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Identification of RAPD markers linked to a major rust resistance gene block in common bean

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Abstract. Rust in bean (*Phaseolus vulgaris* L.), caused by *Uromyces appendiculatus* (Pers.) Unger var. *appendiculatus* [= *U. phaseoli* (Reben) Wint.], is a major disease problem and production constraint in many parts of the world. The predominant form of genetic control of the pathogen is a series of major genes which necessitate the development of efficient selection strategies. Our objective was focused on the identification of RAPD (random amplified polymorphic DNA) markers linked to a major bean rust resistance gene block enabling marker-based selection and facilitating resistance gene pyramiding into susceptible bean germplasm. Using pooled DNA samples of genotyped individuals from two segregating populations, we identified two RAPD markers linked to the gene block of interest. One such RAPD, $OF10_{970}$ (generated by a 5′-GGAAGCTTGG-3′ decamer), was found to be closely linked (2.15 ± 1.50 centi Morgans) in coupling with the resistance gene block. The other identified RAPD, $OI19_{460}$ (generated by a 5′-AATGCGGGAG-3′ decamer), was shown to be more tightly linked (also in coupling) than $OF10_{970}$ as no recombinants were detected among 97 BC_6F_2 segregating individuals in the mapping population. Analysis of a collection of resistant and susceptible cultivars and experimental lines, of both Mesoamerican and Andean origin, revealed that: (1) recombination between $OF10_{970}$ and the gene block has occurred as evidenced by the presence of the DNA fragment in several susceptible genotypes, (2) recombination between $OI19_{460}$ and the gene block has also occurred indicating that the marker is not located within the gene block itself, and (3) marker-facilitated selection using these RAPD markers, and another previously identified, will enable gene pyramiding in Andean germplasm and certain Mesoamerican bean races in which the resistance gene block does not traditionally exist. Observations of variable recombination among Mesoamerican bean races suggested suppression of recombination between introgressed segments and divergent recurrent backgrounds.

Key words: *Phaseolus vulgaris* L. – Bean rust resistance – Polymerase chain reaction (PCR) – DNA markers

Introduction

Rust in bean (*Phaseolus vulgaris* L.), caused by *Uromyces appendiculatus* (Pers.) Unger var. *appendiculatus* [= *U. phaseoli* (Reben) Wint.], is a major bean disease problem and production constraint in many areas of the world (Stavely and Pastor-Corrales 1989). Pathogenic variability of the fungus is extensive, with over 250 races reported and isolated worldwide, 78 of which from the USA alone (Stavely and Pastor-Corrales 1989; Stavely et al. 1989b; Stavely and McMillan 1992). Although resistance to rust in bean cultivars has been effective in reducing losses from *U. appendiculatus*, pathogenic variability of the fungus has necessitated an extensive search for comprehensive resistance sources and the development of efficient strategies for their manipulation and deployment. One of the more stable and broadly-effective sources of vertical resistance known is that first identified in "Mexico 309" (Echavez and Freytag 1982) and subsequently incorpo-

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rated into "B-190" (USDA and Ag. Exp. Stn. Univ. Puerto Rico 1979). B-190 is a semi-vining, black-seeded, high-yielding, tropical dry bean breeding line whose resistance response is expressed as tiny uredinia less than 0.3 mm diameter with 41 races of *U. appendiculatus* (resistant, R) and as tiny necrotic of chlorotic spots (hypersensitive, HR) with races 38, 39, 75, 77, and 79–81 (Stavely 1984b; Stavely et al. 1989b; Stavely unpublished). B-190 is susceptible to races 49–51, 58, 65, 67, 73, 78, 85, and 86, which are rarely found in the USA. Studies on the nature of the resistance in B-190 have indicated that this broad-based resistance response (R and HR) is controlled by several dominant genes tightly linked in coupling and thus inherited as a major gene block when introgressed into susceptible germplasm (Stavely 1984a).

