_3$.856	1.00	1.00	1.00	0.88	0.99
B17	YR $(GACA)_3$.759	1.00	1.00	1.00	1.00	0.99
B19	YR $(GACA)_3$.715	0.00	0.06	0.10	0.00	0.62
B20	YR $(GACA)_3$.680	0.00	0.91	0.97	0.04	0.07
B23	YR $(GACA)_3$.634	0.00	0.03	0.03	0.04	0.79
B25	YR $(GACA)_3$.591	0.98	0.68	0.05	1.00	0.04
B26	YR $(GACA)_3$.568	1.00	1.00	1.00	1.00	0.18
B27	YR $(GACA)_3$.551	0.00	0.52	0.04	0.04	0.99
B31	YR $(GACA)_3$.438	0.98	1.00	0.99	0.12	0.38
B33	YR $(GACA)_3$.253	0.05	0.97	0.99	0.96	0.98
C6	$(GACAC)_2$.963	1.00	1.00	0.95	0.00	0.09
C7	$(GACAC)_2$.947	0.00	0.02	0.06	1.00	0.48
C8	$(GACAC)_2$.887	1.00	1.00	1.00	1.00	0.95
C10	$(GACAC)_2$.821	1.00	0.99	0.98	1.00	1.00
C11	$(GACAC)_2$.743	0.00	0.08	0.01	1.00	0.04
C12	$(GACAC)_2$.727	1.00	1.00	1.00	1.00	1.00
C14	$(GACAC)_2$.623	0.00	0.03	0.02	1.00	0.12
C18	$(GACAC)_2$.479	0.02	0.10	0.04	0.00	0.99
C20	$(GACAC)_2$.424	0.00	0.98	0.97	0.00	0.30
D1	$(ACTG)_3$ RG.1208	1.00	0.02	0.05	0.00	0.87
D2	$(ACTG)_3$ RG.1181	0.02	0.99	0.99	1.00	0.32
D5	$(ACTG)_3$ RG.1013	1.00	1.00	1.00	1.00	0.96
D9	$(ACTG)_3$ RG.795	0.15	0.08	0.10	0.00	0.92
D12	$(ACTG)_3$ RG.652	1.00	0.97	0.09	0.00	0.36
D14	$(ACTG)_3$ RG.638	0.00	0.04	0.03	0.10	0.36
D16	$(ACTG)_3$ RG.635	0.97	0.98	1.00	0.80	0.94
D18	$(ACTG)_3$ RG.621	1.00	0.99	1.00	1.00	0.60
D19	$(ACTG)_3$ RG.604	1.00	0.99	1.00	1.00	0.79
D25	$(ACTG)_3$ RG.485	0.00	0.27	0.95	1.00	0.04
D26	$(ACTG)_3$ RG.479	1.00	0.89	0.11	0.03	0.98
D27	$(ACTG)_3$ RG.434	1.00	0.90	0.05	0.00	0.03
D28	$(ACTG)_3$ RG.420	0.02	0.07	0.04	0.00	0.85
D29	$(ACTG)_3$ RG.415	0.03	0.16	0.89	1.00	0.10
D32	$(ACTG)_3$ RG.345	1.00	0.99	1.00	0.97	0.47
D33	$(ACTG)_3$ RG.333	0.00	0.04	0.04	0.07	0.85

† B = Beige, NB = Negro Brillante, NO = Negro Opaco, R = Rojo, W = wild.

‡ The fragment name consists of the primer sequence followed by the amplified fragment size expressed in bp.

The beige and red landraces did not have unique markers associated with them, but were missing some of the bands observed in the other three accessions. Within each population, markers were generally almost completely fixed. Their frequency was either close to one or zero, especially in the domesticated populations, reflecting the codominant nature of ISSRs (Fig. 4). Among domesticated populations, most of the variation was distributed among and not within populations as shown by a G_{ST} value (Nei, 1973) of 0.70 (H_T = 0.23; H_S = 0.07). Adding the wild population increased total genetic diversity but did not change the distribution of genetic diversity markedly (G_{ST} = 0.67; H_T = 0.30; H_S = 0.10). These results are consistent with the predominantly selfing nature of the species, as further confirmed by the low values for *Nm*, the average number of migrants per generation (around 0.24). The *Nm* values of 1 are generally considered to be the threshold above which migration will counteract population differentiation (Neigel, 1997). The wild population, on one hand, and all the domesticated populations, considered as a single group, showed low differentiation (F_{ST} = 0.06) and, concurrently, a high *Nm* value of 8.47. In contrast, among the different domesticated populations, a much higher differentiation was observed, ranging from F_{ST} = 0.49 between the two black-seeded populations to $F_{ST} \approx 0.85$ among the Beige, Negro Opaco, and Rojo populations (Table 3).

