Theor Appl Genet (1994) 88: 865–870

A.F. Adam-Blondon · M. Sévignac · H. Bannerot · M. Dron

SCAR, RAPD and RFLP markers linked to a dominant gene (*Are*) conferring resistance to anthracnose in common bean

Received: 13 September 1993 / Accepted 21 December 1993

Abstract Anthracnose, caused by the fungus *Colletotrichum lindemuthianum*, is a severe disease of common bean (*Phaseolus vulgaris* L.) controlled, in Europe, by a single dominant gene, *Are*. Four pairs of near-isogenic lines (NILs) were constructed, in which the *Are* gene was introgressed into different genetic backgrounds. These pairs of NILs were used to search for DNA markers linked to the resistance gene. Nine molecular markers, five RAPDs and four RFLPs, were found to discriminate between the resistant and the susceptible members of these NILs. A backcross progeny of 120 individuals was analysed to map these markers in relation to the *Are* locus. Five out of the nine markers were shown to be linked to the *Are* gene within a distance of 12.0 cM. The most tightly linked, a RAPD marker, was used to generate a pair of primers that specifically amplify this RAPD (sequence characterized amplified region, SCAR).

Key words Resistance gene · Molecular markers *Colletotrichum lindemuthianum* · *Phaseolus vulgaris* L. Near-isogenic lines (NILs)

Introduction

Anthracnose is considered to be one of the most severe diseases of common bean (*Phaseolus vulgaris*) in the tropical areas of Latin America and Eastern Africa where this staple crop constitutes one of the major sources of protein (Lenné 1992). Anthracnose is caused by *Colletotrichum lindemuthianum*, a specialized hemibiotrophic fungus (Pastor-Corrales and Tu 1989). Fourteen different races of this fungus were identified by screening a set of differential cultivars (Menezes and Dianese 1988). The existence of at least seven dominant and independent resistance loci in common bean, allowing resistance to different races of the fungus, was demonstrated by genetic analysis (Beebe and Pastor-Corrales 1991). These data, together with a strict race/cultivar specificity, strongly suggest a gene-for-gene interaction between *C. lindemuthianum* and *P. vulgaris*. The lack of sexuality in this fungus, however, has hindered any study of the inheritance of avirulence in the pathogen.

In Europe (Fouilloux 1979) and North America (Park et al. 1987), anthracnose is controlled by a single dominant gene called *Are* (Mastenbroek 1960). Interestingly, the *Are* gene has not been overcome in these areas for 30 years whereas it has never controlled the disease in tropical areas (Pastor-Corrales and Tu 1989).

Breeding resistant varieties to anthracnose still represents a major effort and a long-term investment (Beckmann and Soller 1983). Marker-assisted breeding will certainly provide a major improvement for seed companies. This technique presents major advantages. For example, the use of tightly-linked genetic markers flanking the gene to be introgressed should considerably accelerate the breeding process (Young and Tanksley 1989). When resistance genes are to be introduced in susceptible inbred lines, marker-based techniques avoid cumbersome pathogenicity tests.

The development of molecular markers has enhanced the development of saturated genetic maps for major crops (Tanksley et al. 1989; Michelmore et al. 1992; Gardiner et al. 1993) including common bean (Vallejos et al. 1992; Adam et al. 1993; Nodari et al. 1993 a). In addition, the use of different types of randomly-dispersed molecular markers (RAPD, RFLP, STS, SCARs) (Michelmore et al. 1992; Paran and Michelmore 1993; Williams et al. 1993) in combination with appropriate genetic material (di-haploids, bulks of F_2 individuals, near-isogenic lines or NILs,

The research was supported by the CNRS and the Ministère Français de l'Education Nationale

Communicated by J. Beckmann

A.F. Adam-Blondon · M. Sévignac · M. Dron (✉)
Dépt Biol Mol Végétale, Bat 430, Université Paris Sud, 91405 Orsay Cedex, France

H. Bannerot
Station Amél Plantes, INRA-CNRA, Route de Saint-Cyr, 78026 Versailles Cedex, France

aneuploids) has favored the saturation of genetic regions near particular genetic loci (Beckmann and Soller 1983; Tanksley et al. 1989; Michelmore et al. 1992). These experimental approaches are also a prerequisite to clone genes of agronomic interest, such as resistance genes, for use with genome walking strategies (Debener et al. 1991; Martin et al. 1991; Michelmore et al. 1992).

