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Coupling- and repulsion-phase RAPDs for marker-assisted selection of PI 181996 rust resistance in common bean

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Abstract The Guatemalan black bean (*Phaseolus vulgaris* L.) plant introduction (PI) 181996 is resistant to all known US races of the bean rust fungus *Uromyces appendiculatus* (Pers. ex Pers.) Unger var. *appendiculatus* [syn. *U. phaseoli* (Reben) Wint.]. We report on two random amplified polymorphic DNA (RAPD) markers $OAC20_{490}$ tightly linked (no recombinants) in coupling phase and $OAE19_{890}$ linked in repulsion phase (at 6.2±2.8 cM) to PI 181996 rust resistance. These RAPDs, generated by single decamer primers in the polymerase chain reaction, were identified in near-isogenic bulks of non-segregating resistant and susceptible BC_4F_2 (NX-040*4/PI 181996) lines. Linkage of the RAPD markers was confirmed by screening 19 BC_4F_2 and 57 BC_4F_3 individuals segregating for PI 181996 resistance. Utility of the RAPDs $OAC20_{490}$ and $OAE19_{890}$ was investigated in a diverse group of common bean cultivars and lines. All cultivars into which the PI 181996 resistance was introgressed had the RAPD $OAC20_{490}$. A RAPD similar in size to $OAC20_{490}$, observed in some susceptible common bean lines, was confirmed by Southern blotting to be homologous to the RAPD $OAC20_{490}$. Use of the RAPDs $OAC20_{490}$ and $OAE19_{890}$ in marker-assisted selection (MAS) is proposed. The coupling-phase RAPD is most useful for MAS of resistant BC_nF_1 individuals during traditional backcross breeding. The repulsion-phase RAPD has greatest utility in MAS of homozygous-resistant individuals in F_2 or later-segregating generations.

Key words *Phaseolus vulgaris* L.
Uromyces appendiculatus · Gene pyramiding
Near-isogenic lines (NILs) · Genetic markers

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Introduction

The bean rust fungus consistently reduces yield in dry and snap beans. The areas most severely affected are humid tropical and subtropical regions of Africa and temperate regions of North and South America. In some areas, periodic rust epidemics reduce bean yields and result in the loss of a major source of dietary protein. In Puerto Rico, yield losses of 75% in rust-susceptible cultivars were observed as a result of induced rust epidemics (Velez-Martinez et al. 1989).

Over 200 races of *U. appendiculatus* are known (Stavely and Pastor-Corrales 1989; Stavely et al. 1989; Stavely and Batra 1991). The most economic form of control to the bean rust pathogen is the genetic resistance present in the host. In common bean, resistance to rust is controlled by single dominant genes (Stavely, 1984; Luann Finke et al. 1986; Stavely and Pastor-Corrales 1989).

Pyramiding monogenic resistance has been proposed as a means of providing the most stable and economical form of disease control in crop plants. Epistasis between rust resistance genes (Kolmer and Groth 1984) complicates pyramiding efforts. Thus, construction of a pyramid of resistance genes can be expensive and time-consuming (Pedersen and Leath 1988; Melchinger 1990) because of the required progeny testing.

Molecular markers, such as random amplified polymorphic DNAs (RAPDs), can be used for indirect selection of progeny with pyramided resistance genes (Haley et al. 1993 a; Miklas et al. 1993). However, marker-assisted selection (MAS) is limited by the need for tight linkage between resistance and marker loci. The simple RAPD pro-

tocol coupled with the use of near-isogenic lines (NILs), bulked segregant analysis (Michelmore et al. 1991), or both (Barua et al. 1993; Haley et al. 1993 a; Miklas et al. 1993), provide the opportunity to quickly identify molecular markers tightly linked to genes for resistance.

To-date, RAPDs have been identified linked to the bean rust resistance genes *Up-2* (Miklas et al. 1993), *Ur-3* (Haley et al. 1994 a), and the B-190 gene block (Haley et al. 1993 a). All RAPDs were in coupling-phase linkage to the respective resistance gene. The RAPD markers $OAC20_{490}$ in coupling and $OAE19_{890}$ in repulsion-phase linkage to the PI 181996 resistance are reported here. These RAPD markers were used together to differentiate homozygous and heterozygous rust-resistant genotypes in the backcross population (NX-040*4/PI 181996) segregating for PI 181996 resistance.