A UPGMA dendrogram resulting from a SAHN cluster analysis based on the Dice similarity index is depicted in Fig. 3. It includes 329 individuals that were evaluated with the four ISSR primers and had a complete data set. The phenogram contains six clusters, while only five populations were included in this study. All the beige individuals (n = 51) grouped in a single cluster, which also included two red-seeded individuals (R23 and R24). With the exception of the latter two individuals, all the red-seeded individuals grouped in a single cluster, which did not contain any individuals from the other populations. Most of the individuals of the wild population grouped in a single cluster that included also two individuals from the Negro Brillante population (NB84 and NB85). The Negro Brillante cluster contained two Negro Opaco individuals (NO51 and NO56) and the Negro Opaco cluster included one Negro Brillante individual (NB29). The mixed cluster contained seven Negro Brillante (NB24, 49, 63, 64, 73, 81, 83), nine Negro Opaco (NO16, 58, 64, 66, 67, 82, 83, 85, 92), and two wild individuals (W62, 66). Thus, there were 25/339 (8%) individuals for which the seed phenotypes did not match the cluster membership based on a UPGMA analysis of molecular data.

DISCUSSION

Inter simple sequence repeats have been used to explore genome structure, to assess genetic diversity within germplasm collections as well as to determine the frequency of simple sequence repeats in several species (Blair et al., 1999; Gilbert et al., 1999; Prevost and Wilkinson, 1999). The ISSRs have proven to be a reliable, easy to generate, and versatile set of markers that do not require previous knowledge of the genome sequence to generate DNA markers, unlike SSRs (Zietkiewicz et al., 1994; Gupta et al., 1994). The flexibility to design primers containing a di-, tri-, or tetra-nucleotide repetitive motif anchored by one or more nucleotides at the 3′ or 5′ end make them ideal to explore the genome of any species, including those without previous knowledge of DNA sequence. Several reports have compared the level of polymorphism detected using RFLPs, RAPDs, and ISSRs (Nagaoka and Ogihara, 1997), and more recently ISSRs and AFLPs. The consensus is that ISSRs are very powerful to detect polymorphism, scan the whole genome, are inexpensive, and easy to generate. They can detect even more polymorphism than RFLPs (Kantety et al., 1995) and more than AFLPs in rice (Blair et al., 1999). However, it is now becoming clear that the poly-

