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Identification of DNA probes that reveal polymorphisms among closely related *Phaseolus vulgaris* lines

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Summary

Analyses of genetic diversity within populations could be of great benefit to plant genetic resources conservation. In order to identify genetic markers that are variable within populations, the genome of *Phaseolus vulgaris* was screened with several DNA sequences in order to identify hypervariable sequences. Polymorphisms were observed between Middle American and Andean cultivars using the protein III tandem repeat of the M13 phase and the 33.15 human minisatellite. Extensive differences were observed when the DNA of two divergent lines – BAT93 and Jalo EEP558, of Middle American and Andean origin, respectively – were digested with *Hin*fI, *Taq*I, *Hae*III and hybridized with the 33.15 human minisatellite. Similarly, numerous polymorphisms were observed when the M13 protein III tandem repeat region was hybridized with *Taq*I digests of these cultivars. Polymorphism was also detected among sister lines of two F_6 backcross materials involving Middle American and Andean lines when genomic DNA was digested with *Taq*I and hybridized with M13 tandem repeat region. In addition, polymorphism was observed among Porrillo cultivars that resulted from selection within a single landrace population. Whereas only one isozyme difference had been observed previously among the Porrillo cultivars, eleven restriction fragments detected by the M13 protein III tandem repeat sequence differentiated these cultivars. Ribosomal DNA also hybridized to several polymorphic bands on *Taq*I and *Eco*RI genomic Southern blots of the F_6 backcross material. Only one polymorphism was observed with *Eco*RI-digested genomic DNA of BAT93 and Jalo EEP558 was hybridized with microsatellite $(GACA)_4$. This probe might be useful in ascertaining relationships at the species and subspecies level, and as a marker in mapping studies. Our results show that both the human 33.15 minisatellite and M13 should be useful probes to detect within-population variability in common bean.

Introduction

Extensive studies of phaseolin and isozyme variability have been conducted in *Phaseolus* (e.g., Gepts et al., 1986; Koenig & Gepts, 1989; Singh et al., 1991b; Gepts, 1993). Most biochemical markers used in these studies were not variable enough, however, to probe diversity at the population level. For example, Singh et al. (1991b) identified 76 genotypes with allozyme data in a sample of 83 wild and 227 cultivated *P. vulgaris* accessions. Several large groups of accessions were identified that could not be distinguished by their allozyme phenotypes, although they had considerable diversity for some morphological traits such as seed size and color. The authors concluded that most of the cultivars with the same allozyme genotype, following their origination from a common ancestor, had undergone further diversification for morphological traits but not for allozyme markers. Unlike biochemical markers, however, morphological traits are less desirable as population genetic markers because less is known about their genetic control and they can be subject to environmental influences as is the case in common bean (Leakey, 1988).

Minisatellite sequences have been used to study population dynamics in humans (Jeffreys et al., 1985; Vassart et al., 1987), animals (Wetton et al., 1987; Reeve et al., 1990) and plants (Dallas, 1988; Rogstad et al., 1988). Most of these studies used either the human minisatellite clones (33.6 and 33.15) or M13 bacterio-

phage as probes. The hypervariable regions consist of tandem repeats of short sequences that are dispersed in the genome. These sequences undergo frequent mutations in length due either to unequal recombinations or slippage replication (Jeffreys, 1987), which result in loss or gain of repeat units. Recent data suggests that variability is produced by unequal crossing over between sister chromatids (Wolff et al., 1989). In plants, Dallas (1988) was able to distinguish between cultivars of rice using the human minisatellite probe 33.6. However, differences between individual plants within varieties were not observed. Nybom & Schaal (1990) were able to identify 15 genotypes among 20 sexually reproducing plants of *Rubus occidentalis* and 5 genotypes out of 20 plants in the apomictic *Rubus pensilvanica* using the M13 protein III tandem repeat as a probe.

Other sequences hybridize to hypervariable loci. Oligonucleotide sequences, such as $(CAC)_5$ and $(GACA)_4$, hybridize to hypervariable, microsatellite sequences in humans (Schafer et al., 1988) and in plants (Akkaya et al., 1992; Weising et al., 1992). The ribosomal DNA spacer region in *Vicia faba* is hypervariable (Rogers & Bendich, 1987).