Materials and methods

Plant material

The Guatemalan (Middle American gene pool) black bean PI 181996 is resistant (tiny uredium Type-3 resistance, hypersensitive necrotic Type-2 resistance without sporulation, or a Type-1 immune reaction) to each of the 65 races of *U. appendiculatus* identified and maintained at Beltsville, Md. (Stavely et al. 1992). The resistance of PI 181996 appears to be controlled by a series of tightly linked dominant genes (Stavely 1990). This resistance was introgressed into the navy bean market class by traditional backcross breeding using NX-040 (a sister line of 'Norstar' navy bean; Grafton et al. 1993) as the recurrent parent at Beltsville, Md. The genotypes of 19 BC_4F_2 (NX-040*4/PI 181996) plants, segregating for PI 181996 rust resistance, was determined by progeny testing approximately 18 $BC_4F_{2:3}$ individuals from each F_2 plant (Stavely 1984). To increase the size of our segregating population for estimating linkage distances, 57 $BC_4F_{2:3}$ progeny were obtained from one BC_4F_2 individual heterozygous for PI 181996 resistance. The genotype of each $BC_4F_{2:3}$ plant was identified by progeny testing approximately 20 $BC_4F_{3:4}$ individuals.

DNA extraction

DNA was extracted from newly opened trifoliate leaf tissue using a modified Afanador et al. (1993) mini-prep procedure. An electronic screwdriver adapted to accommodate disposable pestles (Kontes Glass, Vineland, N.J.) produced uniform maceration in a much shorter time.

The DNA was quantified by fluorometry (Hoefer TKO 100, Hoefer Scientific, San Francisco, Calif.) and standardized to 10 ng/µl. DNA yields ranging from 100–300 µg/ml were obtained from the newly opened leaves of 2-week-old plants.

Bulked segregant analysis and PCR protocols

Based on progeny tests, contrasting DNA bulks were formed from equal volumes of standardized DNA from four homozygous-resistant and five homozygous-susceptible BC_4F_2 plants. The DNA bulks, representing near-isogenic lines (NILs), and the parents were screened with 130 decamer primers (Operon Technologies, Alameda, Calif.) in the polymerase chain reaction (PCR) to identify polymorphism between the bulks.

Amplification was done in 25-µl reactions using the Haley et al. (1993 b) reduction in denaturation and annealing times protocol. Amplified products were separated for 5 h at 3 V/cm constant voltage on 1% agarose gels containing 30 µg of ethidium bromide.

Polymorphisms either in coupling- or repulsion-phase linkage to PI 181996 resistance were confirmed by screening the segregating populations of 76 lines. Once established, linkage distances were estimated by maximum likelihood using the program Linkage-1 (Suiter et al. 1983).

Determination of RAPD sequence homology

A RAPD marker identical in size to a RAPD linked in coupling was observed in susceptible common bean. To determine if there was DNA sequence homology between this RAPD and the coupling RAPD, DNA hybridization studies were conducted by Southern blotting. Products amplified with the decamer primer for the coupling RAPD, as previously described, were blotted by Southern transfer to a Biodyne A nylon membrane following the manufacturer's instructions (Bethesda Research Laboratories, Bethesda, Md.). The coupling RAPD probe was isolated from three homozygous-resistant BC_4F_2 plants using the procedure described by Hansen et al. (1993). It was labeled with digoxigenin (DIG)-11-dUTP according to the DNA Random Labeling procedures in the Genius System (Boehringer Mannhem Corp. Indianapolis, Ind.) User's Guide. Hybridization was performed in a solution of 50% (v/v) formamide, 5× SSC buffer (750 mM NaCl, 75 mM sodium citrate; pH 7.0), 0.02% SDS, 0.1% N-laurylsarcosine, 2% Genius Blocking Reagent at a stringency of T_m– 8 °C for 24 h. Labeled RAPDs were detected by chemiluminescence with anti-DIG Fab fragments conjugated to alkaline phosphatase using Lumi-Phos 530 as a substrate (Detection of DIG-labeled nucleic acids: Genius System User's Guide).