In recent years, marker-facilitated selection has received attention as a viable method for the improvement of major-gene disease and insect pest resistance in crop plants (see review by Melchinger 1990). One form of marker, restriction fragment length polymorphism (RFLP), has been used extensively to tag useful genes in tomato [(*Lycopersicon esculentum* L.) Young et al. 1988; Klein-Lankhorst et al. 1991; Sarfatti et al. 1991], rice [(*Oryza sativa* L.) Yu et al. 1991], soybean [(*Glycine max* L.) Muehlbauer et al. 1991; Diers et al. 1992], and maize [(*Zea mays* L.) Bentolila et al. 1991]. A recent modification of the polymerase chain reaction (PCR; Saiki et al. 1988) has resulted in the availability of a relatively-new form of molecular marker: the random amplified polymorphic DNA (RAPD) marker (Welsh and McClelland 1990; Williams et al. 1990). Genetic mapping using RAPD markers has several advantages over other methods (Williams et al. 1990): (1) a universal set of primers can be used and screened in a short period of time, (2) no isolation of cloned DNA probes or preparation of hybridization filters is required, and (3) only small quantities of DNA are needed, allowing the use of simple and rapid methods (Edwards et al. 1991) for genomic DNA isolation. Since their inception, RAPDs have been used to tag major-gene disease resistances in tomato (Klein-Lankhorst et al. 1991; Martin et al. 1991), common bean (Miklas et al. 1993), and lettuce [(*Lactuca sativa* L.), Michelmore et al. 1991; Paran et al. 1991], and to assist in the development of comprehensive genetic maps in several plant species (Williams et al. 1990; Klein-Lankhorst et al. 1991; Quiros et al. 1991; Echt et al. 1992; Reiter et al. 1992).

In most instances, the identification of RFLPs or RAPDs linked to important resistance genes has been dependent on the availability of pairs of backcross-derived near-isogenic lines (NILs) for the identification of genomic regions linked to the gene of interest (Young et al. 1988), the basic objective being the identification of markers located in the linkage block surrounding the introgressed gene (Melchinger 1990). The development of pairs of NILs for economically important genes is costly and time consuming and, more importantly, few are presently available for marker-based applications in common bean. An alternative strategy, bulked segregant analysis (Michelmore et al. 1991), was recently proposed to allow the rapid development of populations useful for the identification of RFLP or RAPD markers linked to important plant genes. Using this method, Michelmore et al. (1991) identified three RAPD markers linked to major genes using contrasting DNA-bulks composed of F_2 individuals of known genotype for the genes of interest. Miklas et al. (1993) have provided additional support for the utility of the bulked segregant method using a backcross-derived population segregating for a major bean rust resistance gene. This combination of backcross introgression, characteristic of traditional NIL development, and bulked segregant analysis serves to enhance the identification of markers that are tightly linked to the gene of interest (Michelmore et al. 1991; Miklas et al 1993).

In this paper we report on the identification of two RAPD markers tightly linked to the rust resistance derived from B-190. These RAPDs were identified using a combination of the NIL and bulked segregant approaches, where DNA samples from individuals derived from backcross introgression and segregating for the resistance of B-190 were pooled to form materials suitable for identifying linked RAPDs. The identification of these RAPD markers, in addition to one previously identified (Miklas et al. 1993), will now enable marker-facilitated pyramiding of rust resistance genes into certain bean market classes of Mesoamerican origin (Gepts and Bliss 1985; Gepts and Debouck 1991) where the resistance factors are not typically found and where bean rust epiphytotics are a common problem.