Various questions in population genetics and breeding of common bean could be addressed with hypervariable sequences. In the research reported here, we have investigated the potential of several probes – human minisatellites, M13, ribosomal DNA, $(GACA)_4$, and a common bean repetitive sequence – to identify hypervariable sequences, i.e. sequences that are variable among closely related genotypes.

Materials and methods

Plant materials

To screen for possible polymorphism, three different types of materials – with increasing degree of genetic relatedness – were analyzed. In a first stage, two divergent cultivars – BAT93 and Jalo EEP558 from the Middle American and Andean gene pools, respectively (Singh et al., 1991a) – were assayed. In the second stage, two sets of more closely related F_6-derived backcross lines were used to screen for variation among them. Within each set, the lines appeared to be phenotypically identical except for their growth habit: lines showed either a bush, erect growth habit or a viny growth habit. The following lines were studied: lines 104 (bush growth habit) and 109 and 115 (viny habit) in cross ((Red Kidney × Redkote) × Gloria × (Red Kidney × Redkote) and lines 136 (bush) and 134 (viny) in cross (Early Pink × (Red Kidney × Redkote)) × (Red Kidney × Redkote). Red Kidney and Redkote are Andean materials and Early Pink is a Middle American material (Singh et al., 1991a). Thirdly, a group of Porrillo cultivars was also used to screen for polymorphism among lines selected within a single population: Porrillo No. 1, Porrillo 70, and Porrillo Sintético are closely related selections in the Middle American landrace Santa Clara (Voysest, 1983).

DNA probes

Probes were obtained from different sources. The M13 probe was obtained from the 781 base pair *BsmI*/*ClaI* fragment of the bacteriophage protein III gene. There were three sources for the human minisatellite 33.6 probe. We obtained a cloned probe containing the sequence from Dr Howard Judelson (University of California, Davis). This probe has a 360 bp insert containing the diverged repeat unit of the 33.6 probe as $(AGGGCTGGAGG)^N$, where N = ca. 33. Another 33.6 probe was an oligonucleotide that was constructed at the Protein Structure Research Laboratory of UC Davis. Its sequence is 5'TGGAGGAAGGGCTGGAGGAGGG3'. The third source was a 33.6 probe supplied by Cambridge Research Biochemicals (ICI). This probe was a purified insert from the original Jeffreys 33.6 clone (Jeffreys et al., 1985).

The human minisatellite probe, 33.15, was obtained from two sources. A purified insert from the original 33.15 clone of A.J. Jeffreys (Jeffreys et al., 1985) was obtained for Cambridge Research Biochemicals. The insert was a tandem repeat of 5'AGAGGTGGGCAGGTGG3'. The other probe was an oligonucleotide (5'AGAGGTGGGCAGGTGG3') that was constructed at UC Davis as was the $(GACA)_4$ oligonucleotide, which had been shown to hybridize to hypervariable sequences in the human genome (Schafer et al., 1988). A pea ribosomal DNA probe, pHA2, was obtained from W.F. Thompson (Jorgensen et al., 1987). Finally, one clone (D0128) from a *Phaseolus vulgaris Eco*RI-*Bam*HI library (Nodari et al., 1992) was also used to screen for hypervariable sequences.

Labeling of probes

Isolated inserts from clones were labeled with the random primer method (Feinberg & Vogelstein, 1983,

1984). The oligonucleotides were labeled by the addition of either a poly A or poly C tail (using either α^{32}P-dATP or α^{32}P-dCTP (800 Ci/mM)) with terminal transferase (Ratliff, 1981) in 0.020 mM potassium cacodylate, 25 mM Tris-HC1, and 25 mM bovine serum albumin at 37° C. Under our experimental conditions, the presence of the labeled poly-A or poly-C tail does not lead to non-specific hybridizations as we observed the same pattern of hybridization with M13 oligonucleotides labeled by the terminal transferase or with M13 labeled by random priming (T. Stockton & P. Gepts, unpubl. results). The absence of non-specific hybridizations with oligonucleotides labeled by terminal transferase has also been observed by Schmitz et al. (1991) and Schubert et al. (1992). The rDNA clone, pHA2, was labeled using nick translation (Rigby et al., 1977).