Results

The RAPD marker $OAC20_{490}$, generated by decamer 5′-ACGGAAGTGG-3′, was linked in coupling with PI 181996 resistance (Fig. 1). No recombination between the marker and the PI 181996 resistance was observed in the segregating population (Table 1). The RAPD marker $OAE19_{890}$ amplified by decamer 5′-GACAGTCCCT-3′, was linked in repulsion (Fig. 2) with the resistance locus at an estimated distance of 6±3 cM (Table 1). The PI 181996 resistance and the RAPD markers segregated as expected in the 76 lines (Table 2).

The selection efficiency for RAPDs $OAC20_{490}$ and $OAE19_{890}$, used separately and together as a codominant

Fig. 1 Amplification products of OAC20, 5′-ACGGAAGTGG-3′, obtained in the parents and BC_4F_2 (NX-040*4/PI 181996) lines segregating for PI 181996 resistance and the coupling-phase linked RAPD $OAC20_{490}$, indicated by an *arrow head*. *Lanes 1 and 2* are the recurrent parent NX-040. *Lanes 3 and 4* contain the donor parent PI 181996. *Lanes 5 and 6* are homozygous-resistant lines. *Lanes 7 and 8* contain homozygous-susceptible lines. *Lanes 9 and 10* are heterozygous lines. *M* is the DNA molecular size marker *Eco*RI/*Hin*dIII digest of λ DNA

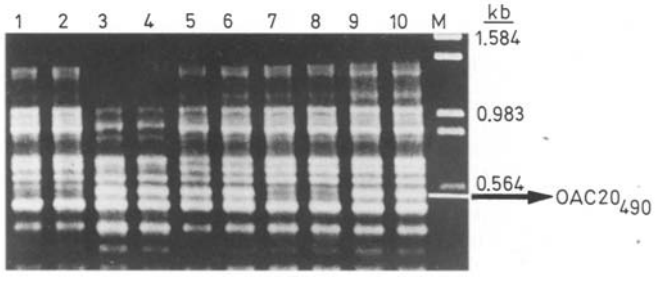

Table 1 Summary of linked loci, observed frequencies, χ^2 values for goodness of fit to expected ratios, and linkage distances between the PI 181996 resistance locus, coupling RAPD $OAC20_{490}$ and repulsion RAPD $OAE19_{890}$

Loci	Observed frequencies						χ^2	P	Linkage distance (cM)
	3	6	3	1	2	1[a]			
PI 181996 $OAC20_{490}$	10	43	0	0	0	23	76.0	0.00	–[b]
PI 181996 $OAE19_{890}$	0	41	22	10	2	1	55.8	0.00	6 ± 3[c]
	9	3	3	1[a]					
$OAC20_{490}$ $OAE19_{890}$	41	12	22	1			3.8	0.05	25 ± 11

[a] Expected frequencies
[b] No recombinants found between PI 181996 and $OAC20_{490}$
[c] Repulsion-phase linkage distance for PI 181996 rust resistance and $OAE19_{890}$

Table 2 Segregation ratios for PI 181996, $OAC20_{490}$ and $OAE19_{890}$

	Observed			Expected			Ratio	χ^2	P
PI 181996	10	43	23	19	38	19	1:2:1	5.76	0.06
$OAC20_{490}$	53		23	57		19	3:1	1.12	0.29
$OAE19_{890}$	63		13	57		19	3:1	2.53	0.11

Table 3 Percent selection efficiency expected with the coupling RAPD $OAC20_{490}$ and the repulsion RAPD $OAE19_{890}$ in MAS for homozygous-resistant individuals for PI 181996 rust resistance in the 19 BC_4F_2 and 57 BC_4F_3 lines

Genotypic frequency	No. of recombinants	Coupling[a] $OAC20_{490}$	Repulsion[b] $OAE19_{890}$	Codominant $OAC20_{490}$+ $OAE19_{890}$
RR-10	0	19	77	83
Rr-43	2	81	15	17
rr-23	1	0	8	0
Number selected		53	13	12

[a] Selection for the coupling-phase RAPD $OAC20_{490}$
[b] Selection against the repulsion-phase RAPD $OAE19_{890}$

pair to determine the genotypes of the 76 lines, is compared in Table 3. Selection for the RAPD in coupling results in selection of all 53 resistant progeny. Selection against the repulsion RAPD results in selection of one susceptible progeny, because of a recombination event at both susceptible loci in this individual. Selection efficiency is improved using both RAPDs as a codominant pair (Table 3) as the susceptible double-recombinant individual is not selected. In the absence of double recombinants, Haley et al. (1994 b) showed that selection efficiency is relatively equal for repulsion and paired codominant RAPD markers.

