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Molecular Tagging of the bc-3 Gene for Introgression into Andean Common Bean

William C. Johnson, Pablo Guzmán, D. Mandala, A. B. C. Mkandawire, S. Temple, R. L. Gilbertson, and P. Gepts*

ABSTRACT

Alternative genes for resistance to Bean Common Mosaic Virus in common bean (Phaseolus vulgaris L.) are necessary as a result of the recent introduction of necrosis-inducing strains of this virus into the USA. The recessive bc-3 gene confers resistance against all known strains of this pathogen. We describe here experiments to develop a relatively easy-to-use procedure to introgress the bc-3 gene into elite bean cultivars. First, we employed bulked segregant analysis to identify RAPD markers linked to the bc-3 locus. The ROC11/359/420 marker was codominant with the bc-3 gene and the ROC20/460 marker was dominant and linked in trans. A survey of cultivated materials allowed us to identify the likely evolutionary origin of the bc-3 resistance allele as a member of the Mesoamerican gene pool, probably of race Mesoamerica. Polymorphism of the RAPD markers in a Davis common bean mapping population (BAT93 × Jalo EEP58) allowed us to map the markers and, by inference, the bc-3 gene to linkage group D6. Second, we used sequence information from the cloned RAPD fragments to design longer, more reliable PCR primers that differentiate individuals homozygous for the resistance allele from susceptible genotypes in segregating populations of Andean origin. Third, we developed a marker tagging system that used a simplified DNA extraction technique and a PCR-based assay to identify the genotype of common bean plants at the bc-3 disease resistance locus. This simplified marker assisted selection system is expected to eliminate the need for costly quarantines and progeny tests in breeding programs for common bean of Andean origin.

Bean Common Mosaic Virus ( BCMV ) causes one of the most serious diseases affecting common bean production. This potyvirus is spread non-persistently by aphids and is also seed-borne (McKern et al., 1992). The symptoms of BCMV can include leaf vein clearing, aphids and is also seed-borne (McKern et al., 1992). Within the Mesoamerican and the Andean (Gepts et al., 1986; Singh et al. 1991b; Khairallah et al., 1992). These gene pools appear to have diverged prior to their independent domestications, as evidenced by numerous studies of morphological and molecular markers in both wild and domesticated lines (Gepts et al., 1986; Singh et al. 1991b; Khairallah et al., 1992). Within the Mesoamerican and the Andean gene pools, six races were identified based on morphological, adaptive, geographic, and agronomic characteristics (Singh et al. 1991a).

In the research reported here, we sought to alleviate the problems associated with the introduction of the recessive bc-3 gene by developing an efficient marker-assisted selection system based on markers that tag the bc-3 gene, a simplified DNA extraction method, and

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1978). The bc-3 gene conditions resistance to all known strains of BCMV. Only one, recessive resistance allele at the locus is known. Presently, it appears that bc-3 is the most effective means of overcoming the virus problem where necrosis-inducing strains of the virus are present. Although the bc-3 gene is widely available to breeders, it has not been extensively deployed in dry bean cultivars widely planted in the regions most seriously affected by necrosis-inducing BCMV strains. Three factors have hampered the introgression of this gene into locally favored cultivars. First, the gene is recessive and requires progeny tests to identify carrier individuals. Second, much of the breeding work is performed outside the present geographical distribution of the necrosis-inducing strains, and inoculation with these necrosis-inducing strains of the virus is necessary to differentiate bc-3-mediated resistance from resistance mediated by other strain-specific resistance genes. Therefore, introduction of these viral strains into these areas can only be carried out under controlled conditions, which are often limited for space. Third, there are large numbers of locally adapted and/or preferred bean cultivars with highly specific geographical distributions. This necessitates the repeated introduction of the bc-3 gene into a large number of bean commercial classes.