Southern blotting and hybridization

Total genomic DNA was isolated from each accession as described in Stockton et al. (1992). The DNA was digested with various restriction endonucleases in overnight digests. Electrophoresis, Southern transfer, and hybridization were done as described by Stockton et al. (1992). The hybridization buffer (5 × SSPE (Saline-sodium phosphate EDTA), 7% sodium dodecyl sulfate, and 1% bovine serum albumin) is a modification of the buffer reported by Westneat et al. (1988).

Quantification of polymorphism

Pairwise comparisons were made between RFLP banding patterns using the similarity coefficient D_{AB} (Wetton et al., 1987) which calculates the probability that a fragment observed in A is also observed in B (D_{AB} = 2 X number of shared fragments/(Number of fragments A + number of fragments B). The probability that two accessions share all fragments was calculated as the average D_{AB} raised by the average number of fragments per genotype assuming independence among fragments (see Discussion).

Results

Several potential sources of probes were assayed in order to identify hypervariable markers. These include the M13 bacteriophage (Vassart et al., 1987), human minisatellites 33.6 and 33.15 (Jeffreys et al., 1985), ribosomal DNA, the $(GACA)_4$ microsatellite, and a repetitive random genomic *Eco*RI-*Bam*HI probe of common bean (Nodari et al., 1992). In a first step, hybridizations were performed with each of these probes to determine whether they revealed complex banding patterns necessary for fingerprinting of individual genotypes. In a second step, polymorphisms were analyzed to determine the probability of obtaining matching restriction fragment patterns.

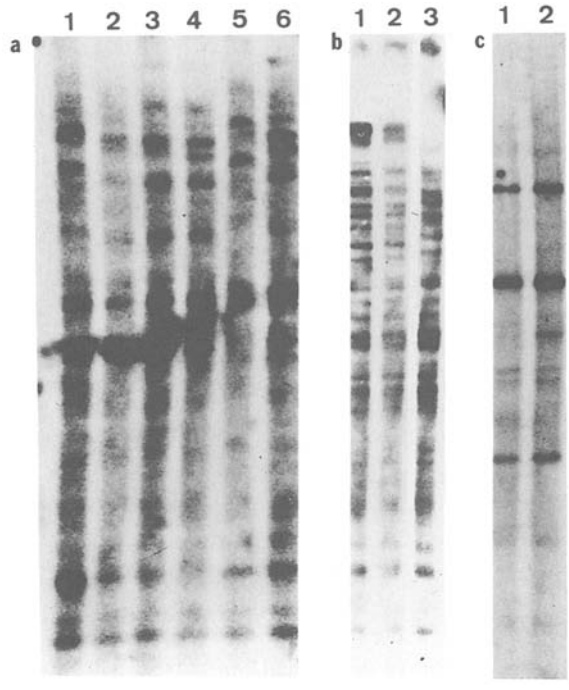

Fig. 1. Hybridization of total DNA of *Phaseolus vulgaris* with potential fingerprinting probes. *a* Total genomic DNA digested with *Taq*I and hybridized with the 781 bp *BsmI*/*Cla*I of the M13 protein III gene. Population (Early Pink × (Red Kidney × Redkote)) × (Red Kidney × Redkote): lane 1: line 136 (bush); 2: line 135 (bush); 3: line 134 (vine). Population ((Red Kidney × Redkote) × Gloria) × (Red Kidney × Redkote): lane 4: line 115 (vine); 5: line 109 (vine); 6: line 106 (bush). *b* Total genomic DNA from Porrillo cultivars digested with *Taq*I and hybridized with the 781 bp *BsmI*/*Cla*I of the M13 protein III gene. Lane 1: Porrillo Sintético; 2: Porrillo No. 1; 3: Porrillo 70. *c* Hybridization of microsatellite $(GACA)_4$ to *Eco*RI-digested total genomic DNA of *Phaseolus vulgaris* cultivars. Lane 1: BAT93; 2: Jalo EEP558.

Polymorphism observed with the M13 probe

The 781 bp *BsmI*/*Cla*I fragment of the M13 protein III gene (henceforth called M13) showed useful polymorphism within or among cultivars. M13 hybridized to between 10 and 16 informative restriction fragments on *Taq*I blots in all materials examined (Fig. 1a, b).

