Theor Appl Genet (1994) 88:337–342

S. D. Haley · L. K. Afanador · P. N. Miklas
J. R. Stavely · J. D. Kelly

Heterogeneous inbred populations are useful as sources of near-isogenic lines for RAPD marker localization

Received: 15 April 1993 / Accepted: 9 September 1993

Abstract The development and use of RAPD markers for applications in crop improvement has recently generated considerable interest within the plant breeding community. One potential application of RAPDs is their use for "tagging" simply-inherited (monogenic) pest-resistance genes and enabling more efficient identification and selection of genotypes carrying specific combinations of resistance genes. In this report, we propose and describe the use of heterogeneous inbred populations as sources of near-isogenic lines (NILs) for targeting RAPD markers linked to major pest resistance genes. The development of these NILs for RAPD marker analyses involved a sequence of line and mass selection during successive generations of inbreeding. DNA bulks derived from the NILs were used to identify a RAPD marker (designated $OK14_{620}$, generated by 5′-CCCGCTACAC-3′ decamer) that was tightly linked (2.23 ± 1.33 centiMorgans) to an important rust [*Uromyces appendiculatus* (Pers.) Unger var. *appendiculatus*] resistance gene (*Ur-3*) in common bean (*Phaseolus vulgaris* L.). The efficiency of this approach was demonstrated by a low rate of false-positives identified, the tightness of the linkage identified, and the ability to detect polymorphism between genomic regions that are representative of the same gene pool of common bean. This method of deriving NILs should find application by researchers interested in utilizing marker-assisted selection for one or more major pest resistance genes. The identification of $OK14_{620}$ should help to facilitate continued use of the *Ur-3* resistance source and will now enable marker-assisted pyramiding of three different bean rust resistance sources (two previously tagged) to provide effective and stable resistance to this important pathogen.

Key words *Phaseolus vulgaris* L. · Bean rust resistance · Polymerase chain reaction (PCR) · DNA markers · Near-isogenic lines (NILs)

Research supported in part by the grant DAN 1310-G-SS-6008-00 from the USAID Bean/Cowpea Collaborative Research Support Program, the Michigan Agricultural Experiment Station, and the USDA-ARS. Mention of a trademark or a proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable

Communicated by A. L. Kahler

S. D. Haley[1] · L. K. Afanador · J. D. Kelly (✉)
Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824, USA

P. N. Miklas
USDA-ARS, Tropical Agricultural Research Station, P.O. Box 70, Mayaguez 00681, Puerto Rico

J. R. Stavely
Microbiology and Plant Pathology Laboratory, Plant Sciences Institute, USDA-ARS, Beltsville, MD 20705, USA

Present address:
[1] Department of Plant Sciences, South Dakota State University, Brookings, SD 57007, USA

Introduction

The concept of "marker-assisted selection", as applied to monogenic disease or other pest resistances, dictates that selection for one or more such resistance genes is practiced by selection for a marker (or two flanking markers) tightly linked to the gene of interest (Melchinger 1990). The use of this approach with morphological markers has been greatly limited primarily due to the low numbers of such markers available in most crop species, their major effects on plant phenotype (often deleterious mutants), and the inability to score multiple morphological-mutant traits in a single segregating population (Paterson et al. 1991). To a certain extent, these limitations have been relieved with the development of markers based on allelic variants of specific enzymes (isozymes; Tanksley and Orton 1983) and, most notably, those based on variation in the length of DNA fragments obtained by digestion with restriction endonucleases (RFLPs; Botstein et al. 1980). RFLP

markers offer significant advantages with respect to increased numbers of loci and alleles detectable, their overall phenotypic neutrality, and the ability to score such markers at any stage of plant development. Although RFLP-based genetic linkage maps have been constructed recently in several crop species (Paterson et al. 1991), including common bean (*Phaseolus vulgaris* L.) (Vallejos et al. 1992; Nodari et al. 1993), these efforts are laborious and intensive and do not generally offer the opportunity to efficiently target tight linkages to specific loci of interest. Furthermore, the choice of parents for developing mapping populations in most crop species has often been based more on optimizing RFLP variation and less on the basis of polymorphism between parents for traits of agronomic interest.