Common bean germplasm can be easily separated into two gene pools, Mesoamerican and Andean, based on the level of divergence at the molecular level (reviewed in Gepts, 1993) and the existence of reproductive isolation (Sprecher and Khairallah, 1989; Coyne, 1965; Koinange and Gepts, 1992). These gene pools appear to have diverged prior to their independent domestications, as evidenced by numerous studies of morphological and molecular markers in both wild and domesticated lines (Gepts et al., 1986; Singh et al. 1991b; Khairallah et al., 1992). Within the Mesoamerican and the Andean gene pools, six races were identified based on morphological, adaptive, geographic, and agronomic characteristics (Singh et al. 1991a).

In the research reported here, we sought to alleviate the problems associated with the introduction of the recessive bc-3 gene by developing an efficient marker-assisted selection system based on markers that tag the bc-3 gene, a simplified DNA extraction method, and

Abbreviations: BCMV, bean common mosaic virus; bp, base pairs; BSA, bulked segregant analysis; cM, centimorgans; MAS, marker-assisted selection; PCR, polymerase chain reaction; PEX, potassium ethyl xanthogenate; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; RIP or RIL, recombinant inbred population or line; SCAR, sequence-characterized amplified region; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Tm, annealing temperature.
more reliable PCR (polymerase chain reaction) amplification protocols through the development of sequence characterized amplified regions (SCARs, Paran and Michelmore 1993). A survey of common bean germplasm reveals that the marker tags identified here appear to be useful for introduction of the bc-3 gene from its Mesoamerican background into the Andean gene pool.

MATERIALS AND METHODS

Population Development and Germplasm Screening

The potential bc-3 donors considered were breeding lines (Kornegay, 1992) developed by CIAT (Centro Internacional de Agricultura Tropical, Cali, Colombia). All the potential bc-3 recipients were East African landraces collected in 1992 in Malawi. Seed proteins were extracted as described in Gepts et al. (1986) and one-dimensional SDS-PAGE electrophoresis was performed as in Laemmli (1970) as modified by Ma and Bliss (1978). Isozymes were analyzed by the methods of Koenig and Gepts (1989). The marker profile for phaseolin, malic enzyme, rubisco, shikimate dehydrogenase, diaphorase-1, and diaphorase-2 were used to determine the gene pool origins of potential parents (Mesoamerican vs. Andean: Singh et al. 1991b). Potential parents were screened for photoperiod sensitivity by observation under long-day conditions and for BCMV susceptibility by inoculation with BCMV strain NL3 (Drijfhout, 1978). Photoperiod-insensitive BCMV susceptible parents were chosen on the basis of viability and fecundity under long-day greenhouse conditions at Davis. We developed five F2 populations segregating for bc-3 by hybridizing CIAT donor males with Malawian recipient females (Table 1). Two of these populations were crosses between Andean-derived donors and recipients (populations C and E), two were crosses between Mesoamerican-derived donors and recipients (A and D), and one was an inter-gene pool cross with a Mesoamerican derived recipient (B). F1 and F2 generations were grown in the greenhouse under insect-free conditions, attempting to maximize seed set. We collected and stored leaf samples at -70°C separately for each F2 plant. One of the Andean × Andean F2 populations (E) failed to produce sufficient seed for statistically significant screening of most F3 families.

BCMV Inoculation and Scoring

For each of the four populations (A, B, C, and D, Table 1) used in bulked segregant analysis (BSA, Michelmore et al. 1991), a minimum of 47 F2 plants were grown to maturity. A minimum of 24 individual seedlings from each F2-derived F3 family were then used to determine the resistance genotype of each F2 parent. F3 family seedlings were grown in trays and inoculated by rubbing young unifoliate leaves with gauze dipped in a sap prepared from BCMV strain NL3-infected tissue by grinding tissue in 0.01 M K3PO4 pH 8.0 buffer with celite (an abrasive powder). Greenhouse temperatures were maintained above 25°C during the daytime to encourage the development of necrotic symptoms in population A, B, and D, which were simultaneously segregating for the f gene. Beginning 10 d after inoculation, seedlings showing clear signs of mosaic or systemic necrosis were counted and removed. Beginning 20 d after inoculation, plants clearly not affected by necrosis or mosaic were counted and removed. This process was continued until all F3 progeny were unequivocally scored for their reaction to BCMV strain NL3.