The usefulness, distribution, and degree of recombination between the RAPDs and PI 181996 resistance were investigated using an array of Andean and Middle American common bean genotypes (Table 4). The RAPD $OAC20_{490}$ was observed in all common bean lines into which PI 181996 resistance had been introgressed. A RAPD of similar size to $OAC20_{490}$ was prevalent in Middle American germplasm without PI 181996 resistance. RAPDs of similar sizes to those for the *Up-2* and *Ur-3* genes and the B-190 gene block were also observed in common bean germplasm without the corresponding genes (Haley et al. 1993 a; Haley et al. 1994 a; Miklas et al. 1993).

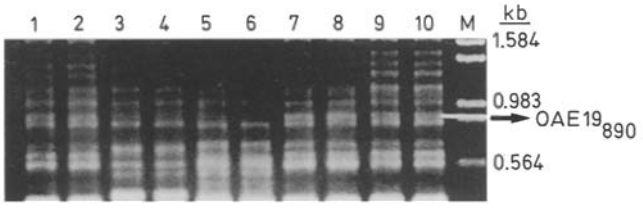

Fig. 2 Amplification products of OAE19, 5′-GACAGTCCCT-3′, obtained in the parents and BC_4F_2 (NX-040*4/PI 181996) lines segregating for PI 181996 resistance and the repulsion-phase linked RAPD $OAE19_{890}$, indicated by an *arrow*. *Lanes 1 and 2* are the recurrent parent NX-040. *Lanes 3 and 4* contain the donor parent PI 181996. *Lanes 5 and 6* are homozygous-resistant lines. *Lanes 7 and 8* contain homozygous-susceptible lines. *Lanes 9 and 10* are heterozygous lines. *M* is the DNA molecular size marker *Eco*RI/*Hin*dIII digest of λ DNA

Fig. 3 In **A** DNA fragments amplified by the primer OAC20 in common bean are shown. The RAPD $OAC20_{490}$ is indicated by a *solid arrow head*. The lanes marked by an "*X*" indicate common bean which contains PI 181996 resistance. In **B** the Southern blot probed with the RAPD $OAC20_{490}$ is shown. *Lanes 1 to 3* contain the kidney beans CELRK and Montcalm and the snap bean Eagle, respectively, all of Andean ancestry. *Lanes 4 to 6* contain the pinto lines BelMiDak RR-2, -5 and -6 respectively, of Middle American lineage. *Lane 7* is the snap bean BelJersey RR-15. *Lanes 8 and 9* contain homozygous-resistant lines. *Lanes 10 and 11* contain heterozygous-resistant and homozygous-susceptible lines respectively. The lines are those used in this study which are segregating for PI 181996 resistance. *Lane 12* is the pinto bean Arapaho of Middle American ancestry. *Lane 13* is the snap bean Early Gallatin of Andean parentage. *Lanes 14 and 15* are the plant introductions PI 151388 and PI 195369 classified as black beans of Middle American origin. *Lanes 16 and 17* contain the pinto lines BelDakMi RR-2 and -3 of Middle American lineage. *M* is the DNA molecular size marker λ *Eco*RI/*Hin*dIII digest