To alleviate these potential limitations, two major strategies have emerged for targeting DNA markers tightly linked to specific loci ("gene tagging"). The first of these, using backcross-derived near-isogenic lines (NILs) has proven extremely useful for targeting tightly-linked RFLP markers in several important crop species (Young et al. 1988). A newer form of DNA marker, the random amplified polymorphic DNA (RAPD) marker (Welsh and McClelland 1990; Williams et al. 1990), has also proven useful for efficiently targeting tightly-linked markers using backcross-derived NILs (Martin et al. 1991). In common bean, few such pairs of backcross-derived NILs are presently available and, furthermore, their development solely to enable gene tagging with RFLP or RAPD markers is generally not possible for more than a few different resistance sources.

The second major strategy for targeting tightly-linked DNA markers has been referred to as "bulked segregant analysis" (Michelmore et al. 1991). Bulked segregant analysis alleviates the problems associated with the unavailability of backcross-derived NILs by utilizing DNA-bulks of resistant and susceptible individuals in a segregating population to screen for polymorphisms that are linked to the target locus. Successes have been realized using RAPD markers and conventional bulked segregant analysis (Michelmore et al. 1991) as well as bulked segregant analysis in combination with backcross-derived NILs (Haley et al. 1993; Miklas et al. 1993). Widespread use of this method is expected and many reports are likely to be forthcoming as appropriate populations are developed.

Many conventional breeding procedures have been used for the improvement of oligogenic pest resistances in autogamous crop species. Of these, the backcross breeding method has been used widely in many crop species to introgress resistance genes from unadapted sources into the background of a susceptible, but otherwise desirable, cultivar or experimental line. Many oligogenic resistances, however, are commonly found in germplasm or in experimental lines that are themselves products of selective improvement and are desirable in terms of adaptation or productivity. For these situations, breeders have most often chosen other common breeding methods (pedigree, modified-pedigree, bulk breeding methods, or some combination thereof) for handling segregating generations during inbreeding. These methods have proven very useful in applied breeding efforts for oligogenic pest resistances and many resistant cultivars have been produced by these procedures. Although the end-products of these breeding methods are fundamentally different, each serves to fix homozygosity at regions unlinked to the resistance locus. In backcross breeding, the maintenance of heterozygosity at the resistance locus, while systematically progressing toward homozygosity elsewhere, is the fundamental reason why backcross-derived NILs have proven so useful for targeting tightly-linked DNA markers. Other common breeding methods that serve to maintain heterozygosity at the resistance locus and fix homozygosity elsewhere should provide genetic materials that may be used in the same fashion as backcross-derived NILs.

In this paper, we propose and describe the use of heterogeneous inbred populations as sources of NILs for targeting RAPD markers tightly-linked to major resistance genes. The method entails the development of a form of NIL through a process characteristic of cultivar and line development in many autogamous crop species: inbreeding through self-pollination rather than backcrossing. Using this method, we identified a RAPD marker tightly linked to a major bean rust [*Uromyces appendiculatus* (Pers.) Unger var. *appendiculatus*] resistance locus (*Ur-3*; Ballantyne 1978; Stavely et al. 1989). This form of NIL should find widespread application by researchers who are involved in breeding for major oligogenic resistance traits using marker-assisted selection.

Materials and methods

Plant material

The bean rust resistance utilized and tagged in our studies was originally derived from tropical black bean germplasm (through chemical mutagenesis of cultivar 'San Fernando'; Moh 1971) via the small, white-seeded cultivar 'NEP-2'. NEP-2 is a rust-resistant differential cultivar that develops distinct small necrotic spots or flecks (HR; grade 2 or $2,2^+$) with 26 races and small sporulating pustules (R; < 0.3 mm diameter, grade 3) with four races in the standard bean rust collection (Stavely 1984 b; Stavely et al. 1989). The resistance from NEP-2 was incorporated into upright, Type II dry bean cultivars with the development of 'C-20' navy bean (Kelly et al. 1984) which was subsequently used by Kelly and Adams (1987) in the development of 'Sierra' pinto (Kelly et al. 1990) and 'Alpine' Great Northern (Kelly et al. 1992). This resistance is commonly referred to as the 'Aurora gene' after the rust-resistant differential cultivar 'Aurora' and has been given the symbol *Ur-3* (Ballantyne 1978). Evidence suggests that this resistance consists of a tightly-linked block of completely dominant resistance genes (Stavely et al. 1989), as has been shown with other rust resistance sources in common bean (Stavely 1984 a).

