

Random Amplified Polymorphic DNA (RAPD) Marker Variability between and within Gene Pools of Common Bean

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Abstract. The objective of this study was to evaluate the degree of RAPD marker variability between and within commercially productive market classes representative of the Andean and Middle American gene pools of common bean (*Phaseolus vulgaris* L.). Six sets of near-isogenic lines were screened with oligonucleotide primers in the polymerase chain reaction-based RAPD assay. Simultaneous analyses with at least three sets of lines enabled us to score RAPD markers between the two major gene pools, races within the same gene pool, and different genotypes of the same race (within race). A “three-tiered” pattern of polymorphism was observed: between gene pools > between races > within races. The overall level of polymorphism between the Andean and Middle American gene pools was 83.4%. The overall level of polymorphism between races within the same gene pool was similar for Andean races (60.4%) and Middle American races (61.7%). The level of polymorphism between related commercial navy bean lines was 39.2% and between related commercial snap bean lines was 53.6%. The inherent simplicity and efficiency of RAPD analyses, coupled with the number of polymorphisms detectable between related commercial genotypes, should facilitate the construction of RAPD-based genetic linkage maps in the context of populations representative of most bean breeding programs.

The common bean is characterized by an extensive range of genetic variation. Biochemical, physiological, and morphological evidence strongly suggests that this variation is not distributed at random but is associated with two distinct centers of diversity commonly known as the Andean and Middle American gene pools (Gepts and Bliss, 1985; Gepts et al., 1986). Each of these gene pools has been subdivided into races based primarily on comprehensive analyses of germplasm within the Andean and Middle American centers (Singh et al., 1991a). Bean breeders have often confined hybridization within specific races or seed types (Adams, 1972; Zaumeyer, 1972). Long-term genetic improvement of common bean will likely require methods that allow for more efficient use of the vast array of genetic resources available. Constructing comprehensive genetic linkage maps using DNA markers may promote the use of genetic variation in breeding programs (Pateron et al., 1991).

Genetic linkage maps in several crop species have recently been constructed based largely on restriction fragment length polymorphism (RFLP) loci (Pateron et al., 1991). In certain autogamous crop species, most notably tomato (*Lycopersicon esculentum*

Mill.) (Miller and Tanksley, 1990) and soybean (*Glycine max* L.) (Keim et al., 1989), limited RFLP-based genetic diversity among commercially adapted genotypes has led to map construction in the context of interspecific crosses that show higher levels of variation. Recent reports suggest that a similar situation may exist in common bean with respect to the ability to detect RFLPs among commercially adapted materials (Chase et al., 1991; Guo et al., 1991; Nodari et al., 1992). In addition to potential difficulties in detecting RFLPs among commercially adapted genotypes, RFLP technology remains extremely laborious and perhaps too costly for many breeding problems. The recently identified RAPD genetic marker (Welsh and McClelland, 1990; Williams et al., 1990) shows promise for many plant breeding applications primarily due to its inherent simplicity of analysis.

RAPD markers are heritable differences in the presence of DNA fragments amplified from genomic DNA using single oligonucleotide primers of arbitrary sequence in the polymerase chain reaction (PCR; Saiki et al., 1988). Polymorphism between two individuals is generally scored as the presence or absence of an amplified DNA fragment as visualized in agarose or polyacrylamide gels. The absence of a particular DNA fragment (nonamplification) may result from deletion of a priming site, insertions rendering priming sites too distant to support amplification, insertions that change the size of a DNA segment without preventing its amplification, or simple base changes in either or both primer sites bounding the DNA fragment (Williams et al., 1990). The main advantages of RAPD technology over RFLP technology are its inherent simplicity of analysis and the ability to conduct PCR tests with extremely small quantities of tissue for DNA extraction (Edwards et al., 1991; Welsh et al., 1991). Although the dominant inheritance of RAPD markers does not favor their use for genetic mapping in F_2 populations (Allard, 1956),

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linkage analysis with other types of populations will minimize this perceived limitation, as recently demonstrated in *Arabidopsis thaliana* (L.) Heynh. (Reiter et al., 1992).