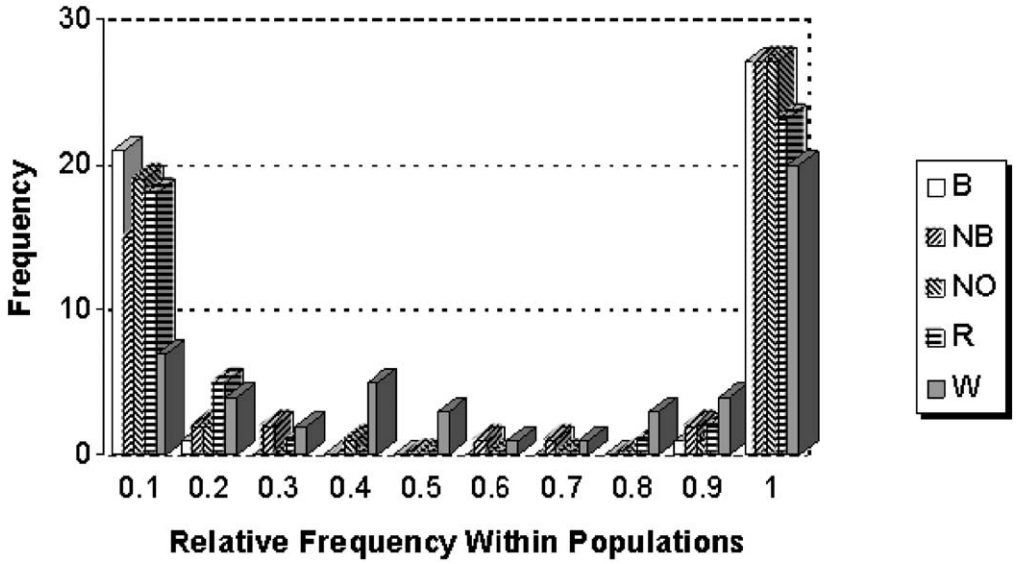

Fig. 4. Frequency distribution of markers within each of the five populations analyzed (B, Beige; NB, Negro Brillante; NO, Negro Opaco; R, Rojo; W, wild). The bimodal nature of the distributions reflects the codominant nature of the inter simple sequence repeat markers.

morphism detected by ISSRs is dependent on the plant species being investigated as well as the type of simple sequence repeat incorporated in the ISSRs primer used to amplify PCR products. Our results confirm these earlier observations. Several characteristics make ISSRs also a very useful marker in common bean. They are able to distinguish among closely related common bean populations. The ISSR primers amplify fragments that are dispersed throughout the genome as demonstrated by mapping ISSR markers in the F_8 generation of a recombinant inbred population. The ISSR markers were able to detect individual resulting from potential introgression among wild and domesticated populations (although independent evidence is necessary for confirmation, see below).

Microsatellite fingerprinting in the genus *Phaseolus* was investigated by Hamann et al. (1995), who concluded that the repetitive element $(GATA)_4$ and, to a lesser extent, $(GACA)_4$ were well represented in *P. vulgaris* and *P. lunatus*. They also concluded that that the dinucleotide motif $(CA)_8$ seemed to be less represented in the of *P. vulgaris*. On this basis, several of our primers contained $(GACA)_3$ and $(GATA)_3$ as the repeat sequence. Among the five accessions tested in this study, we found that the primers containing $(GACA)_3$ as the repetitive motif produced better amplification and higher polymorphism than those containing $(GATA)_3$. Although a nonexhaustive effort was attempted to optimize and characterize all the primers designed for amplifying ISSRs, all the primers tested amplified fragments in beans, indicating the potential of this technique to measure genetic variability in bean germplasm collections. The combination of the four ISSR primers was sufficient to distinguish all the populations included in this study. Generation of ISSR bands was based on four primers, two of which included $(GACA)_4$ as the repeat sequence anchored either at the 3′ or 5′ end (Table 1). The double pentanucleotide motif $(GACAC)_2$ was not anchored but produced a very reliable and useful fingerprinting that differentiated all the populations included in this study. Coverage of the whole genome by ISSRs has been claimed previously by others (Blair et al., 1999; Fang and Roose, 1999). Nevertheless, ISSRs were difficult to map in einkorn wheat (Kojima et al., 1998). In our mapping, we found that a sample of some 50 markers mapped to 9 of the 11 linkage groups of the existing core genetic map of bean (Freyre et al., 1998). Although a tendency for clustering was observed among some of the markers (Fig. 2), this has been also observed in some attempts to map AFLPs markers in beans (R. Papa and P. Gepts, 2004, unpublished results).

Table 3. Genetic differentiation (F_{ST}) and number of migrants (*Nm*) among populations included in this study.†

Populations	Beige	Negro Brillante	Negro Opaco	Rojo	Wild
Beige		0.24	0.10	0.08	0.22
Negro Brillante	0.68		0.52	0.19	0.25
Negro Opaco	0.83	0.49		0.17	0.19
Rojo	0.86	0.72	0.75		0.27
Wild	0.69	0.67	0.73	0.65	

† Above the diagonal: *Nm*; below the diagonal: F_{ST}.

There was a strong association between molecular haplotypes and seed color as 304/329 (92%) of the individuals clustered with individuals showing the same seed color (Fig. 3). Conversely, 25/329 (8%) of the individuals grouped with individuals with seeds of a different color. A strong association is expected in predominantly selfpollinated species (Hedrick et al., 1978). This reproductive system leads to multilocus associations, whether the loci involved are linked or not. Our results show that ISSR markers in common bean are distributed over the entire bean genome, although individual markers can be linked (Fig. 2). Seed color and color pattern genes are also distributed throughout the bean genome (McClean et al., 2002). Thus, in the absence of outcrossing and the ensuing recombination, multilocus associations between seed color and ISSR markers can be expected.