RAPD Analysis

For F2 individuals identified as homozygous recessive or homozygous dominant at the bc-3 locus, genomic DNA was isolated from the retained leaf samples as described in Gepts and Clegg (1989) and Gepts et al. (1992). DNA was quantified with a Hoefer TKO 100 DNA Fluorimeter (Hoefer Scientific Instruments, San Francisco, CA). RAPD reactions were performed in an Ericomp Twinblock thermal cycler (Ericomp, San Diego, CA). Reaction parameters were similar to those of Williams et al. (1990), but with 10 to 20 ng of genomic DNA in a total reaction volume of 25 μL. The thermal profile we found most useful was 1 cycle of 2 min at 94°C; 3 cycles of 1 min at 94°C/1 min at 35°C/2 min at 72°C; 32 cycles of 10 s at 94°C/30 s at 35°C/1 min at 72°C; and 1 cycle of 5 min at 72°C. RAPD markers were labeled as RO (RAPD of Operon Technologies, Inc., Alameda, CA) followed by a letter and two digits (the Operon primer number), a slash, and the approximate size of the amplified fragment in base pairs.

Bulked Segregant Analysis

Each homozygous F2 individual was used in a RAPD reaction to verify acceptable DNA quality before contrasting equimolar bulks were constructed of homozygous susceptible and resistant individuals for each of the four segregating populations. The contrasting bulks were screened with 175 decamer primers (Operon Technologies). When amplification patterns from contrasting bulks were not identical the reactions were performed two more times to verify repeatability of the observed polymorphism. If the polymorphism persisted, the same primer was used to amplify each individual from the contrasting bulks to verify uniformity.

Segregation of RAPD marker genotypes for primers ROS13, ROC11, and ROC20 was compared by χ2 analysis (P < 0.01) and Mapmaker (Lander et al., 1987) with the existing BAT93 × Jalo EEP558 recombinant inbred population (RIP) marker data set and with each other to identify linkages. Although this population does not segregate for the bc-3 gene, it has shown a high frequency of polymorphisms and has become a standard mapping population in common bean (Gepts et al., 1993).

Table 1. Population design for identification of bc-3 linked markers.

<table>
<thead>
<tr>
<th>Population</th>
<th>Number of F2 individuals</th>
<th>Bulk composition†</th>
<th>Malawian bc-3 recipient</th>
<th>CIAT bc-3 donor</th>
<th>bc-3 linked RAPD markers segregating</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>48</td>
<td>8S/8R</td>
<td>1-1 (M)‡</td>
<td>MCM 3031 (M)</td>
<td>ROS13/660</td>
</tr>
<tr>
<td>B</td>
<td>60</td>
<td>11S/7R</td>
<td>6-5 (M)</td>
<td>MCR 2204 (A)</td>
<td>none</td>
</tr>
<tr>
<td>C</td>
<td>47</td>
<td>8S/8R</td>
<td>21-5 (A)</td>
<td>MCR 2205 (A)</td>
<td>ROC20/460 and ROC11/420</td>
</tr>
<tr>
<td>D</td>
<td>48</td>
<td>9S/9R</td>
<td>Namajengo (M)</td>
<td>MCM 3031 (M)</td>
<td>none</td>
</tr>
<tr>
<td>E</td>
<td>52</td>
<td>3S/1R</td>
<td>11-1 (A)</td>
<td>MCR 2205 (A)</td>
<td>ROC20/460 and ROC11/420</td>
</tr>
</tbody>
</table>

† Total number of F2 individuals comprising S (susceptible: BC-3/BC-3) and R (resistant: bc-3/bc-3) bulks.
‡ A: Andean; M: Mesoamerican.
SCAR Development