One of our objectives for using RAPD markers is to construct a RAPD-based genetic linkage map in the context of commercial common bean germplasm for potential application to bean breeding problems. Knowing the relative levels of RAPD-based polymorphism would help to plan such mapping efforts and help to assess the feasibility of constructing maps for specific breeding objectives within certain market classes. The main objective of our study was to evaluate the levels of RAPD-based variation between and within commercially adapted market classes representative of the Andean and Middle American gene pools of common bean.

Materials and Methods

Plant materials. Our work with RAPD markers has focused on identifying markers linked to major disease resistance genes using near-isogenic lines (NILs) developed from resistant and susceptible individuals in a segregating population or from resistant and susceptible cultivars or breeding lines (Haley et al., 1993; Miklas et al., 1993). Six sets of NILs were used (Table 1): DNA bulks from segregants in, two backcross-derived snap bean populations (snap-1 and snap-2) (Haley et al., 1993); DNA bulks from segregants in three advanced-generation (F₉) kidney bean breeding populations (kidney); DNA bulks from segregants in a backcross-derived Type II navy bean population (navy-1) (Miklas et al., 1993); DNA bulks from two Type II and six Type I navy bean cultivars (navy-2); and DNA bulks from segregants in one advanced-generation (F₂) Type II pinto bean breeding population (pinto). Data for RAPD-marker variability in this study were obtained from simultaneous analyses with at least three sets of NILs and a common oligonucleotide primer. Because some NILs represented single-gene introgression between races or gene pools, only those RAPDs that were present in both members of one NIL and absent in both members of another NIL were tabulated.

DNA extraction and RAPD analysis. DNA was extracted from plants following procedures described previously (Miklas et al., 1993). Some DNA extractions were done using a mini-prep method (Edwards et al., 1991) with slight modifications: a CTAB extraction buffer was used (Miklas et al., 1993), an RNA digest step was added (Sambrook et al., 1989), and final DNA resuspension was done in TE_{0.1} [10 mM tris-HCl (pH 8.0), 0.1 mM EDTA]. Regardless of extraction method, DNA samples were standardized to a uniform concentration (10 ng-μl⁻¹) by DNA fluorometry (TKO 100, Hoefer Scientific, San Francisco). The PCR procedure reported by Miklas et al. (1993) was followed with minor modifications. About 25 ng genomic DNA template and 25 ng single

decamer primer(kits A through Y; Operon Technologies, Alameda, Calif.) were used in a 25-μl reaction that contained two units of Stoffel fragment polymerase (Perkin ElmerCetus, Norwalk, Conn.), 1X buffer [10 mM tris-HCl (pH 8.3), 10 mM KCl; provided with the polymerase], 5.0 mM MgCl₂, and 200 mM each dNTP (Perkin Elmer Cetus), overlaid with 25 μl mineral oil before amplification. Amplification was carried out in a DNA thermal cycler (model 480; Perkin Elmer Cetus) programmed for three cycles of 1 min/94C, 1 min/35C, and 2 min/72C; 34 cycles of 1 min/94C, 1 min/40C, and 2 min/72C (the final step extended by 1 sec for each of the 34 cycles); and one final extension cycle of 5 min/72C. Following amplification, ≈ 20 μl of the completed reaction was run in 1.4% agarose gels containing ethidium bromide (0.5 μg-ml⁻¹), 40 mM tris-acetate, and 1 mM EDTA. A permanent photographic record was obtained for each PCR analysis.