The development of near-isogenic lines for RAPD marker analyses involved a sequence of line and mass selection during successive segregating generations of inbreeding. In 1986, a cross was made between a rust-resistant (P86300; full-sib of Sierra) and a rust-susceptible (P86295) breeding line. This cross was advanced to the F_2 generation in the greenhouse and space-planted in the field (Saginaw, Mich.) in 1987. Five single-plant selections were made (for seed type,

upright growth habit, and agronomic adaptation) and subsequently grown in a winter nursery in 1987–1988 (Puerto Rico). One of these $F_{2:3}$ lines was mass-selected for desirable pinto seed characteristics and advanced to the F_4 generation. In 1988, this $F_{2:4}$ line was grown in the field (Saginaw, Mich.) and again mass-selected for desirable agronomic and seed characteristics. A winter nursery in 1988–1989 (Puerto Rico) was used to advance this line without selection to the F_6. In 1989, the $F_{2:6}$ generation line was planted in the field (Saginaw, Mich.) for preliminary yield trials and also for rust evaluations (as described below). Seven single-plant selections were made based on agronomic traits, seed characteristics, and resistance to rust. Five of these single-plant selections were advanced to the F_8 (as mass selected $F_{6:8}$ lines) in a winter nursery in 1989–1990 (Puerto Rico). These were again grown in yield trials and a rust nursery (Saginaw, Mich.) in 1990. Line number 90T-4042 was observed to be segregating [HR: grade 2, 2^+; S: grade 6 (sporulating pustules > 0.8 mm diameter)] for reaction to rust while the other four $F_{6:8}$ generation sibs were uniformly resistant. The heterogeneous reaction to rust must have arisen from a heterozygous $F_{2:6}$ generation plant selected for rust resistance and maintained as heterogeneous mass-selected line until the F_8 generation. Two near-isogenic lines within 90T-4042 were selected (resistant 90T-4042R; susceptible 90T-4042S) based on greenhouse rust evaluations on remnant $F_{6:8}$ generation materials.

The material used for RAPD marker analyses consist of a bulked segregant population derived from the cross of 90T-4042R with 90T-4042S. An F_2 population of 70 individual plants was developed (coded A4513) from this cross and evaluated for rust resistance under greenhouse conditions (described below). Separate DNA bulks, constructed using the DNA of four resistant and four susceptible F_2 individuals, were screened with random decamer primers to identify RAPD markers tightly linked to the *Ur-3* locus (described below). Progeny testing was not done *a priori* to distinguish homozygous resistant and heterozygous resistant F_2 plants for composition of the resistant DNA bulk.

A second population was developed to evaluate linkage in a cross representative of our pinto bean breeding program. The parents of this cross, 'Olathe' pinto (Wood and Keenan 1982) and Sierra, show contrasting respones to rust races that are used to distinguish *Ur-3*. Crosses were made and advanced to the F_2 generation in the greenhouse. Field and greenhouse inoculations (described below) were used to determine the rust resistance reaction of 125 individual F_2 plants that were subsequently used for segregation analyses.

Rust evaluations

All rust evaluations were done using spore suspensions of rust race 53. This rust race facilitates unambiguous identification of the presence of the *Ur-3* gene block by producing characteristic large uredinia (grade 6) on susceptible plants and characteristic hypersensitive flecks or spots on resistant (HR) plants (Stavely 1984b; Stavely et al. 1989). Greenhouse inoculations were conducted as described previously (Haley et al. 1993). Field inoculations were conducted (for the development of 90T-4042 and the Olathe/Sierra population) in space-planted nurseries at the third-trifoliate stage and again after 2 weeks. A portable, battery-powered sprayer (atomizer) was used to deliver an inoculum suspension calibrated to approximately 2×10^4 spores ml^{-1}. Disease ratings were made 12 days after inoculation when typical large uredinia had developed on the leaves of the susceptible Olathe parent. Progeny tests of both segregating populations were conducted in the greenhouse with ten plants from each $F_{2:3}$ line to confirm the disease rating of the F_2 plants.