Materials and methods

Genetic materials and disease evaluation

The segregating populations used to locate, confirm, and map the resistance gene block originated by backcross introgression of the *U. appendiculatus* resistance of B-190 from the Mesoamerican gene pool into the Andean gene pool. The Mesoamerican and Andean gene pools in common bean are widely considered to have arisen from seperate domestication events (Singh and Gutiérrez 1984; Gepts and Bliss 1985; Gepts and Debouck 1991), leading to considerable variation between the two pools at the agronomic (Singh and Gutiérrez 1984; Singh et al. 1991a), biochemical (Gepts and Bliss 1985; Sprecher 1988; Singh et al. 1991a, b), and DNA levels (Chase et al. 1991; Khairallah et al. 1991; Nodari et al. 1992). Two separate segregating populations were used: (1) the BBL-47 population: BC_6F_2 population of 97 individuals (originating from four individual BC_6F_1 plants) from the cross "BBL-47" *6//"Green Giant 447"/B-190 and (2) the Slenderette population: BC_5F_2 population of 13 individuals (originating from one individual BC_5F_1 plant) from the cross "Slenderette" *5/3/"Eagle"//Green Giant 447/B-190. In the development of these populations, all progeny from crosses, back-

crosses, and self-pollinations were tested for rust resistance in each generation using 8–12 differentiating races of *U. appendiculatus* (Stavely 1983; Stavely and McMillan 1992).

The rust resistance phenotype of each individual in the BBL-47 and Slenderette populations was determined and then its genotype confirmed with 10 F_3 progenies derived from each F_2 individual. Inoculum of race 53 of *U. appendiculatus* was used in greenhouse tests to distinguish resistant (R) from susceptible (S) plants based on the production of characteristic small uredinia (< 0.3 mm diameter; grade 3) on R plants and characteristic large uredinia (0.5–0.8 mm diameter; grade 5) (Stavely 1984b) on S plants. At the third-trifoliate stage, an inoculum suspension (calibrated to approximately 2×10^4 spores ml^{-1}) was sprayed evenly onto both the upper and lower leaf surfaces in a controlled dew chamber. Inoculated plants were transferred to a greenhouse room one day post-inoculation. Disease expression was scored 12 days after inoculation when typical uredinia had developed on the leaves of BBL-47 and B-190 (parent cultivars).

Bulk-NIL composition and RAPD analysis

DNA was extracted from plants (each individual in the segregating populations, the parent cultivars, and a range of bean cultivars with and without the resistance derived from B-190) following slight modifications of the procedure of Saghai-Maroof et al. (1984) as described by Miklas et al. (1993). All DNA samples were standardized to a uniform concentration (10 ng μl^{-1}) by DNA fluorometry (Hoefer TKO 100, Hoefer Scientific, San Francisco, Calif.).

The bulk-NIL populations used for RAPD identification were composed of bulked DNA samples from S and nonsegregating R individuals (based on rust evaluations as described above) in the two segregating populations. The BBL-47 pair (designated BBL-47 S and BBL-47 R) were composed of equal quantities of DNA from individuals tracing to different F_1 plants (four individuals per bulk-NIL). The bulk-NILs from the Slenderette population (designated Slenderette S and Slenderette R) were composed similarly except for the Slenderette S bulk-NIL which included the DNA from the only S individual in the population.

The PCR procedure reported by Williams et al. (1990) was followed with minor modifications. Approximately 25 ng of genomic DNA template and 25 ng of single decamer primer (mainly kits F through Y, Operon Technologies, Alameda, Calif.) was used in a 25 µl reaction that contained 2 units of Stoffel Fragment Polymerase (Perkin Elmer Cetus, Norwalk, Conn.), 1X buffer [10 mM TRIS-HCL (pH 8.3), 10 mM KCl; provided with the polymerase], 5.0 mM $MgCl_2$, and 200 mM of each dNTP (Perkin Elmer), overlaid with 25 µl of mineral oil prior to amplification. To ensure that potentially linked amplification products were not primer artifacts (Williams et al. 1990), genomic DNA was omitted from a control reaction included for each primer examined.

Amplification was carried out in a Perkin Elmer Cetus DNA Thermal Cycler 480 programmed for three cycles of 1 min/94° C, 1 min/35° C, and 2 min/72° C, and then 44 cycles of 1 min/94° C, 1 min/40° C, and 2 min/72°C, the final step extended by 1 s for each of the 44 cycles. These amplification conditions provided easily-visualized records of potential polymorphisms. Upon preliminary identification of the RAPDs reported herein, the following PCR conditions were used to enhance the quality of the amplified product: five cycles of 1 min/94° C, 2 min/40° C, and 3 min/72° C, 34 cycles of 1 min/94° C, 2 min/50° C, and 3 min/72° C, followed by one final extension cycle of 7 min/72°C. Approximately 20 µl of the completed amplification reaction were 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 run. A total of 306 primers was screened, 178 with the BBL-47 bulk-NILs alone and 128 with the addition of the Slenderette bulk-NILs.