We performed large volume (100 μL) amplifications of the putative bc-3 linked RAPD markers from BAT93 and Jalo EEP558 and used the methods of Danner (1982) to isolate individual polymorphic bands from polyacrylamide gels. Cloning of the RAPD bands was performed by means of the TA cloning system (Invitrogen Corporation, San Diego, CA), following the manufacturer’s instructions. Sequencing of the cloned RAPD fragments was performed using the manufacturer’s instructions for Sequenase version 2.0 DNA sequencing kit (United States Biochemical, Cleveland, OH). PCR primers were designed with the University of Minnesota Webprimers software (available at http://alces.med.umn.edu/bin/webprimers). Criteria for designing primers were (in order of importance) (i) the shortest primer possible with an estimated Tm above 56°C, (ii) terminating in a 3' C or G residue, (iii) avoiding possible loopback structures, and (iv) including the original RAPD primer sequence. Based on this analysis, SCAR primers were manufactured by Operon Technologies or Gibco BRL (Gaithersburg, MD) (Table 2). The primers derived from the ROC11 RAPD markers were paired in various combinations until reliable, repeatable polymorphisms were obtained.

Several thermal cycle profiles were used in an attempt to identify the fastest profile with clearly distinguishable amplification products. The thermal profile which was chosen as the most effective was as follows: 1 cycle of 2 min at 94°C, 30 cycles of 10 s at 92°C/10 s at 53°C/25 s at 72°C, and 1 cycle of 5 min at 72°C.

RESULTS

Identification of Markers Tagging the bc-3 Gene

Of the two RAPD markers (ROS13/660 and ROAD19/690) identified by Haley et al. (1994) as being linked to the bc-3 gene, none was polymorphic in the five populations tested (Table 1) with the exception of marker ROS13/660 in population A. Through bulked segregant analysis, we identified an additional RAPD polymorphism linked to bc-3 (ROC11/350/420 and ROC20/460). The ROC20/460 marker was linked in trans to the recessive resistance allele of bc-3, and segregated as expected of a typical dominant RAPD marker. The ROC11/350/420 marker segregated codominantly, with the 350-bp product (linked in cis to the resistance allele) not as strongly amplified as the 420-bp product (linked in trans to the resistance allele). These markers were polymorphic in the two Andean × Andean populations (C and E). All three markers were confirmed by verifying polymorphism in the parents and in each of the individuals comprising the contrasting bulks. None of the individuals comprising the bulks showed banding patterns indicating recombination between the bc-3 allele and any of the 3 RAPD markers, suggesting tight linkage.

In an effort to develop a simplified MAS system based on these RAPD markers, we attempted to use DNA obtained via the PEX (Potassium Ethyl Xanthogenate) extraction method of Williams and Ronald (1994) to amplify the RAPD markers linked to bc-3. Unfortunately, this method was unsuitable for RAPD analysis because the amplification patterns were unclear and inconsistent, particularly for the ROS13/660 and ROC11/350/420 markers. However, the method produced DNA of sufficient quality and quantity for PCR amplification of the phaseolin sequences with 21-bp primers (Kami et al. 1995), suggesting that longer primers may be more suitable if one desires to take advantage of this very fast and simple DNA extraction method.

Mapping of bc-3 Molecular Tags

When markers differentiating susceptible and resistant bulks were verified as reproducible and uniform in each bulk, we screened the parents of the main Davis mapping population for common bean, BAT93 and Jalo EEP558, with each of the four known bc-3 linked RAPD markers. Although bc-3 is not segregating in the recombinant inbred population previously used for constructing a genetic map of common bean (Nodari et al. 1993; Gepts et al. 1993), the ROC11/350/420, ROC20/460, and ROS13/660 RAPD markers were polymorphic, but the ROAD19/690 RAPD marker was not. The 75 recombinant inbred lines of this population were screened for all three polymorphic RAPD markers, enabling us to

Table 2. Phaseolin and RAPD genotype survey in cultivated germplasm.