In this paper, we present the identification of several molecular markers linked to the *Are* resistance gene from common bean. Four pairs of near-isogenic lines (NILs) were used to screen for polymorphic DNA markers putatively linked to *Are*. The markers (RAPD and RFLP) revealing polymorphisms for one or more of the four pairs were used to check for linkage to the *Are* gene using a backcross progeny. A SCAR marker was developed from a RAPD marker close to the *Are* gene.

Materials and methods

Plant and fungal material

Four pairs of NILs, 'CocoR/S', 'EyR/S', 'P12R/S' and 'SWR/S', carrying the *Are* gene in four different genetic backgrounds ('Coco', 'Early-wax', 'Processor' and 'Slender-White', respectively) were used. The *Are* gene was introduced from 'Cornell49242' into the Processor genetic background. During the construction of the P12 NILs, back cross steps were used as parental sources for the construction of the other pairs of NILs (EyR/S, CocoR/S, SWR/S). P12 NILs were the products of 12 backcrosses and 14 selfings. The *Are* gene was introduced from P12 BC3 in the SW NILs. Nine backcrosses by Slender-White and 12 selfing steps were involved in obtaining the SWR/S pair. In the Coco NILs, the donor parent of the *Are* gene was P12 BC7. Consecutively, five backcrosses by Coco and 13 selfings were carried out. The Ey lines were derived from a cross between SW BC2 and Early-Wax, one backcross by Early-wax, followed by 22 selfings. At each selfing step, progeny tests were performed in order to select heterozygotes at the *Are* locus (*Are*, *are*). Finally, the resistant (*Are*, *Are*) and susceptible (*are*, *are*) isolines of each pair were derived from these heterozygotes (Fouilloux and Bannerot 1977). After all these steps, both members (resistant/susceptible) of each pair of NILs were phenotypically indistinguishable. Monospore cultures of *C. lindemuthianum* race1 were used to check for the presence of *Are*. The *Are* gene was present in each of the resistant members of the NILs and absent in the corresponding susceptible isolines (Fouilloux et Bannerot 1977; Mahé et al. 1993).

A backcross progeny (BC1) of 120 individuals was obtained from a cross between 'EO_2' and 'Corel' (recurrent parent). EO_2 possesses the *Are* resistance gene (introgressed from Cornell 49242) and Corel is susceptible. Plants were grown in a greenhouse under controlled conditions as described by Gantet et al. (1991). A single spore isolate of *C. lindemuthianum* race 1 was provided by F. Legrand and J. Tailler (Laboratoire de Cryptogamie, Université d'Orsay, France). It corresponds to race α, previously described by Charrier and Bannerot (1970). Culture of the fungus and in-vitro tests of pathogenicity on leaves were performed on the backcross progeny as described by Gantet et al. (1991).