RAPD analyses

The general procedures for RAPD and statistical analyses [Chi-square (χ^2) and linkage] were as previously described (Haley et al. 1993, 1994; Miklas et al. 1993). Genomic DNA was extracted from greenhouse-grown tissue of the A4513 population (Miklas et al. 1993) while DNA from the field-grown Olathe/Sierra population was collected, prior to inoculation with rust spores, using a modified "mini-prep" procedure reported previously (Haley et al. 1994). The RAPD fragment identified herein was originally detected using the contrasting DNA bulks described previously and a polymerase chain reaction (PCR) cycling profile (as reported by Miklas et al. 1993) that lacked consistency and repeatability for this particular primer-DNA combination. Several modifications of our basic PCR cycling program were made in attempts to enhance the quality of the putatively-linked DNA fragment. The following cycling profile in a Perkin Elmer Cetus DNA Thermal Cycler 480 was eventually developed: one cycle of 3 min/94 °C, 1 min/35 °C, and 90 s/72 °C; 2 cycles of 1 min/94 °C, 1 min/35 °C, and 90 s/72 °C; 34 cycles of 1 min/94 °C, 1 min/40 °C, and 90 s/72 °C; and one final extension cycle of 5 min/72 °C. This cycling profile provided much enhanced repeatability and appeared to preferentially enhance the amplification of smaller DNA fragments. We also explored the use of the Perkin Elmer Cetus DNA Thermal Cycler 9600 in attempts to optimize the repeatability of the PCR for the putatively-linked RAPD fragment. Amplification reactions were prepared as with the PE 480. The cycling profile included one cycle of 4 min/94 °C; three cycles of 15 s/94 °C, 15 s/35 °C, and 75 s/72 °C; 34 cycles of 15 s/94 °C, 15 s/40 °C, and 75 s/72 °C; and one final extension cycle 5 min/72 °C. the duration of the transition period (ramping) between template denaturation and primer annealing was set at 45 s and was extremely critical for the success of the reactions.

Results and discussion

Efficiency of NILs derived from heterogeneous inbreds

The search for a RAPD marker tightly linked to the *Ur-3* locus involved screening of 365 total random decamer primers against the resistant and susceptible screening bulks. Of these, only six primers (6/365 = 1.6%) generated a DNA fragment that was polymorphic between the screening bulks yet did not cosegregate closely (< 30 centiMorgans) with the *Ur-3* locus. We previously reported the identification of such "false-positives" using screening bulks derived from a combination of bulked segregant analysis and backcross-derived NILs (Haley et al. 1993; 12/306 primers = 3.9%). We note that the reduced occurrence of false-positives in the present study was observed using NILs that had been selected only twice (in F_6 and F_8 generations) for the *Ur-3* resistance, as opposed to successive screenings as is required for backcross-derived NILs.

We believe that this improved efficiency in targeting tightly-linked markers may be explained on the basis of three main factors. First, we have demonstrated that greater levels of RAPD-based variability are observed between more-genetically-dissimilar germplasm sources of common bean (Haley et al. 1994). In this study, our NILs originated by within-gene pool introgression of *Ur-3* from race Mesoamerica to race Durango, a much narrower introgression than between gene pools (Andean and Middle American; Singh et al. 1991) as utilized previously (Haley et al. 1993; Miklas et al. 1993). A reduced occurrence of false-positives should therefore be observed with narrower genetic divergence where fewer polymorphisms are expected. A second, and likely very important, factor was that the NILs developed in our study were highly inbred (F_8) and thus the level of residual heterozygosity unrelated to the *Ur-3* locus was likely to be less than that present in the backcross-derived NILs used previously (Haley et al. 1993; Miklas

et al. 1993). Melchinger (1990) indicated that the probability of false-positives decreases with increased inbreeding (backcrossing with selection for the resistance allele) because the length of the introgressed segment is reduced at a lower rate than those segments not linked to the target gene. A third potential explanation is our bulking strategy which may have served to mask any residual heterozygosity present between the original 90T-4042R and 90T-4042S NILs. It would be interesting to determine if this bulking strategy actually improved our efficiency or if direct screening of 90T-4042R and 90T-4042S would have resulted in the same number of false-positives.