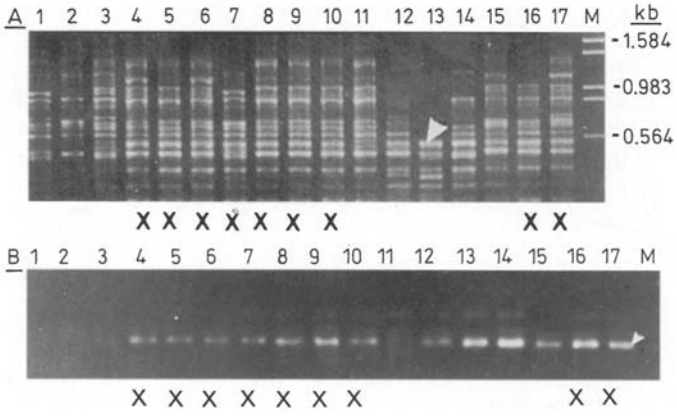

Table 4 The occurrence of RAPDs $OAC20_{490}$ and $OAE19_{890}$ in common bean

Type[a]	$OAC20_{490}$	$OAE19_{890}$	Type[a]	$OAC20_{490}$	$OAE19_{890}$
Kidney beans (A)			Black beans (MA)		
3M-152	–	–	B 85003	+	–
CDRK	–	–	B-190	+	+
CELRK	–	–	La Vega	+	+
Linden	–	–	XAN 176	–	–
Montcalm	–	–			
Royal Red	–	–			
Plant Introductions (A)			Plant Introductions (MA)		
PI 151385	+	–	PI 181996	+	–
PI 151388	+	–	PI 195369	+	–
			PI 195376	+	–
			PI 201486	+	–
			PI 311159	–	–
			PI 451895	+	–
Snap beans (A + MA)			Red beans (MA)		
*BelJersey RR-14	+	+	8437-95	–	–
*BelJersey RR-15	+	–	DOR 364	–	+
*BelJersey RR-16	+	+	Ecuador 299	–	–
*BelJersey RR-17	+	–			
Eagle	–	–			
Early Gallatin	+	–			
Sprite	+	–			
Pinto beans (MA)			Navy beans (MA)		
Arapaho	+	–	*BelMiDak RR-1	+	–
Bill Z	+	–	*BelMiDak RR-2	+	–
ND 88-065-42	–	+	*BelMiDak RR-3	+	+
Othello	+	+	*BelMiDak RR-4	+	+
P 90521	+	+	*BelMiDak RR-5	+	–
PX-057	+	–	*BelMiDak RR-6	+	–
*2-2186	+	+	C 20	–	+
*3-4510	+	+	NX-040	–	+
3-4537	+	–	Mayflower	–	+
BelDakMi RR-1	+	–	Sanilac	+	+
*BelDakMi RR-2	+	–	Schooner	–	+
*BelDakMi RR-3	+	–	Seafarer	+	–
*BelDakMi RR-4	+	–	Arroyo Loro	–	+

[a] A = Andean, MA = Middle American, RR = Rust resistant
+ = Present, – = Absent
* = Germplasm into which PI 181996 resistance was introgressed

DNA hybridization analysis has been suggested as a way to establish sequence homology between similar-sized RAPD fragments observed in individuals of undefined relationships (Weeden et al. 1992). Stringent Southern-blot analyses, with $OAC20_{490}$ as a probe, resulted in the hybridization of the $OAC20_{490}$ probe to the RAPD observed in common bean lacking PI 181996 resistance. This indicates that, in addition to being of similar size, the sequences of the RAPD observed in susceptible germplasm and the RAPD $OAC20_{490}$ are homologous (Table 4; Fig 3). Apparently, the RAPD $OAC20_{490}$ is ubiquitous in common bean of the same Middle American origin as PI 181996, but rare in material of Andean origin (Table 4).

Discussion

To be useful in MAS, a molecular marker must fulfil the following criteria. The technique used to generate the markers must be reliable, relatively simple to perform, and capable of processing a large number of samples per unit time. In addition, selection for the marker must signify selection for the gene if progeny testing, to identify individuals with the desired genes, is to be reduced.

The RAPD marker procedures are simple, quick, relatively inexpensive and can process many samples per day compared to restriction fragment length polymorphisms (RFLPs). Thus, RAPD procedures are potentially more readily incorporated into a plant breeding program than RFLPs. Indeed, the *Up-2* RAPD marker $OA14_{1100}$ was used to identify the *Up-2* gene, in the presence of PI 181996 resistance to which it is hypostatic, in developing the rust-resistant lines BelMiDak RR-7 (Kelly et al. 1993) and BelMiDak RR-8 and -9 (Stavely et al. 1994). Utilization of RAPDs for MAS will depend on marker orientation with the resistance allele and the type of population under analysis.

Haley et al. (1994 b) and our results support the use of RAPDs in repulsion-phase linkage to the target allele for

increased efficiency in MAS. In BC_nF_2 and F_2 or later-generation populations, selection against a RAPD marker linked in repulsion phase to a dominant gene for resistance, will separate homozygous-susceptible and heterozygous-resistant progeny from the homozygous-resistant individuals. Conversely, in BC_nF_1 (x% Rr:y% rr) populations of traditional backcross breeding, selection against a repulsion-phase RAPD will eliminate all progeny. Thus, during a traditional backcross breeding program only coupling-phase RAPDs are necessary to obtain the resistant individuals by MAS. Selection efficiency is improved by using coupling- and repulsion-RAPDs together as a codominant pair of markers, in an F_2 or later-segregating population, when double recombinants occur.