DNA extraction, RFLP and RAPD analysis

Isolation of total DNA was as described by Dellaporta et al. (1983) with the addition of a CsCl density-gradient step. For RFLP analysis, bean DNA was digested by *Eco*RI and *Hin*dIII restriction enzymes according to the manufacturer (GIBCO BRL, Gaithersburg, Md., USA). The DNA fragments were separated on 0.8% (w/v) agarose gels in Tris-Borate EDTA (TBE) buffer (Maniatis et al. 1989) and blotted onto Positive^{TM} nylon membranes (Appligène, Illkirch, France) by a modified alkaline procedure (Reed and Man 1985). After depurination, gels were incubated for 45 min in 0.4 N NaOH and then sprayed with 20X SSC during the vacuum transfer. Sources of RFLP probes were random low-copy-number sequences from a bean genomic library of *Mbo*I fragments inserted into a pBluescript IIsk vector (Stratagene Cloning Systems, La Jolla, Calif., USA). Inserts were obtained by a PCR procedure. *E. coli* clones were grown on Petri dishes of 2XTY-agar medium (Maniatis et al. 1989). A single colony was transferred into a sample tube containing 50 µl of PCR buffer and was overlayed with one drop of mineral oil. The PCR buffer composition was: 1X enzyme buffer (Appligène), 50 µM of each dNTP, 0.1 µM of each primer and one unit of *Taq* polymerase (Appligène). One of the primers was the M13 sequencing primer and the other was derived from the M13 reverse sequence primer (5′ GGAAACAGCTATGACCATG 3′). Amplification was performed in a Braun thermocycler (Braun Diessel Biotech GmbH, Melsungen, Germany) programmed for 35 cycles of 1 min at 94 °C, 1 min and 30 s at 45 °C, and 2 min at 72 °C. Probes were labeled by primer extension with random hexanucleotide primers (Maniatis et al. 1989). Prehybridization and hybridizations were performed at 62 °C in a solution containing 7% SDS, 250 mM phosphate buffer (pH 7.2) and 1 mM EDTA (Church and Gilbert 1984). Three washing steps of 30 min each were performed at 55 °C in a solution containing 40 mM phosphate buffer (pH 7.2) and 1% SDS. X OMAT-AR Kodak films (Rochester, N.Y., USA) were then exposed to the filters for 3–4 days at −80 °C with an intensifying screen. RAPD analyses were carried out with 10-mer primers (Operon Technologies Inc., Alameda, USA) according to the procedure of Operon, except that two units of *Taq* DNA polymerase (Appligène) were used in each sample. In this case, the thermocycler was programmed as follows: 5 min at 92 °C and 35 steps of 1 min at 92 °C, 1 min at either 35 °C or 42 °C (depending on primer), and 1 min at 72 °C. Products were analysed on 1.4% agarose gels in TBE and revealed by ethidium bromide staining.

SCAR analysis

An individual band corresponding to a marker to be cloned was purified on a 5% acrylamide gel in 1X TAE (Maniatis et al. 1989). After ethidium-bromide staining, the band was excised and purified as described by Maniatis et al. (1989). A sample of this purified fragment was re-amplified using RAPD-PCR conditions as above, except that the primer concentration was reduced by two-fold. Filling and phosphorylation of the fragment termini were performed using the Klenow fragment of *E. coli* and T_4 DNA kinase according to Maniatis et al. (1989). After purification by chromatography on a Sepharose^{R} column (Pharmacia, Uppsala, Sweden), it was blunt-end ligated into the *Sma*I site of a pBluescript plasmid. The *Rec-E. coli* strain DH5α was transformed (Maniatis et al. 1989). Four clones were selected and tested as probes to detect polymorphism between the four pairs of NILs (on Southern blots of amplification products).

Double-stranded sequencing of the cloned PCR product was carried out (^{T7}Sequencing^{TM} kit, Pharmacia) by the dideoxychain-termination method using the T7 and KS primers. Two specific oligonucleotides were then synthesized. Their sequences contain the RAPD primer at the 5′ termini followed by the 14 adjacent nucleotides (Table 1). These two 24-bp oligonucleotides were used in a PCR procedure which was performed as described by Paran and Michelmore (1993) except that the annealing temperature of the PCR reaction was 65 °C. Synthetic oligonucleotides and *Taq* polymerase were supplied by Appligène.

Table 1 Sequence of the two 24-mer oligonucleotide primers derived from the sequence of RoH20

Locus	Primers	Sequence[a]
RoH20	SCH20-1	5′GGGAGACATCCATCAGACAACTCC3′
	SCH20-2	5′GGGAGACATCTTCATTTGATATGC3′

[a] The underlined sequence represents the initial RAPD primer (H20 from Operon Technologies Inc.)