Results and Discussion

DNA amplification using the PCR for RAPD analyses resulted in an average of 5.4 (range 1–12) discernible DNA fragments per oligonucleotide primer-DNA template combination. The quality of DNA amplification and readability of DNA bands in agarose gels varied considerably among primers. Many of the primers (≈ 20%) produced either no amplification or generally unreadable amplification patterns (DNA “smear”) and could not be scored for RAPDs. Innis and Gelfand (1990) have suggested that such problems (particularly DNA smears) maybe a result of nonspecific amplification during initial PCR cycles and subsequent preferential amplification of nonspecific products during later PCR cycles (“plateau effect”). Williams et al. (1990) reported that smears could be minimized by reducing either the polymerase or the genomic DNA in reactions. Our optimization experiments have indicated that improved consistency, repeatability, and DNA fragment clarity may be obtained with such modifications as 1) using two primer annealing stringency levels, 2) reducing total cycle number (≈ 37), and 3) including a final extension cycle of 5 min/72C.

Many of the oligonucleotide primers identified more than one RAPD marker for a given comparison. Therefore, we chose to calculate the level of variability as a function of the occurrence of at least one RAPD per functional primer, consistent with variability estimates for RFLPs with single-copy probes. These analyses of RAPD-based variation revealed the highest level of polymorphism between the two major gene pools (Table 2). Seven comparisons were made between Andean and Middle American materials, with polymorphism levels ranging from 80.7% to 85.1% (overall average 83.4%). Although the number of primers screened varied among these comparisons, no clear differences in levels of polymorphism were observed.

Table 1. Near-isogenic line (NIL) designation, gene pool, race, and derivation or background of materials used.

| NIL | Gene pool ^a | Race ^b | Derivation or background |
|--------|------------------------|-------------------|---|
| Snap-1 | A | S | BBL-47*6//Green Giant 447/B-190 (Haley et al., 1993) |
| Snap-2 | A | S | Slenderette*5/3/Eagle//Green Giant 447/B-190 (Haley et al., 1993) |
| Kidney | A | NG | Advanced generation (F ₉) kidney breeding lines K86506, K86002, K86012 |
| Navy-1 | MA | M | C-20*6/Early Gallatin (Miklas et rd., 1993) |
| Navy-2 | MA | M | Seaforth, Harofleet, Harokent, OAC Rico Seafarer, Fleetwood, Kentwood, Bunsu |
| Pinto | MA | D | Advanced generation (F ₉) pinto breeding line, P89419 |

^aA = Andean gene pool, MA = Middle American gene pool.

^bD = Durango race, M = Mesoamerica race, NG = Nueva Granada race, S = Snap bean race.

Table 2. Levels of RAPD-based polymorphism between and within Andean and Middle American gene pools of common bean.

| Comparison | Gene pools ^z | Race designation | Primers tested | Polymorphic primers ^y | Percent polymorphism |
|---------------|-------------------------|---------------------------|----------------|----------------------------------|----------------------|
| Snap-1/navy-1 | A/MA | Snap/Mesoamerica | 124 | 105 | 84.7 |
| Snap-1/navy-2 | A/MA | Snap/Mesoamerica | 275 | 222 | 80.7 |
| Snap-2/navy-2 | A/MA | Snap/Mesoamerica | 108 | 89 | 82.4 |
| Snap-1/pinto | A/MA | Snap/Durango | 297 | 249 | 83.8 |
| Snap-2/pinto | A/MA | Snap/Durango | 107 | 89 | 83.2 |
| Kidney/navy-2 | A/MA | Nueva Granada/Mesoamerica | 67 | 56 | 83.6 |
| Kidney/pinto | A/MA | Nueva Granada/Durango | 67 | 57 | 85.1 |
| Snap-1/kidney | A/A | Snap/Nueva Granada | 67 | 45 | 67.2 |
| Snap-1/snap-2 | A/A | Snap/snap | 110 | 59 | 53.6 |
| Pinto/navy-1 | MA/MA | Durango/Mesoamerica | 145 | 98 | 67.6 |
| Pinto/navy-2 | MA-MA | Durango/Mesoamerica | 278 | 155 | 55.8 |
| Navy-1/navy-2 | MA/MA | Mesoamerica/Mesoamerica | 102 | 40 | 39.2 |

^zA= Andean gene pool, MA = Middle American gene pool.^yIndicates number of primers showing at least one RAPD for indicated comparison.