<table>
<thead>
<tr>
<th>Cultivar name (Origin)</th>
<th>Gene pool†</th>
<th>Race‡</th>
<th>Phs§</th>
<th>ROC11/420¶</th>
<th>ROC20/460¶</th>
<th>ROS13/660¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinto UI 114 (USA)</td>
<td>M</td>
<td>Durango</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yolano (USA)</td>
<td>M</td>
<td>Durango</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gloria (USA)</td>
<td>M</td>
<td>Durango</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Black Turtle Soup (USA)</td>
<td>M</td>
<td>Mesoamerica</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Sal 778 (USA)</td>
<td>M</td>
<td>Mesoamerica</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>BAT93 (Colombia)</td>
<td>M</td>
<td>Mesoamerica</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Flor de Mayo (Mexico)</td>
<td>M</td>
<td>Jalisco</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Jalo EEP558 (Brazil)</td>
<td>A</td>
<td>Nueva Granada</td>
<td>T</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Montcalin (USA)</td>
<td>A</td>
<td>Nueva Granada</td>
<td>T</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CA Early Light Red Kidney (USA)</td>
<td>A</td>
<td>Nueva Granada</td>
<td>T</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sacramento (USA)</td>
<td>A</td>
<td>Nueva Granada</td>
<td>T</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CA Dark Red Kidney (USA)</td>
<td>A</td>
<td>Nueva Granada</td>
<td>T</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>White Kidney (USA)</td>
<td>A</td>
<td>Nueva Granada</td>
<td>T</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MI Improved Cranberry (USA)</td>
<td>A</td>
<td>Nueva Granada</td>
<td>T</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Linden (USA)</td>
<td>A</td>
<td>Nueva Granada</td>
<td>T</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bola (Ecuador)</td>
<td>A</td>
<td>Peru</td>
<td>T</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coscorrón (Chile)</td>
<td>A</td>
<td>Chile</td>
<td>C</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tórtola (Chile)</td>
<td>A</td>
<td>Chile</td>
<td>C</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

† M: Mesoamerican, A: Andean.
‡ Race designations as per Singh et al. (1991a).
§ Phs: Phaseolin genotypes. S: Sanilac; T: Tendergreen; C: Contender.
¶ Genotypes of RAPD markers: +: band present; -: band absent.
locate them via $\chi^2$ analysis and Mapmaker (Lander et al., 1987) on linkage group D6 (Gepts et al., 1993; Johnson and Gepts, 1994) (Fig. 1).

The ROC11/350/420 and ROC20/460 RAPD markers showed no recombination in the BAT93 × Jalo EEP558 RIP ($n = 75$), suggesting a linkage distance of $0 \pm 1.0$ centimorgans (cM; 95% confidence interval) between the two markers. These two markers and the ROS13/660 marker are $2.9 \pm 19.2$ cM apart in a population derived from a cross between two Mesoamerican derived breeding lines, N84004 navy bean and B85009 black bean. The lack of recombination between the ROC11/350/420 and ROC20/460 markers and the $bc-3$ gene in 20 individuals homozygous for $bc-3$ in populations C and E gives an estimate of a $0.0 \pm 7.5$ cM between the markers and the $bc-3$ locus.

Germplasm Survey

We screened 18 cultivars (11 Andean, seven Mesoamerican) for their marker genotypes to determine the genetic backgrounds in which the ROC11/420, ROC20/460, and ROS13/660 RAPD markers might be useful for MAS (marker assisted selection) (Table 2 and Fig. 2). For the ROC11/350/420 and ROC20/460 markers, all 11 cultivars of Andean origin presently lacking $bc-3$ were positive for the ROC11/420 and ROC20/460 RAPD bands and negative for the ROC11/350 RAPD band, suggesting that these RAPD markers will be useful to introduce the $bc-3$ gene into germplasm of Andean origin. All the screened cultivars of Mesoamerican origin were negative for the ROC20/460 and ROC11/420 RAPD bands and positive for the ROC11/350 RAPD band, suggesting that these RAPD markers will not be useful in germplasm of Mesoamerican origin. Marker ROS13/660, which is linked in trans to $bc-3$, was found to be absent from all cultivars of Andean origin, as well as from Middle American cultivars Sutter Pink, Gloria, Yolano (all of race Durango), and Flor de Mayo (race Jalisco). This marker was present only in Middle American cultivars Black Turtle Soup, BAT93, and Sal 778 (all of race Mesoamerica).