Linkage analysis

The suspected linkage between genetic markers and the segregation ratio (1:1 for a single locus) were checked using the GeneLink software (X. Montagutelli, Institut Pasteur, France) which performs chi-square analysis (P=0.01). Marker order and map distances were estimated using MapMaker version 1.0 (Lander et al. 1987). RAPD markers were first ordered with a LOD score equal to, or above, three. RFLP markers were then introduced with a LOD score equal to, or above, two. Recombination fractions were transformed by the Kosambi map function to estimate the map distance (Kosambi 1944).

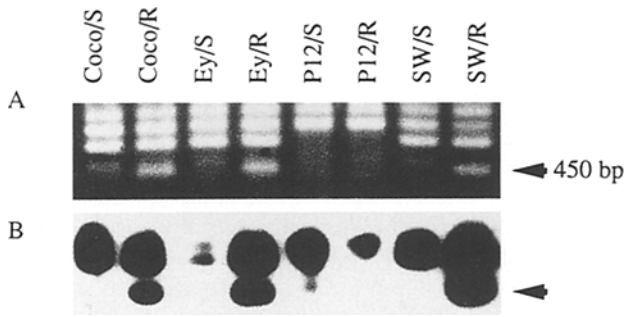

Fig. 1A, B Identification of a RAPD marker linked to the *Are* gene. **A** Amplification products of the four pairs of near-isogenic lines (CocoR/S, EyR/S, P12R/S and SWR/S) using the primer H20. The marker discriminating resistant (*R*) and susceptible (*S*) lines, RoH20, is indicated by an *arrow*. **B** Hybridization of a Southern blot containing the amplification products of the NILs shown in **A**, with the cloned RoH20 used as a probe (the *arrow* indicates the 450 bp fragment)

Results

Establishment of a genetic linkage group around *Are*

In addition to the number of successive backcrosses and selfings, the excellent phenotypic homogeneity of the four pairs of NILs suggested that they should also be very homogeneous at the genomic level. Thus, any polymorphic genetic marker should be linked to the introgressed *Are* gene. However, as some introgressions may occur without phenotypic effects, this should be verified afterwards The isogenicity of these four pairs of NILs was analysed with RFLP and RAPD techniques. One-hundred and thirty-seven probes were tested on DNA blots of the four pairs of NILs. Out of 234 probe × enzyme combinations only four RFLPs were observed (Mahé et al. 1993). These four probes differentiated the two members of the SW pair of NILs but not the members of the three other pairs. Because few polymorphisms were provided through RFLP analysis, a search for RAPD markers was undertaken. In our experience, this technique reveals on average two markers per primer in the case of bean (Adam et al. 1993). Further RAPD analysis was carried out on SW NILs and the parents of the backcross progeny (EO_2, Corel). Only the RAPDs identified between the SW isolines were subsequently used to test the three other pairs of NILs. Two-hundred and thirty-eight primers were tested. Five RAPDs were detected by comparing SW/S and SW/R. They were also informative between EO_2 and Corel. Because of the direction of the backcross, only dominant and codominant genetic markers for EO_2 could be scored. Only one of these five RAPDs, RoH20, was polymorphic between the resistant and susceptible members of the two other pairs of NILs (Ey and Coco, Fig. 1 A). Neither the RFLP probes nor the RAPD primers tested were informative for the P12 NILs. These data further demonstrate the high isogenicity level of these lines.

The linkage of these nine markers to the *Are* locus was analysed in a backcross progeny. RAPD analysis was carried out with 120 individuals and RFLP analysis was done with 72 individuals. Four markers (two RAPDs and two RFLPs) were unlinked to the *Are* gene. Thus, the final linkage group near *Are* contains five of the nine markers revealed by the NILs analysis (Fig. 2). As expected, RoH20, the polymorphic marker for three pairs of NILs, is the most tightly linked to *Are* (0.5 cM). Its orientation with respect