Segregation analyses

To confirm cosegregation of marker and disease phenotype, putative linked RAPDs were analyzed against the parents (BBL-47, Slenderette, B-190) and a small subset of individuals (10–15) of known resistance genotype from the BBL-47 and Slenderette populations. Chi-square tests were used to confirm simple dominant inheritance of the disease phenotype and the linked RAPD markers identified (Steel and Torrie 1980). The recombination frequency between the markers identified and the gene block conferring resistance was calculated with Linkage-1 (Suiter et al. 1983) using the 97 individuals from the BBL-47 population.

Results

Identification of $OF10_{970}$ and $OI19_{460}$ RAPDs

The screening of 306 decamer primers against the BBL-47 R and S bulk-NILs resulted in the amplification of 1658 discernible DNA fragments ranging from 2000 to 100 bp, corresponding to an average of 5.4 fragments per primer. Of the primers screened, 14 generated a fragment that was present in the R bulk-NIL but not in the S bulk-NIL. Twelve of these primers (12/306 = 3.9%) were subsequently shown to generate a DNA fragment that did not cosegregate closely (> 30 centiMorgans) with resistance when analyzed against the parents (BBL-47, B-190, Slenderette) and a subset of F_2 individuals in the segregating populations (of known rust resistance genotype). Although we did not verify their linkage because of the lack of close cosegregation, these "false-positives" could reflect residual donor chromosomal segments unrelated to the resistance gene block (see discussion by Yu et al. 1991). Three primers (1%) generated polymorphic fragments that were apparently linked to susceptibility and thus not pursued. The inclusion of the Slenderette S and R bulk-NILs served as a convenient check for linked polymorphisms; both linked RAPD polymorphisms reported herein were identified in experiments containing both the BBL-47 and Slenderette bulk-NILs.

Two primers generated polymorphic DNA fragments that were shown to be linked to the resistance gene block. These RAPDs were scored against the 97 segregating individuals from the BBL-47 population; their inheritance, as well as that of the rust resistance phenotype, was shown to fit a pattern of simple dominant transmission (Table 1). One of these RAPDs, $OF10_{970}$ (generated by a 5′-GGAAGCTTGG-3′ decamer), was found to be closely linked (2.15 ± 1.50 cM) in coupling with the resistance gene block from B-190. The other identified RAPD, $OI19_{460}$ (generated by a 5′-AATGCGGGAG-3′

Table 1. Loci, phenotypic frequencies, χ^2 values for goodness of fit to 3:1 (single point; χ^2 A, χ^2 B) and 9:3:3:1 (two-point; χ^2 AB) expected ratios, and recombination frequency between loci

Locus		Phenotypes[a]				χ^2 A	χ^2B	χ^2AB	Recombination frequency
A	B	AB	Ab	aB	ab				
B-190	$OF10_{970}$	74	0	2	21	0.03	0.42	76.32**	2.15±1.50
B-190	$OI19_{460}$	74	0	0	23	–	0.03	90.62**	0.00±–[b]
$OI19_{460}$	$OF10_{970}$	74	0	2	21	–	–	76.32**	2.15±1.50

** Significant at the 0.01 probability level
[a] Phenotypes: AB: rust-resistant and shows RAPD; Ab: rust-resistant without RAPD; aB: rust-susceptible and shows RAPD; ab: rust-susceptible without RAPD
[b] Standard Error not defined

Table 2. Survey of Andean genotypes (snap and kidney) for $OF10_{970}$ and $OI19_{460}$ RAPD markers (S, susceptible; R, resistant; +, marker present; −, marker absent)