Table 1. Comparison of average distances and match probabilities in three populations of *Phaseolus vulgaris* probed with M13

Plant material	Average no. of fragments observed	Average no. of fragments shared	Average D_{AB}	Probability of sharing all fragments
BAT93, Jalo EEP558	26.0	19.0	0.36	1.7×10^{-5}
Porrillo cultivars	23.0	20.7	0.82	1.0×10^{-2}
F_6 backcross	22.4	17.5	0.83	1.5×10^{-2}

The similarity value (D_{AB}) between BAT93 and Jalo EEP558 was 0.66. Within the F_6 backcross material (Fig. 1a), similarity values (D_{AB}) ranged from 0.69 to 0.92 and had an average value of 0.83 (Table 1). The similarity values (D_{AB}) ranged between 0.7 to 1 between Porrillo cultivars. Porrillo Sintético and Porrillo 1 had identical restriction fragment patterns (Fig. 1b) and a D_{AB} value of 1. Porrillo 70 differed from the other two Porrillo cultivars and had a D_{AB} value of 0.70 with the former two Porrillo cultivars. The probability that two accessions share all fragments was estimated to be 1.5×10^{-2} for members of the F_6 backcross material and 1.0×10^{-2} between the Porrillo cultivars, respectively (Table 1). Thus, approximately 1% of the accessions in these closely related cultivars were likely to be identical by descent when probed with M13 tandem repeat on *Taq*I genomic Southern blots. More distantly related lines, BAT93 and Jalo EEP558, had a much lower probability of sharing all fragments (1.7×10^{-5}) (Table 1).

Polymorphism observed with human minisatellite probes

Hybridization with the human minisatellite probe 33.6 did not reveal a restriction fragment pattern with distinct bands, but rather a smear (result not shown). Southern hybridizations with this probe were therefore discontinued in favor of hybridizations with the human minisatellite probe 33.15.

The level of polymorphism observed when BAT93 and Jalo EEP558 were hybridized with 33.15 was greater than that observed with M13 (results not shown). The D_{AB} observed between these accessions was 0.36 with the 33.15 probe and 0.66 with the M13 protein III tandem repeat as a probe. These observations indicate that 33.15 may provide more information at the population level than M13.

Hybridization with other probes

Variation was found in ribosomal DNA among accessions in *P. vulgaris*. Polymorphism was observed on *Taq*I blots and *Eco*RI blots in 4 F_6 backcross plants (not shown). Similar to *V. faba* (Rogers & Bendich, 1987), there was a distinct restriction fragment of approximately 3.8 kb and several variable fragments around 6.7 kb. However, extreme variability has been found in the spacer region of the ribosomal DNA cistron in *Vicia faba*. *P. vulgaris* did not appear to be as variable as *V. faba* although only a small sample of variability was sampled in *P. vulgaris*. The microsatellite $(GACA)_4$ showed several distinct bands on Southern blots (Fig. 1c), regardless of the restriction enzyme used to digest the DNA. Only one polymorphism, however, distinguished BAT93 and Jalo EEP558. The *Pst*I genomic probe D0128, hybridized to multiple bands with every restriction digest (not shown). However, no polymorphism was detected within the F_6 material, among the Porrillo cultivars, or between BAT93 and Jalo EEP558.

Discussion

Although approximately 1% of accessions were likely to be identical when probed with M13 tandem repeat region on *Taq*I blots (Table 1), this probe/enzyme combination detected more variability than any other marker analyzed so far in common bean. For example, 11 restriction fragments differentiated Porrillo 70 from the other two Porrillo cultivars examined (Table 1, Fig. 1b), whereas only one isozyme allele (Malic acid enzyme) differentiated these cultivars (Singh et al., 1991b). Similarly, hybridization with M13 produced 15 restriction fragment polymorphism between BAT93 and Jalo EEP558, whereas these cultivars were only distinguished by 6 isozyme polymorphisms (*Aco-*

Table 2. D_{AB} values among sister lines of the F_6 backcross population digested with *Taq*I and probed with the M13 protein III tandem repeat region