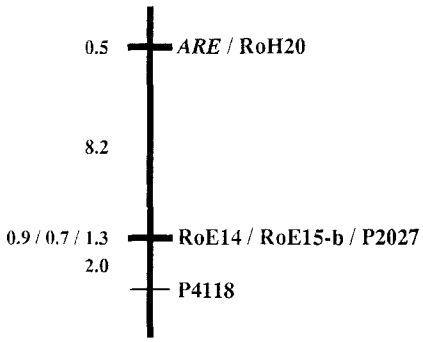

Fig. 2 The *Are* linkage group. "Ro" represents RAPD markers. The name of the Operon primer follows this prefix. Several products can be amplified with each primer. The "*b*" of RoE15-b indicates a specific element (850 bp) of the amplification fragments produced with the primer E15. "*P*" stands for RFLP markers. The fragment length of RoE14 is 1 800 bp. P2027 hybridized to a 2600-bp fragment on *Hin*dIII digestions whereas P4018 hybridized to a 3100-bp fragment on *Eco*RI digestions. Map distances, on the left, are in centiMorgans. They have been estimated using the Kosambi map function. *Large bars* indicate that the relative order of the loci could not be determined. In this case, 0.9 cM corresponds to the map distance between RoE14 and RoE15-b; 0.7 cM stands for the map distance between RoE15-b and P2027; 1.3 cM is the map distance between RoE14 and P2027

to *Are* could not be determined with a minimum LOD score of two. The four other markers were located on the same side of the *Are* locus, dipersed through a distance of 12 cM. Surprisingly, the four unlinked markers to *Are* are tightly linked together and map to another linkage group (data not shown). As these latter markers revealed polymorphism only between the two members of SW NILs, it is unrealistic to assume that they represent a genomic region epistatic to the resistance gene. This independent introgression event might rather be the consequence of outcrossing during SW NILs breeding or, by chance, of introgression of a genomic region without obvious phenotypic effect.

Converting the RoH20 RAPD marker into a SCAR marker

RoH20 is a dominant marker (450-bp amplified fragment) discriminating EO_2 from Corel (Fig. 3 A). In order to stabilize this amplification product, a SCAR was produced. The polymorphic band RoH20 was cloned as described in Materials and methods. When used as a probe, this clone revealed a fragment of 450-bp only in the resistant members of three pairs of NILs (Coco, Ey, SW) as expected from the RAPD RoH20 data (Fig. 1 B). P12/R did not show this fragment, which suggested that the introgressed region is smaller in P12/R than in the other resistant genotypes (Coco/R, SW/R, Ey/R). An additional band (550-bp) was present in all samples. This might represent another amplified locus conserved in all members of these four pairs of NILs. Preliminary data from hybridization of EO_2 and Corel genomic DNA blots suggest that RoH20 corresponds to a moderately-repeated sequence (data not shown).

The cloned fragment was sequenced from both ends. The sequence did not reveal any palindromic structure except for the termini (10-bp) which correspond exactly to the RAPD primers used in the original amplification experiments. A computer analysis did not reveal any open reading frame or any homology with any previously described sequence in the data banks [Genebank(R) and EMBL data libraries]. Specific primers of 24-mer, coded SCH20-1 and SCH20-2, corresponding to both ends of the insert, including the primer sequence H20, were synthesized (Table 1). Both were more than 50% GC and did not possess any palindromic sequence.

SCH20-1 and SCH20-2 when used as primers did amplify a 450-bp fragment both in the EO_2 and Corel cultivars, suggesting that the polymorphism observed with the H20 primer was caused by a mismatch in one of the two primer-targeted sequences. As described by Paran and Michelmore (1993) 24-mer primers allow stabilization of the annealing of complementary sequences presenting a mismatch. This is not the case for 10-bp RAPD primers. Restriction digests were carried out directly on the amplification products to recover polymorphisms. Eleven restriction enzymes (4-bp cutters) were tested. An informative polymorphism (136-bp, Fig. 3 B) was observed with a *Dde*I digest.

Both RAPD (Fig. 3 A) and SCAR (Fig. 3 B) analysis of the progeny (120 individuals) provided identical conclusions, further demonstrating that the observed polymorphisms with the two techniques correspond effectively to the same locus. The SCAR was much easier to score than the RAPD (Fig. 3).