Genotype	B-190	$OF10_{970}$	$OI19_{460}$	Genotype	B-190	$OF10_{970}$	$OI19_{460}$
Snap Bean				Kidney Bean			
BARC-06	R	+	+	CELRK	S	–	–
BARC-07	R	–	+	Chinook	S	–	–
BARC-10	R	+	+	SVM 29-21	S	–	–
BARC-11	R	+	+	SVM 31-19	S	–	–
BARC-12	R	+	+	SVM 37-16	S	–	–
BARC-13	R	+	+	SVM 40-23	S	–	–
BARC-14	R	+	+	Isabella	S	–	–
BARC-16	R	+	+	K86002	S	–	–
BARC-17	R	–	+	K86012	S	–	–
BARC-18	R	–	+	Montcalm	S	–	–
BARC-19	R	+	+	Redkloud	S	–	–
BARC-21	R	+	+	Sacramento	S	–	–
BARC-22	R	+	+				
BARC-25	R	–	+				
BARC-26	R	–	+				
BARC-27	R	–	+				
BBL-47	S	–	–				
Benton	S	–	–				
Bountiful	S	–	–				
Eagle	S	–	–				
Early Gallatin	S	–	–				
Atlantic	S	–	–				
Hystyle	S	–	–				
Labrador	S	–	–				
Podsquad	S	–	–				
Provider	S	–	–				
Raider	S	–	–				
Rebel	S	–	–				
Resisto	S	–	–				
Slenderette	S	–	–				
Slenderwhite	S	–	–				

decamer), was also shown to be very tightly linked in-coupling with the resistance phenotype. The $OI19_{460}$ marker and the resistance gene block mapped to the same locus as no recombinants were observed between them (Table 1). The amplification of both of these DNA fragments was highly repeatable, enabling clear visualization on agarose gels and unambiguous scoring of their presence or absence (Fig. 1).

Usefulness of $OF10_{970}$ and $OI19_{460}$ RAPDs

Both linked-RAPDs identified were scored across a diverse collection of common bean germplasm of both Andean and Mesoamerican origin. Distinct differences between the two markers were revealed with respect to their potential suitability for reliable marker-facilitated selection. Within Andean germplasm (snap and kidney beans;

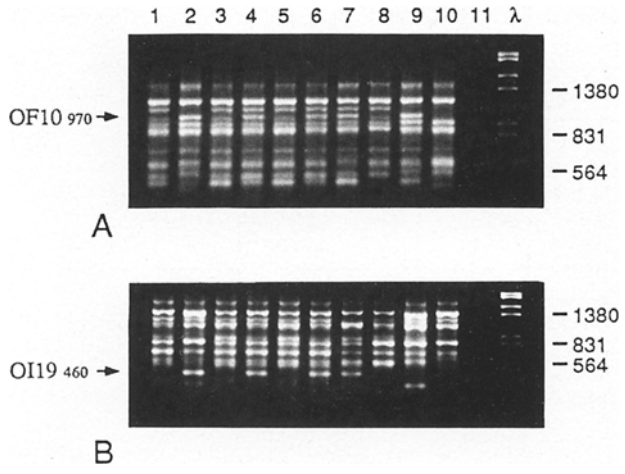

Fig. 1. Ethidium bromide-stained electrophoretic pattern of amplified DNA depicting $OF10_{970}$ (**A**) and $OI19_{460}$ (**B**) RAPD fragments. Key to individuals: (*1*) rust-susceptible BBL-47 parent, (*2*) rust-resistant B-190 parent, (*3*) BBL-47S Bulk-NIL, (*4*) BBL-47R Bulk-NIL, (*5*) rust-susceptible F_2 individual, (*6*) rust-resistant F_2 individual, (*7*) rust-resistant BARC-6 snap bean, (*8*) rust-susceptible Chinook kidney bean, (*9*) rust-susceptible C-20 navy bean, (*10*) rust-susceptible Seafarer navy bean, (*11*) negative control (no template DNA added to PCR amplification), (*12*) molecular weight marker (λ *Hin*dIII/*Eco*RI; size of bands indicated in bp)

Table 2), the presence and absence of both RAPD markers was closely associated with resistance and susceptibility, respectively, except for the absence of $OF10_{970}$ in several snap beans bred to possess the resistance of B-190 (Stavely and McMillan 1992; Stavely et al. 1992). Without exception, $OI19_{460}$ was present in all snap beans with the resistance gene block and absent in those snap and kidney beans without it.