Inheritance and linkage of Ur-3 and $OK14_{620}$

One DNA fragment, designated $OK14_{620}$ (generated by a 5′-CCCGCTACAC-3′ decamer) (Fig. 1), was found to be polymorphic between the screening bulks and then subsequently confirmed to be linked to the *Ur-3* locus in both segregating populations. This DNA fragment was identified quite early in the screening process, yet problems with repeatability of the reaction initially prevented analysis of the RAPD marker within the two segregating populations. Many other primers were subsequently screened in attempts to identify other linked RAPD markers. The lack of other linked RAPDs with additional screening led to attempts at further optimization and refinement of the original PCR protocol (Miklas et al. 1993) which led to the development of the protocol reported herein (see Materials and methods). The PE9600 DNA Thermal Cycler (Fig. 1B) provided highly-repeatable results consistent with amplification reactions on the PE480 Thermal Cycler.

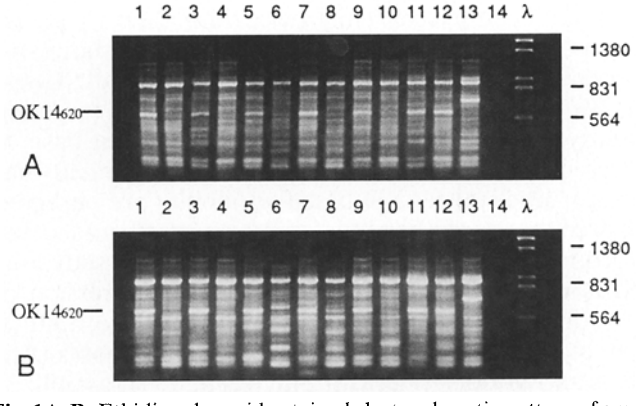

Fig. 1A, B Ethidium bromide-stained electrophoretic pattern of amplified DNA depicting $OK14_{620}$ RAPD fragment from PE 480 (**A**) and PE 9600 (**B**) DNA Thermal Cyclers. Key to Individuals: (*1*) 90T-4042R parent, (*2*) 90T-4042S parent, (*3*) Sierra parent, (*4*) Olathe parent, (*5*) rust-resistant DNA bulk, (*6*) rust-susceptible DNA bulk, (*7*) rust-resistant F_2 (A4513) individual, (*8*) rust-susceptible F_2 (A4513) individual, (*9*) rust-resistant F_2 (Olathe/Sierra) individual, (*10*) rust-susceptible F_2 (Olathe/Sierra) individual, (*11*) BELJERSEY-RR-1 snap bean, (*12*) rust-differential NEP-2, (*13*) PI181996 line, (*14*) negative control (no template DNA added to PCR amplification), (*15*) molecular weight marker (λ *Hin*dIII/*Eco*RI; size of bands indicated in bp)

Table 1 Chi-square (χ^2) and linkage analyses[a]

Locus	Expected ratio	Observed frequency	χ^2	*P*
Ur-3(A4513)	3:1	52:18	0.00	1.00
Ur-3 (Olathe/Sierra)	3:1	85:40	2.90	0.08
$OK14_{620}$(A4513)	3:1	52:18	0.00	1.00
$OK14_{620}$(Olathe/Sierra)	3:1	86:39	2.24	0.13
Ur-3/$OK14_{620}$(A4513)	9:3:3:1	52:0:0:18	70.00	0.00
Ur-3/$OK14_{620}$(Olathe/Sierra)	9:3:3:1	84:1:2:38	111.50	0.00
Linkage estimates				
A4513	No recombinants			
Olathe/Sierra	2.23 ± 1.33 cM			