An additional polymorphic band (260 bp) emerged from the *Dde*I analysis (Fig. 3 B). It is present in Corel and absent in EO_2. As shown in Fig. 3, this polymorphism is not informative for the analysis of backcross progeny. SCH20 will also be useful in F2 progenies analysis, as it is a co-dominant marker.

Discussion

NILs are considered useful biological tools when searching for genetic markers, such as molecular markers, linked to an introgressed locus. The theoretical mean proportion of donor genome retained in the 11 chromosomes (assumed to all have the same length, 100 cM) after t generations of backcross breeding without selection was calculated for each pair (Stam and Zeven 1981; Muehlbauer et al. 1988, 1991). This proportion is 0.043 for Coco/R, 0.285 for Ey/R, 0.014 for P12/R, and 0.019 for SW/R. Among 117 tested probes in the RFLP analysis, approximately five probes should have detected polymorphism between CocoS/R, 33 between EyS/R, one between P12S/R, and two between SWS/R. These results might represent overestimations because they did not consider any selection process during the breeding programs (Muehlbauer et al. 1988). Obviously, classical selection on phenotypic characters did enhance the recovery of non-targeted regions, at least for Coco and Ey NILs. The difficulty in predicting the size of the introgressed region is in agreement with the observations of Young and Tanksley (1989). They showed that, under classical selection, variations in the size of the introgressed fragment around the $Tm2^2$ resistance gene were very large. In the present case, the classical backcross scheme provided better levels of isogenicity than expected, except for SW NILs in which a region independent from *Are* was introgressed. The number of additional selfings (between 12 and 22) stabilized the non-selected loci; very few polymorphisms were observed with the analysis of several hundred putative loci (Mahé et al. 1993; present data).

Young and Tanksley (1989) also demonstrated that marker-assisted breeding during backcross programs could largely reduce both the number of required steps and the length of introgressed fragments. Additional molecular markers flanking *Are* will be sought and then used to test

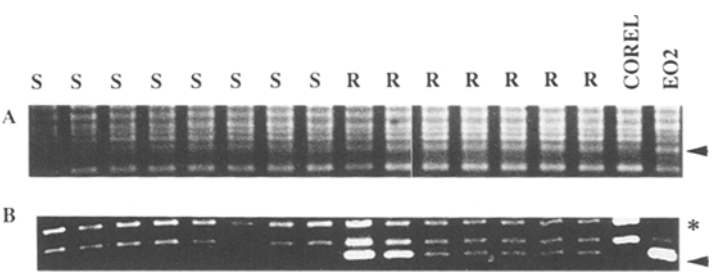

Fig. 3A, B Parents and representative individuals of the backcross progeny (*R* for resistant and *S* for susceptible) were scored either for the RAPD marker RoH20 (**A**) or for the corresponding SCAR marker SCH20 (**B**). The RAPD (450-bp) and SCAR (136-bp) markers are indicated by *arrows*. An additional amplified fragment contributed by Corel is indicated by a *star* (260 bp)

the advantage of using linked markers in marker-assisted breeding (Young and Tanksley 1989; Hospital and al. 1992). Distances between markers in this region will also be re-estimated using a set of recombinant inbred lines derived from a cross between EO_2 and Corel (Bannerot, unpublished results). Because RAPD technology might lead to irreproducibility among laboratories, it was decided to convert the RAPD marker linked to *Are*, in this case RoH20, into a Sequence Characterized Amplified Region. The advantages of SCAR over RAPD were largely discussed by Paran and Michelmore (1993). In the present case, SCH20 could be employed for the localisation of the *Are* region on the bean genetic map (Vallejos et al. 1992; Gepts 1993; Gepts et al. 1993). SCAR markers can also provide useful information related to the sequence organization at a RAPD locus. SCAR markers will certainly be very useful for any laboratory interested in marker-assisted breeding.