Given this expectation, what is the origin of the individuals for which the association broke down? We sug-

gest that there are two possible reasons. First, these individuals could represent incomplete lineage sorting, the latter being defined as the progressive loss of polymorphisms in related lineages of populations eventually leading to the absence of shared polymorphisms. In the transitional period between the separation of the populations from their common ancestor and complete lineage sorting, certain polymorphisms will remain shared unless they arose after the separation such as the seed color alleles. The landraces included in this study are probably not derived directly from the sympatric wild population as the Mesoamerican bean domestication has been traced genetically to the west-central part of Mexico (Jalisco and Guanajuato; Gepts, 1988). The archaeological record of common bean in Mesoamerica is currently not older than 2300 yr (Kaplan and Lynch, 1999). Thus, on an evolutionary time scale, the separation between wild and domesticated beans and among the different landraces may have been too short to achieve full lineage sorting.

An alternative explanation is that the unusual individuals actually result from hybridization one or more generations before the observations reported in this study. On the basis of the dendrogram, the extent of introgression would reach about 8%. However, dendrograms may underestimate the frequency of hybrids because separate clusters only appear when a sufficient number of markers show significant differences in frequency. For example, the two most closely related populations (Negro Opaco and Negro Brillante; Table 3) could be distinguished by six markers (B25, B27, D12, D26, D27, and D29) with a frequency difference of at least 0.48 (Table 2). Introgression, however, may affect only a minor portion of the genome, which would only be detected with a small number ($\geq$1) of markers. These would not necessarily be detected in a dendrogram. A different way of analyzing this issue is to consider individual markers, especially those occurring at low frequency ($<$0.10 or $<$0.05) in the different populations, but occur at a higher frequency in the other populations analyzed (for examples, see Fig. 4). Individuals exhibiting one or more of these markers could have resulted from introgression (although incomplete lineage sorting cannot be excluded, as pointed out earlier). At the more stringent level (5%), some 20 to 25% of the individuals carried one or more loci with low-frequency markers. For the less stringent criterion of 10%, the frequency ranged between 23% for the Beige population to 58% for the wild population (data not shown). Overall, these estimates of potentially outcrossed or introgressed individuals based on individual loci were therefore higher that the estimates based on the entire set of loci, obtained either statistically or graphically.

Common bean is considered a self-pollinated species, but several lines of evidence suggest that outcrossing may be occasionally important (Wells et al., 1988). Ibarra-Pérez et al. (1997) recently summarized the literature reporting on experiments conducted to directly measure the level of outcrossing. Although the outcrossing rates were generally below 5%, some studies reported higher levels, reaching 60 to 80% in some instances (Table 1 in Ibarra-Pérez et al., 1997). Differences in outcrossing rates appeared to vary according to environmental conditions prevailing during the study, the bean genotypes involved, and the presence of pollinating insects. Debouck et al. (1993) and Freyre et al. (1996) identified several cases of gene flow from domesticated to wild beans in Colombia, Ecuador, and Bolivia. Beebe et al. (1997) mentioned that extensive wild-weedy-domesticated complexes of *P. vulgaris* were observed in regions of Peru and Colombia where wild and domesticated beans are sympatric. In the Mesoamerican gene pool, Vanderborght (1983) described the existence of weedy forms in Mexico and introgression rates of up to 50% in wild populations. Crosses between wild and domesticated common beans yield viable and fertile progenies (Burkart and Brücher, 1953; Miranda Colín, 1979; Evans, 1980; Koenig and Gepts, 1989; Kornegay et al., 1993; Singh et al., 1995; Pereira et al., 1996; Mumba and Galwey, 1998, 1999). With the exception of a preliminary report by Triana et al. (1993), all estimates of outcrossing in beans involved cultivars. Although the Triana et al. (1993) study involved wild as well as domesticated beans, their study was conducted on a research station outside the natural distribution area of wild beans. Thus, further investigations into levels of outcrossing in common bean in different locations and years is of great interest. Additional research is needed to determine whether the individuals in which the correlation between seed color and ISSR markers has apparently been broken or which carry unusual markers result from incomplete lineage sorting or hybridization, or a combination of both.

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