[a] Standard error not defined

Monogenic inheritance of the *Ur-3* and $OK14_{620}$ loci was confirmed in both segregating populations (Table 1). The close fit observed in the A4513 population strongly suggests that the original resistant NIL parent in this cross, 90 T-4042R, was homozygous for the *Ur-3* resistance (90 T-4042R was not originally progeny-tested). Cosegregation analyses revealed a tight linkage between the *Ur-3* and $OK14_{620}$ loci in the A4513 population (Table 1). No recombinants were observed among 70 individuals segregating in this population. The analysis of the Olathe/Sierra population, however, revealed three recombinants (two susceptible plants with $OK14_{620}$ and one resistant plant without $OK14_{620}$) among 125 individuals segregating in the population. The recombination frequency within this population (2.23 ± 1.33 cM) demonstrates the closeness of linkage in a population representative of many typical crosses in our breeding program.

Germplasm survey

In previous studies (Haley et al. 1993; Miklas et al. 1993), we determined the potential utility of linked RAPD markers not only through linkage analysis but also by examination of the linkage relationship in a collection of common bean germplasm. These analyses have revealed definite patterns of utility for the RAPD markers, either being limited to use within certain gene pools (Miklas et al. 1993) or to use within certain races or market classes of the same gene pool (Haley et al. 1993). To assess possible gene pool or race specificity for the utility of $OK14_{620}$, we screened a collection of 105 common bean genotypes that are representative of most major bean market classes (Table 2). Several interesting findings are apparent from this survey.

In our survey, no gene pool or race specificity was observed with respect to the utility of $OK14_{620}$. With very few exceptions, the linkage relationship between *Ur-3* and $OK14_{620}$ appears to have remained intact during different introgression or meiotic events. This RAPD marker was found to be present in nearly all genotypes bred for the *Ur-3* resistance and absent in

Table 2 Survey of genotypes for presence of the *Ur-3* locus and the $OK14_{620}$ RAPD marker

Genotype[a]	*Ur-3*[b]	$OK14_{620}$[b]	Genotype	*Ur-3*	$OK14_{620}$
4-5753 (N)	R	+	L-226-10 (N)	R	+
51051 (RD)	R	+	L-227-1 (N)	R	+
90T-4042 R (P)	R	+	Mayflower (N)	R	+
90T-4042 S (P)	S	–	Mexico 235 (RD)	R	+
Alpine (GN)	R	+	Mexico 309 (B, RD)	S	–
Arapaho (P)	S	–	Midnight (B)	R	+
Aurora (N, RD)	R	+	Montcalm (K)	S	–
Aztec (P)	S	–	NEP-2 (N, RD)	R	+
B-190 (B)	S	+	OAC Rico (N)	S	–
BARC-RR (S)[c]	S	–	Olathe (P, RD)	S	–
BBL-47 (S)	S	–	Othello (P)	S	–
BELDAK-RR (P)[c]	R	–	Ouray (P)	S	–
BELDAKMI-RR (P)[c]	ND	–	P86263 (P)	R	+
BELJERSEY-RR (S)[c]	R	+	P86295 (P)	S	–
BELJERSEY-RR-8 (S)	R	–	P86300 (P)	R	+
BELMIDAK-RR (N)[c]	ND	+	PI151385	ND	–
BELNEB-RR (GN)[c]	S	–	PI151388	ND	–
Beryl (GN)	S	+	PI151395	ND	–
Black Magic (B)	R	+	PI181996	ND	–
Blackhawk (B)	S	–	PI189016	ND	–
BTS (B)	R	+	Pindak (P)	S	–
Bunsi (N)	R	–	Redkloud (K)	S	–
C-20 (N)	R	+	Sacramento (K)	S	–
CELRK (K)	S	–	Sanilac (N)	S	–
Charlevoix (K)	S	–	Seafarer (N)	S	–
Chinook (K)	S	–	Seaforth (N)	S	–
CNC (B, RD)	S	–	Sierra (P)	R	+
Crestwood (N)	S	–	Starlight (GN)	R	+
Domino (B)	R	+	SVM 29-21 (K)	S	–
Ecuador 299 (RD)	R	+	SVM 31-19 (K)	S	–
Fiesta (P)	S	–	SVM 37-16 (K)	S	–
Fleetwood (N)	S	+	SVM 40-23 (K)	S	–
Harofleet (N)	S	+	T-39 (B)	R	+
Harokent (N)	S	–	UI-114 (P)	S	–
Isabella (K)	S	–	UI-537 (PK)	S	–
Kentwood (N)	S	–	UI-59 (GN)	S	–