Evolutionary Origin of $bc-3$

We obtained seeds of the original source of $bc-3$ (PI 181954) courtesy of Richard Hannan (USDA/ARS, Western Regional Plant Introduction Station, Pullman, WA). For this accession, seed protein analysis revealed an S type phaseolin characteristic of the Mesoamerican gene pool (Gepts et al., 1986). Morphological seed characters were consistent with members of race Mesoamerica within the Mesoamerican gene pool.

Development of SCARS

After isolating, cloning, and sequencing the polymorphic RAPD markers, we designed longer PCR primers (15-25 bases) to specifically amplify the linked markers. Despite repeated attempts with varying reaction conditions we were unable to recover the polymorphism observed in the RAPD reactions with the ROS13/660 primer pair (Table 3). The fragments amplified by the ROS13/660 primer pair was of the expected size, but did not appear to be useful because there was no observable polymorphism in the genotypes previously displaying the RAPD polymorphism. The various combinations of ROC11/350F and ROC11/350R primers amplified products of the expected size, but which also failed to display a polymorphism. In addition, they amplified additional products of larger size. These larger products matched...
some of the original RAPD bands but were sensitive to reaction conditions and did not co-segregate with the RAPD marker polymorphism. The ROC20/460F1 + ROC20/460R1 and the ROC11/420F2 + ROC11/420R3 primer pairs, however, did amplify a dominant, single-band polymorphism. The polymorphic SCAR primer pairs were then used to amplify DNA from the RILs of the mapping population to verify that the observed polymorphisms correspond to the same markers observed in the RAPD analysis. With SCAR markers all 55 RILs observed displayed the same presence-absence of the markers as the corresponding RAPD marker.

**DNA Extraction Simplification**

Several DNA extraction protocols (Cheung et al. 1993; Afanador et al., 1993; Kami et al. 1995; Gepts and Clegg, 1989; Williams and Ronald, 1994) were evaluated for use with these SCARs. As with the aforementioned phaseolin primers, we were able to consistently (24/24 attempts) determine the marker genotypes with DNA obtained by the PEX method of Williams and Ronald (1994).

**DISCUSSION**

While performing BSA, spurious RAPD polymorphisms were frequent. Repetition proved crucial to identifying robust polymorphisms. The ROC11/350/420 co-dominant RAPD polymorphism was particularly susceptible to variations in reaction conditions, and the polymorphism could be considered quantitative. The ROS13/660 RAPD polymorphism was also notably sensitive to reaction conditions. Modified concentrations of Mg$^{2+}$, polymerase, and/or primer were sometimes necessary to ensure amplification of these two markers. Because of these difficulties associated with the RAPD markers, SCARs will be more useful than RAPDs in future efforts (Kelly et al., 1995) to use MAS to pyramid the genes conditioning resistance to BCMV.

In our attempts to develop SCAR markers, the original polymorphisms was preserved for two of the original four RAPD markers, ROC11/420 and ROC20/460. The polymorphism for the ROS13/660 and ROC11/350 markers was lost, i.e., a fragment of similar size was amplified in all genotypes. If the original RAPD polymorphism was due to the presence-absence of the amplified sequence in the contrasting bulks or to insertions-deletions within the amplified sequence, polymorphism would likely have been retained. If, however, the original RAPD polymorphism was due to single base pair differences between the contrasting bulks within the primer regions, then the new primers would likely be less sensitive than the original decamers to incomplete annealing, resulting in a loss of the polymorphism. The amplification of additional, larger bands with the ROC11/350 primers could potentially be attributed to the presence of repetitive sequences, as suggested by the sequence similarity (not identity) of the ROC11/420 and ROC11/350 clones (data not shown).