The difference between the two markers was more evident within germplasm of Mesoamerican origin (Table 3) where the gene block is indigenous. With the lone exception of "Kentwood" (and its backcross-derivative, "Harokent"), no recombination between $OI19_{460}$ and the gene block appears to have occurred. $OI19_{460}$ was absent in all other susceptible germplasm, present in Mexico 309 (resistant parent of B-190), and present in resistant breeding lines and cultivars derived from B-190 ("L226-10" and "L227-1" navy beans, Freytag et al. 1985; "BELNEB RR-1" and "BELNEB RR-2" great northern beans, Stavely et al. 1989a).

The $OF10_{970}$ RAPD was different from $OI19_{460}$ with respect to its usefulness within the Mesoamerican gene pool. $OF10_{970}$ was shown to be present in most susceptible small-seeded genotypes (navies and blacks; Mesoamerican race) and thus of limited potential use within these classes of beans. Within the medium-seeded Durango race, however, $OF10_{970}$ was shown to be potentially useful as it was absent in the majority of susceptible genotypes and present in BELNEB RR-1, -2 great northern beans. The appearance of $OF10_{970}$ in several susceptible genotypes of Durango origin may well be traced to the recent introgression of upright architecture from the small-seeded Mesoamerican race to the medium-seeded Durango race (Kelly and Adams 1987). Nevertheless, the potential utility of $OF10_{970}$ as a marker in pinto and great northern breeding programs was indicated by its absence in standard susceptible medium-seeded (Durango race) germplasm.

Although only a single mapping population was used in this study, the Harokent and Kentwood recombinants provided useful information for the determination of the relative positioning of the three loci. The coincidence of occurrence (of markers and resistance phenotypes) among the Mesoamerican genotypes (Table 3) suggested that both identified RAPDs are positioned on the same side relative to the B-190 gene block and are not in fact positioned on opposing sides. In addition, these observations indicate that the $OI19_{460}$ marker is very tightly linked to the B-190 gene block and is not actually a DNA fragment amplified from within the gene block itself.

Discussion

The primary objective of our work involved the identification of a tightly linked RAPD marker for the broadly-effective resistance of B-190 enabling the application of molecular genetic techniques to applied breeding efforts. The fundamental advantage of tightly linked markers for such resistance genes is the opportunity to efficiently screen genotypes without reliance on test inoculation with the pathogen. This strategy, known as marker-facilitated selection (Paterson et al. 1991), would be particularly effective in the case of breeding for rust resistance in common bean where epistatic interactions among major resistance loci necessitate laborious testcrossing and rust race screening and have greatly hindered the pyramiding of multiple resistance factors into susceptible germplasm. The RAPD markers reported herein, particularly $OI19_{460}$, will now enable marker-facilitated combination of the resistance of B-190 with that from "Early Gallatin" (Up_2 gene; Christ and Groth 1982; Miklas et al. 1993) into susceptible navy, black, pinto, and great northern bean germplasm. In addition, the absolute cosegregation of $OI19_{460}$ and the resistance from B-190 in Andean germplasm supports the application of this RAPD marker for use within this gene pool.