Previously, markers in coupling phase were observed in susceptible bean germplasm without the gene of interest (Haley et al. 1993 a; Miklas et al. 1993). The RAPD $OAC20_{490}$ was also found in common bean lacking PI 181996 resistance. The occurrence of the RAPD without the gene may be explained by either a recombination event between the gene and the RAPD or non-homology of similar-size fragments. The results of our DNA hybridization studies, using the RAPD $OAC20_{490}$ as a probe, showed that the observed RAPD in susceptible common bean shared both size and sequence in common with the RAPD $OAC20_{490}$ linked in coupling phase to PI 181996 resistance.

There are inherent difficulties in the application of RAPDs in MAS, such as band reliability between runs and multiple step protocols. To overcome these problems, sequence-characterized amplified regions (SCARs) (Kesseli et al. 1992; Paran and Michelmore 1993) or allele-specific associated primers (ASAPs) (Weeden et al. 1992) have been described. The SCARs and ASAPs use longer (25-mers) primers in pairs to specifically amplify a DNA fragment linked to a gene of interest. These SCARs and ASAPs are obtained by sequencing the ends of a RAPD, originally amplified by a single short primer (8- to 14-mers) of random sequence. Once developed, SCARs and ASAPs shorten the protocol necessary to identify individuals with the marker, as dye or fluorometric quantification replace electrophoretic detection of the amplified DNA fragment (Weeden et al. 1992). However, the SCAR and ASAP techniques will not overcome the inability, at present, to use RAPDs for MAS of introgressed resistance in susceptible germplasm with coupling or without repulsion markers.

We have used bulked segregant and RAPD analyses to tag the PI 181996 rust resistance in *P. vulgaris*. The coupling- and repulsion-phase RAPDs $OAC20_{490}$ and $OAE19_{890}$ identified, will be useful in the introgression of PI 181996 resistance into bean breeding lines with or without other rust resistance genes. The RAPDs will be most useful in the introgression of the PI 181996 resistance into common bean germplasm either lacking $OAC20_{490}$, such as kidney bean, or possessing $OAE19_{890}$, such as navy bean (Table 4). Similarly, the *Up-2* RAPD is only useful in the Middle American, and not the Andean gene pool which is the source of the gene (Miklas et al. 1993). The gene-pool specificity between our coupling- and repulsion-phase RAPDs and genetic markers previously identified deserves further attention. Perhaps better populations can be developed for obtaining genetic markers with broader utility for MAS.

When applicable, it is envisaged that the coupling-phase RAPD $OAC20_{490}$ will be most useful in MAS of heterozygous-resistant BC_nF_1 individuals for PI 181996 resistance during backcross breeding. The repulsion-phase RAPD $OAE19_{890}$ will have greatest utility in identifying homozygous-resistant individuals for PI 181996 resistance in F_2 and subsequent segregating generations. The use of these and the previously identified RAPDs will reduce the need for progeny testing and the time and cost of developing rust resistant lines and cultivars with multiple rust-resistance genes. However, initial screening for the RAPDs in germplasm to which PI 181996 resistance is to be introgressed is advised.

References

Afanador LK, Haley SD, Kelly JD (1993) Adoption of a "mini-prep" DNA extraction method for RAPD marker analysis in common bean (*Phaseolus vulgaris* L.). Annu Rep Bean Improv Coop 36:10–11

Barua UM, Chalmers KJ, Hackett CA, Thomas WTB, Powell W, Waugh R (1993) Identification of RAPD markers linked to a *Rhynchosporium secalis* resistance locus in barley using near-isogenic lines and bulked segregant analysis. Heredity 71: 177–184

Grafton KF, Chang KC, Venette JR, Vander Wal AJ (1993) Registration of 'Norstar' navy bean. Crop Sci 33:1405–1406

Haley SD, Miklas PN, Stavely JR, Byrum J, Kelly JD (1993 a) Identification of RAPD markers linked to a major rust resistance gene block in common bean. Theor Appl Genet 86: 505–512