[a] Letters (in parentheses) following genotype names indicate market class or seed type: B, black bean; N, navy bean; GN, Great Northern bean; K, kidney bean; P, pinto bean; PK, pink bean; S, snap bean; RD, rust differential

[b] R, has *Ur-3*; S, does not have *Ur-3*; ND, not detectable; +, $OK14_{620}$ present; –, $OK14_{620}$ absent

[c] Germplasm releases: BARC-RR (6, 7, 10, 11, 12, 13, 14, 16, 17, 18, 19, 21, 22, 25, 26, 27); BELDAK-RR (1, 2); BELDAKMI-RR (1, 2, 3, 4); BELJERSEY-RR (1, 2, 3, 4, 5, 6, 7, 9); BELMIDAK-RR (1, 2, 3, 4, 5, 6, 7); BELNEB-RR (1, 2)

nearly all those known to be lacking *Ur-3*. The only observed exceptions were the pesence of $OK14_{620}$ in rust-susceptible 'Fleetwood' navy (and its backcross derivative 'Harofleet'), 'B-190' black bean, and 'Beryl' Great Northern bean cultivars, and the absence of $OK14_{620}$ in the rust-resistant 'Bunsi' navy bean cultivar and certain pinto (BELDAK-RR-1,-2; Stavely and Grafton 1989) and snap bean (BELJERSEY-RR-8; Stavely et al. 1992) germplasm releases bred to carry *Ur-3* resistance.

The germplasm survey for $OK14_{620}$ also serves to answer some pertinent questions about different rust resistance sources in common bean. All five standard bean rust differentials that are thought to possess the *Ur-3* locus based, on rust race screening (NEP-2, Aurora, 'Mexico 235', '51051', and 'Ecuador 299') (Stavely et al. 1989), also possess $OK14_{620}$ (Table 2). The absence of $OK14_{620}$ in all five plant introductions tested (Table 2) strongly suggests that their broad resistance (resistant to all races in the standard collection; Stavely 1988) does not include, at least, that portion of the *Ur-3* gene block that is linked to $OK14_{620}$.

Finally, the presence or absence of $OK14_{620}$ in certain dry bean germplasm releases highlights the usefulness of such RAPD markers in gene pyramiding with the resistance of the PI lines. The BELDAKMI-RR pinto bean and BELMIDAK-RR navy bean germplasm releases were developed using *Ur-3* resistance from various sources and rust resistance from PI lines (PI151388 or PI181996). The PI lines effectively prevent the identification of other nonallelic resistance genes (which are hypostatic to the resistance of the PI lines) when combined in the same genetic background. Had $OK14_{620}$ bean available during the development of these germplasm releases, it could have been used effectively to pyramid *Ur-3* with the resistance from the PI sources (for BELDAKMI-RR pintos which lacked $OK14_{620}$) and document that *Ur-3* was present following pyramiding efforts (for BELMIDAK-RR navies which showed $OK14_{620}$).

Conclusions

In this study we used NILs, derived by inbreeding through self-pollination rather than backcrossing, to identify a RAPD marker tightly linked to an important rust resistance locus in common bean. This form of NIL

differs from backcross-derived NILs previously used to tag resistance genes only to the extent that it bears no strict resemblance to a particular parent (recurrent parent) used in its development. The efficiency of this method was indicated by the low rate of false-positives identified, the tightness of the linkage identified, and the ability to detect polymorphism between genomic regions that are representative of the same gene pool of common bean. This form of NIL should find wide application by researchers concerned with targeting DNA markers, either RFLP or RAPD, tightly linked to important resistance genes. The availability of this form of NIL ultimately depends on the maintenance and eventual detection of heterozygosity at the locus conferring resistance following successive generations of inbreeding.