The observation of the differential utility of $OF10_{970}$ and $OI19_{460}$, presumably as a function of proximity to the rust resistance gene block, is of particular interest. Much of the work on molecular marker identification has involved the use of interspecific introgressions from wild to cultivated species (Young et al. 1988; Paran et al. 1991; Paterson et al. 1991). Although the two major gene pools

Table 3. Survey of Mesoamerican genotypes (navy, black, pinto, and great northern) for $OF10_{970}$ and $OI19_{460}$ RAPD markers (S, susceptible; R, resistant; +, marker present; −, marker absent)

Genotype	B-190	$OF10_{970}$	$OI19_{460}$	Genotype	B-190	$OF10_{970}$	$OI19_{460}$
Navy Bean				Black Bean			
Aurora	S	−	−	91T-4806	S	+	−
90 MS36	S	+	−	91T-4810	S	+	−
91T-1234	S	+	−	B-190	R	+	+
91T-1621	S	+	−	B85003	S	+	−
Bunsi	S	+	−	B90222	S	+	−
C-20	S	+	−	B90223	S	+	−
Fleetwood	S	+	−	Blackhawk	S	+	−
Harofleet	S	+	−	BTS	S	+	−
Harokent	S	+	+	CNC-2	R	+	+
Kentwood	S	+	+	Mex-309	R	+	+
L-226-10	R	+	+				
L-227-1	R	+	+				
Mayflower	S	+	−				
N85120	S	+	−				
NEP-2	S	+	−				
OAC Rico	S	+	−				
Sanilac	S	+	−				
Seafarer	S	−	−				
Seaforth	S	−	−				
Pinto Bean				Great Northern Bean			
90T-4011	S	+	−	91T-2064	S	−	−
90T-4042	S	−	−	91T-2704	S	−	−
90T-4056	S	+	−	Alpine	S	−	−
90T-4066	S	−	−	BELNEB RR-1	R	+	+
Arapaho	S	−	−	BELNEB RR-2	R	+	+
Aztec	S	−	−	Beryl	S	−	−
Fiesta	S	−	−	G91213	S	−	−
Olathe	S	−	−	GN123	S	−	−
Othello	S	−	−	Starlight	S	+	−
Ouray	S	+	−	UI-59	S	−	−
Pindak	S	−	−				
Sierra	S	−	−				
UI-111	S	−	−				
UI-114	S	−	−				

in common bean are within the same species, available information provides evidence of considerable divergence at the molecular level (Gepts and Bliss 1985; Sprecher 1988; Chase et al. 1991; Nodari et al. 1992) which in fact facilitates the identification of DNA sequence divergence (via RFLP or RAPD markers) between the two major gene pools. The observation of variable recombination as a function of the relatedness of the introgressed segment (from B-190) and the rest of the genome appears to provide additional support for previous observations in other crops (Rick 1969; Paterson et al. 1990) of restricted recombination (or linkage disequilibrium) between chromosomal segments of diverse origin. Plans are underway in our laboratory for study in this area using mapping populations that represent within-pool introgression from the Mesoamerican race into the Durango race.

The appearance of $OI19_{460}$ in the susceptible navy bean Kentwood (and its backcross-derivative, Harokent) provides evidence for an extremely rare recombination event within the region of interest and prehaps provides an opportunity for further studies on the fine mapping of the resistance gene block from B-190; previous studies (Stavely 1984a) have indicated that this region is composed of several resistance factors each conferring resistance to different races of the bean rust pathogen. From an applied standpoint, however, this observation stresses the obvious need for confirmation of polymorphism of RAPD markers between resistant and susceptible parents prior to introgression of the resistance into susceptible germplasm.

The identification of $OI19_{460}$ has provided a marker that will apparently be useful for marker-facilitated transfer of the resistance of B-190 across gene pools (Mesoamerican into Andean), across races within the Mesoamerican gene pool (Mesoamerican race into Durango race), and within the Mesoamerican race where the resistance block is indigenous. This apparent usefulness of $OI19_{460}$ is clearly related to the tight linkage between the marker and the resistance gene block. The use of this

marker in conjunction with a tightly-linked marker previously identified for the Up_2 gene (Miklas et al. 1993), which confers hypersensitive resistance to several rust races to which B-190 is susceptible (Christ and Groth 1982; Stavely 1984b; Stavely et al. 1989b), will now enable marker-facilitated gene pyramiding of bean rust resistance genes into traditionally susceptible Mesoamerican bean market classes.

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