Haley SD, Afanador L, Kelly JD (1993 b) Further optimization of random amplified polymorphic DNA (RAPD) analysis in common bean. Annu Rep Bean Improv Coop 36:12–13

Haley SD, Afanador LK, Miklas PN, Stavely JR, Kelly JD (1994 a) Heterogeneous inbred populations are useful as sources of near-isogenic lines for RAPD localization. Theor Appl Genet 88: 337–342

Haley SD, Afanador L, Kelly JD (1994 b) Selection for monogenic pest resistance traits with coupling- and repulsion-phase RAPD markers. Crop Sci 34:1061–1066

Hansen H, Hilmar L, Bodner U (1993) Rapid and simple purification of PCR products by direct band elution during agarose-gel electrophoresis. Biotechniques 14:28–30

Kelly JD, Stavely R, Miklas P, Afanador L, Haley SD (1993) Pyramiding rust resistance genes using RAPD markers. Annu Rep Bean Improv Coop 36:166–167

Kesseli RV, Paran I, Michelmore RW (1992) Efficient mapping of specifically targeted genomic regions and the tagging of these regions with reliable PCR-based genetic markers. Proc Symp Applications of RAPD Technology to Plant Breeding. Joint Plant Breeding Symposia Series. 1 Nov Minneapolis, pp 31–36, ASA, Madison, WI

Kolmer JA, Groth JV (1984) Inheritance of a minute uredium infection type of bean rust in bean breeding line 814. Phytopathology 74:205–207

Luann Finke M, Coyne DP, Steadman JR (1986) The inheritance of resistance to rust, common bacterial blight, plant habit and foliar abnormalities in *Phaseolus vulgaris* L. Euphytica 35:969–982

Melchinger AE (1990) Use of molecular markers in breeding for oligogenic disease resistance. Plant Breed 104:1–19

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance genes by bulked segregant anal-

ysis: a rapid method to detect markers in specific genomic regions using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Miklas PN, Stavely JR, Kelly JD (1993) Identification and potential use of a molecular marker for rust resistance in common bean. Theor Appl Genet 85:745–749

Paran I, Michelmore RW (1993) Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet 85:985–993

Pedersen WL, Leath S (1988) Pyramiding major genes for resistance to maintain residual effects. Phytopathology 26:369–378

Stavely JR (1984) Genetics of resistance to *Uromyces phaseoli* in a *Phaseolus vulgaris* line resistant to most races of the pathogen. Phytopathology 74:339–344

Stavely JR (1990) Genetics of rust resistance in *Phaseolus vulgaris* plant introduction PI 181996 (abstract). Phytopathology 80:1056

Stavely JR, Batra LR (1991) Recently identified new pathogenic variability in *Uromyces appendiculatus* (abstract). Phytopathology 81:704

Stavely JR, Pastor-Corrales MA (1989) Rust. In: Schwartz HF, Pastor-Corrales MA (eds) Bean production problems in the tropics, 2nd edn. CIAT, Cali, Colombia, pp 159–194

Stavely JR, Steadman JR, McMillan RT Jr (1989) New pathogenic variability in *Uromyces appendiculatus* in North America. Plant Dis 73:428–432

Stavely JR, Steinke J, McMillan RT Jr, Grafton KF, Steadman JR, Kelly JD, Coyne DT, Lindgren DT, Silbernagel JM (1992) Rust-resistant bean germplasm releases. Annu Rep Bean Improv Coop 35:228–229

Stavely JR, Kelly JD, Grafton KF (1994) BelMiDak-rust-resistant navy dry bean germplasm lines. HortScience 29:709–711

Suiter AK, Wendel JF, Case JS (1983) LINKAGE-1: a PASCAL computer program for the detection and analysis of genetic linkage. J Hered 74:203–204

Velez-Martinez H, Lopez-Rosa J, Freytag GF (1989) Determination of yield loss caused by rust [*Uromyces phaseoli* (Reben) Wint.] incommon bean (*Phaseolus vulgaris* L) in Puerto Rico. Annu Rep Bean Improv Coop 32:134–135

Weeden NF, Timmerman GM, Hemmat M, Kneen BE, Lodhi MA (1992) Inheritance and reliability of RAPD markers. Proc Symp Applications of RAPD Technology to Plant Breeding. Joint Plant Breeding Symposia Series. 1 Nov Minneapolis, pp 12–17, ASA, Madison, WI