The *Ur-3* rust resistance has been utilized more extensively than other sources in conventional breeding for rust resistance in common bean. This resistance has been introgressed across gene pools into snap bean germplasm (Stavely et al. 1992), across races into pinto (Kelly et al. 1990) and Great Northern (Kelly et al. 1992) cultivars, and across market classes (within race Mesoamerica) into navy bean cultivars (Kelly et al. 1984, 1989) and germplasm (Stavely et al. 1992). The identification of $OK14_{620}$ will most likely promote the continued use of *Ur-3* in several bean market classes and, perhaps equally important, will now enable marker-assisted pyramiding of three different bean rust resistance sources (with two others previously tagged) (Haley et al. 1993; Miklas et al. 1993) to provide broad and stable resistance to the bean rust pathogen.

References

Ballantyne BJ (1978) The genetic basis of resistance to rust, caused by *Uromyces appendiculatus* in bean. PhD Dissertation, Sydney University, Australia

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331

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

Haley SD, Miklas PN, Afanador L, Kelly JD (1994) Random amplified polymorphic DNA (RAPD) marker variability between and within gene pools in common bean. J Am Soc Hort Sci 119:122–125

Kelly JD, Adams MW (1987) Phenotypic recurrent selection in ideotype breeding of pinto beans. Euphytica 36:69–80

Kelly JD, Adams MW, Saettler AW, Hosfield GL, Ghaderi A (1984) Registration of C-20 navy bean. Crop Sci 24:822

Kelly JD, Adams MW, Saettler AW, Hosfield GL, Varner GV, Beaver JS, Uebersax MA, Taylor J (1989) Registration of 'Mayflower' navy bean. Crop Sci 29:1571–1572

Kelly JD, Adams MW, Saettler AW, Hosfield GL, Varner GV, Uebersax MA, Taylor J (1990) Registration of 'Sierra' pinto bean. Crop Sci 30:745–746

Kelly JD, Hosfield GL, Varner GV, Uebersax MA, Miklas PN, Taylor J (1992) Registration of 'Alpine' Great Northern bean. Crop Sci 32:1509–1510

Martin GB, Williams JGK, Tanksley SD (1991) Rapid identification of markers linked to a *Pseudomonas* resistance gene in tomato by using random primers and near-isogenic lines. Proc Natl Acad Sci USA 88:2336–2340

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 analysis: 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

Moh CC (1971) Mutation breeding in seed-coat colors of beans (*Phaseolus vulgaris* L.) Euphytica 20:119–125

Nodari RO, Tsai SM, Gilbertson RL, Gepts P (1993) Towards an integrated linkage map of common bean. 2. Development of an RFLP-based linkage map. Theor Appl Genet 85:513–520

Paterson AH, Tanksley SD, Sorrells ME (1991) DNA markers in plant improvement. Adv Agron 46:39–90

Singh SP, Gepts P, Debouck DG (1991) Races of common bean (*Phaseolus vulgaris*, Fabaceae). Econ Bot 45:379–396

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

Stavely JR (1984b) Pathogenic specialization in *Uromyces phaseoli* in the United States and rust resistance in beans. Plant Dis 68:95–99

Stavely JR (1988) Rust resistance in beans: the plant introduction collection as a resource and resistant development. Annu Rep Bean Improv Coop 31:64–65

Stavely JR, Grafton KF (1989) Registration of BELDAK-Rust Resistant-1 and -2 pinto dry bean germplasm. Crop Sci 29: 834–835

Stavely JR, Steadman JR, McMillan RT (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 DP, Lindgren DT, Silbernagel MJ (1992) Rust-resistant bean germplasm releases. Annu Rep Bean Improv Coop 32:228–229

Tanksley SD, Orton TJ (1983) Isozymes in plant genetics and breeding, parts 1A and 1B. Elsevier Science, Amsterdam, Netherlands

Vallejos CE, Sakiyama NS, Chase CD (1992) A molecular marker-based linkage map of *Phaseolus vulgaris* L. Genetics 131:733–740

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 24:7213–7218

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Wood DR, Keenan JG (1982) Registration of Olathe bean. Crop Sci 22:1259–1260

Young ND, Zamir D, Ganal MW, Tanksley SD (1988) Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked to the *Tm-2a* gene in tomato. Genetics 20: 579–585