Within the Andean gene pool, polymorphism was greater between the snap-1 and kidney lines than between the snap-1 and snap-2 lines (67.2% vs. 53.6%; average 60.4%) (Table 2). Although genetic introgression from the Mesoamerican genotype 'B-190' could have increased the level of between-race polymorphism (snap-1/kidney), the observation of greater variability between snap and kidney (Nueva Granada) bean races is consistent with their divergence for morphological and agronomic characteristics. The high level of polymorphism between the two snap bean genotypes was interesting to note because snap bean germplasm is considered to have been derived from an extremely narrow genetic base, and much of the available variability is the result of deliberate breeding efforts (Zaunmeyer, 1972). A comprehensive analysis of allozyme variability among snap bean genotypes by Weeden (1984) demonstrated that, despite this narrow genetic base, variation at the biochemical level is detectable between otherwise morphologically indistinguishable snap bean cultivars. Our results appear to substantiate those of Weeden (1984) with respect to the ability to differentiate snap bean genotypes that have been derived from a narrow genetic base and are otherwise similar for morphological and agronomic characteristics.

The level of polymorphism between Middle American races was similar to that between the snap and kidney bean races (Table 2). A difference was noted, however, between the comparisons of races Durango and Mesoamerica (67.6% and 55.8%; overall average 61.7%). The pinto line used in our study was a product of a phenotypic recurrent selection program (Kelly and Adams, 1987; Kelly et al., 1990) designed to introgress the Type II growth habit (from tropical black bean germplasm) into the more traditional Type III germplasm characteristic of most pinto bean cultivars grown in the United States. We had thus expected less polymorphism between the navy-1 and pinto lines than between the navy-2 and pinto lines. The lower level of polymorphism observed between the navy-2 and pinto lines, compared to that between the navy-1 and pinto lines, could have resulted from a masking of polymorphisms by including genetically distinct Type II navy cultivars ('Bunsi' and its backcross derivative 'OAC Rico') in the navy-2 DNA bulk.

The high level of polymorphism detected between the navy-1 and navy-2 lines (39.2%; Table 2) can be explained based on the general divergence between the 'C-20' type (Type II indeterminate growth habit; Kelly et al., 1984) and the more traditional Type I navy cultivar grown for many years in the upper Midwestern United States (Type I determinate growth habit). The modification

of plant architecture in navy bean germplasm was based on the architectural ideotype (architype) concept developed and proposed by Adams (1982). This architectural modification, also derived from tropical black bean germplasm ('San Fernando' via 'NEP-2') (Adams, 1982), appears to have resulted in a broadening of the genetic base in navy beans, as evidenced by the high degree of RAPD variability detected in our study.

Our observations of greater variation between more genetically divergent materials generally agrees with other measures of genetic diversity in common bean based on morphological and agronomic traits (Singh et al., 1991b), seed storage proteins (phaseolin) (Gepts and Bliss, 1985), allozyme analysis (Koenig and Gepts, 1989), mitochondrial RFLP analysis (Khairallah et al., 1991), and cluster analysis by pedigree (McClellan et al., 1993) and RAPD data (Skroch et al., 1992). This "three-tiered" pattern of variability (between pools > between races > within races) is also consistent with estimates of nuclear RFLP-based variation showing 80% to 90% polymorphism between gene pools, 50% to 60% polymorphism between races of the same gene pool, and 20% to 30% between genotypes of the same race (Chase et al., 1991; Nodari et al., 1992).

In summary, the inherent simplicity and efficiency of the RAPD assay, coupled with the levels of polymorphism observed in our study, demonstrate the potential for developing RAPD-based genetic linkage maps in common bean using populations developed from between-pool and between-race crosses. Further, the high degree of within-race polymorphism (e.g., in navy and snap beans) supports the notion of genetic mapping in crosses of commercially adapted materials of the same market class.

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