Many breeders are reluctant to adopt MAS strategies based on RAPDs because they are concerned about the lack of reproducibility and the lower level of reliability of this technology. Although the initial development of a SCAR-based MAS system requires a greater investment in the development stages than the use of RAPD markers identified through BSA, the economic advantage of the SCAR markers becomes apparent when the marker system is employed in multiple backcrossing programs. In addition to a much simplified procedure for DNA extraction and marker amplification, these novel SCAR markers can be assayed by the gel-free direct staining method of Gu et al. (1995).

For breeding programs the primary advantages these two polymorphic SCARs provide over the earlier identified RAPD markers (Haley et al., 1994) are a much simplified method to evaluate the genotypes of segregating individuals (see above) and a different range of genotypes (those from the Andean gene pool) in which these markers will be useful. The PEX protocol is the quickest (approximately 6 h per 100 samples) and least labor intensive of the DNA extraction methods tested (see materials and methods). The new ROC20/460 and ROC11/420 markers, described here, should be useful to transfer bc-3 into Andean backgrounds (Tables 1 and 2). Conversely, the ROS13/660, identified previously by Haley et al. (1994), may be useful in some cultivars of Mesoamerican origin presently lacking bc-3 (presumably those belonging to races Durango and Jalisco), but not all. The ROC20/460 and/or ROC11/420 bands, linked in trans to the recessive resistance allele of bc-3, can...
be negatively selected in F2 or later generations of similar crosses, and can also be negatively selected following selfing of backcrosses. In addition to their use in breeding programs, SCAR markers are expected to have additional applications for researchers. These SCAR markers may be useful to initiate map-based cloning of the bc-3 gene, and can also be employed in phenetic analyses of genetic diversity.

Identification of markers linked to the bc-3 gene was expedited by our knowledge of the pattern of genetic diversity in common bean, particularly with reference to the existence of the Mesoamerican and Andean gene pools. Based on the S phaseolin type and seed characteristics of the source accession, and the pattern of molecular marker diversity among cultivars in our survey, the bc-3 gene possibly originated in beans of Mesoamerican origin, probably among members of race Mesoamerica. The bc-3 gene was, however, introduced by hybridization (Kornegay, 1992) into improved germplasm of either Andean or Mesoamerican background acting as donor parents in this study. Because of linkage drag, the genomic region around the bc-3 gene, in which the marker tags lie, is also expected to be of Mesoamerican origin. Hence, it would be easier to identify linked markers in populations involving a recipient and donor parents of Andean origin, the latter containing a Mesoamerican genomic region harboring the bc-3 gene, than in populations involving a Mesoamerican recipient parent and either a Mesoamerican or an Andean donor parent. This observation is consistent with the fact that markers were identified in populations C and E, both of which resulted from Andean × Andean crosses, but not in populations A, B, and D, which resulted from Mesoamerican × Mesoamerican or Mesoamerican × Andean crosses.

Recent studies on angular leaf spot [a disease caused by the fungus Phaeoisariopsis griseola (Sacc) Ferraris] indicate that this pathogen of the common bean, which is quite variable in its pathogenicity spectrum, may have coevolved with each of the bean gene pools separately (Guzmán et al., 1995). As a consequence, resistance genes derived from Mesoamerican materials tend to be more effective against isolates of the pathogen that attack Andean materials and vice versa. Resistance genes for two other variable and important fungal pathogens, anthracnose [Colletotrichum lindemuthianum (Sacc. & Magnus) Lams.–Scrib.] and bean rust [Uromyces appendiculatus (Pers.:Pers.) Unger], also display this property of enhanced resistance based on the source of the resistance gene and the pathogen's origin (Singh et al. 1991a; Steadman et al., 1995; C. Leakey, 1988, personal communication). Because there is a notable degree of variability in the pathogenicity spectrum of different BCMV isolates, it is possible that strains of this virus also coevolved with particular subspecies or races of the common bean. Therefore, in addition to MAS, the availability of linked markers for use as a rapid assay of an individual's genotype will be a valuable tool for future studies of host-pathogen interaction and coevolution.

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