Population	((Red Kidney × Redkote) × Gloria) × (Red Kidney × Redkote)			(Early Pink × (Red Kidney × Redkote)) × (Red Kidney × Redkote)	
Growth habit	Bush	Viny	Viny	Viny	Bush
Line	106	109	115	134	136
106	–	0.83	0.88	0.82	0.86
109		–	0.79	0.79	0.69
115			–	0.92	0.84
134				–	0.88

2, *Diap*-1, *Lap*-3, *Me, Rbcs, Skdh*). Similar observations were made with the human minisatellite probe 33.15. Variation detected by these probes appears to be sufficient to analyze intra-population genetic diversity such as in the mixed bean genotypes grown by farmers in Malawi (Adams & Martin, 1988; Gepts & Bliss, 1988) or wild bean populations in Latin America (Debouck et al., 1993). Isozyme analysis might help in determining the origin and genotype of each landrace, but the evidence might not be conclusive since many closely related cultivars differ by at most one or few isozyme alleles (Singh et al., 1991b). In contrast, the fingerprint produced by hybridization with either 33.15 or the M13 protein III tandem repeat region should provide more distinguishing differences between closely related cultivars (e.g., the Porrillo cultivars) and, thus, be a more reliable indicator of the origin of a landrace. In comparisons such as the Porrillo Sintético-Porrillo No. 1 comparison, which could not be distinguished using a single probe-restriction enzyme combination (M13-*Taq*I), other probe-enzyme combinations may show differences. For example, the 33.15 human minisatellite sequence or other tetranucleotide-recognizing enzymes such as *Hae*III or *Hin*fI may be useful in this respect.

Although these fingerprints might be influenced by introgression, they should be a predictor of maximal genomic similarity of the recipient line and minimal similarity to the donor line in an introgressive hybridization situation (Hillel et al., 1990). This is based on the assumption that these sequences are dispersed in the genome. In another study (T. Stockton et al., unpubl. results), nine markers cross-hybridizing to the M13 protein III tandem repeat region were mapped in an F_2 segregating population between BAT93 and Jalo EEP558. Six of these fragments were unlinked to any previously mapped marker, whereas three fragments mapped to the ends of existing linkage groups. These results suggest that sequences cross-hybridizing with M13 are not randomly distributed in the genome but rather might be located towards the ends of chromosomes. Thus, we are unable to determine if these sequences are a good overall predictor of the presence of the recipient genome after introgression has occurred.

The similarity values within the F_6 progeny and between Porrillo cultivars (Table 1) derived from M13 hybridizations (D_{AB} = 0.82 and 0.83) were similar to those found in inbreeding populations such as inbred mice and naked mole rats (Reeve et al., 1990). In naked mole rats, the mean probability that a band in one individual was present in a member of another colony was 0.89, 0.42 and 0.84 for M13, 33.15, and 33.6 patterns, respectively. In contrast, similarity values less than 0.5 were found between individual house sparrows (Wetton et al., 1987) which are presumably outcrossing.

In contrast, low similarity values (D_{AB} = 0.36) were obtained between BAT93 and Jalo EEP558 when probed with the human minisatellite 33.15 on *Hin*fI blots. This low value confirms the divergence between these two genotypes observed with other markers (isozymes: Singh et al., 1991b; RFLPs: Nodari et al., 1992) which results from independent domestication in Middle America and the Andes, respectively (Gepts, 1993).

The microsatellite $(GACA)_4$, which showed one polymorphism between BAT93 and Jalo EEP558, might be a useful probe for determining the relationships of subspecies and species in the genus *Phaseolus* or as a marker in mapping studies. Similarly, the genomic clone D0128 which hybridized to multiple bands that were not polymorphic among cultivars, might be a useful probe in detemining phylogenetic relationships above the species level.

In conclusion, we have shown that not all hypervariable probes are equally useful for fingerprinting in *P. vulgaris*. Specifically, M13 and the 33.15 human minisatellite are able to reveal polymorphisms even among closely related genotypes of this species. Such probes will be useful to investigate population structure and dynamics in cultivated and natural populations of common bean. Similar studies in other species should follow an analogous screening of fingerprinting probes.

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