The *Are* gene is important because it represents one of the major genetic sources of resistance towards anthracnose in the non-tropical areas where common bean is grown (Fouilloux 1979; Park et al. 1987; Pastor-Corrales and Tu 1989). Additional progenies and molecular markers will be used to saturate this region before initiating a genome walk towards the *Are* gene. This work is in progress for other genes conferring pathogen resistance in plants (Debener et al. 1991; Martin et al. 1993). The molecular characterization of these loci will be of major interest for the understanding of the mechanisms underlying specific pathogen resistance in plants (Debener et al. 1991; Michelmore et al. 1992). Other genes conferring resistance to other bean diseases are also being mapped, such as a major gene for bean rust resistance (Haley et al. 1993) and QTLs explaining a part of common blight resistance (Nodari et al. 1993 b). When a common bean unified map is established, it will be of interest to know whether, as in many other cases (Pryor 1987), these genes, and others involved in specific resistance to bean diseases, are clustered.

Acknowledgements The authors thank the reviewers for their careful reading of this manuscript. J. Grisvard (Département de Biologie Moléculaire Végétale, Orsay, France) was largely involved in many aspects of this work. Stimulating discussions with J. Falquet and F. Creusot (Département de Biologie Moléculaire Végétale, Orsay, France) are gratefully appreciated.

References

Adam AF, Creusot F, Grisvard J, Sevignac M, Choisne N, Dron M (1993) Reverse genetics as an approach to isolate a french bean resistance gene against anthracnose. In: Fritig B, Legrand M (eds) Mechanisms of plant defense responses. Kluwer Academic Publishers, Netherlands, pp 33–36

Beckmann JS, Soller M (1983) Restriction fragment length polymorphism in genetic improvement: methodology, mapping and costs. Theor Appl Genet 67: 35–43

Beebe SE, Pastor-Corrales MA (1991) Breeding for disease resistance in common beans. In: Van Schoonhoven A, Voysest O (eds) Common beans: research for crop improvement. CAB International Oxford, pp 561–610

Charrier A, Bannerot H (1970) Contribution à létude des races physiologiques de l'anthracnose du haricot. Ann Amélior Plant 18: 171–179

Church G, Gilbert W (1984) Genomic sequencing. Proc Natl Acad Sci USA 81: 1991–1995

Debener T, Lehnackers H, Arnold M, Dangl JL (1991) Identification and molecular mapping of a single *Arabidopsis* locus conferring resistance against a phytopathogenic *Pseudomonas* isolate. The Plant J 1: 289–302

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA micro-preparation: version II. Plant Mol Biol Rep 1: 19–21

Fouilloux G (1979) New races of bean anthracnose and consequences on a breeding program. In: Maraite H, Meyer JA (eds) Diseases of tropical foodcrops. Université Catholique Louvain la Neuve, Belgique, pp 221–235

Fouilloux G, Bannerot H (1977) Four pairs of isogenic lines except for the "*ARE* gene". Bean Improv Coop 20: 59–60

Gantet P, Bettini P, Grisvard J, Dron M (1991) Genetic linkage between *MexII*, a specific resistance gene to anthracnose and *Anp*, a gene involved in pod anthocyanin accumulation in bean. Plant Dis 75: 941–942

Gardiner JM, Coe EM, Melia-Hancock S, Hoisington DA, Chao S (1993) Development of a core RFLP map in maize using an immortalized F_2 population. Genetics 134: 917–930

Gepts P (1993) Linkage map of common bean (*Phaseolus vulgaris* L.). In: OBrien SL (ed) Genetic maps. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp 6101–6109

Gepts P, Nodari R, Tsai SM, Koinange EMK, Llaca V, Gilbertson R, Guzman P (1993) Linkage mapping in common bean. Annu Rept Bean Improv Coop 93: 24–25

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

Hospital F, Chevalet C, Mulsant P (1992) Using markers in gene introgression breeding programs. Genetics 132: 1199–1210

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12: 172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly M, Lincoln SE, Newburg L (1987) MapMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1: 174–181

Lenné JM (1992) *Colletotrichum* diseases of legumes. In: Bailey JA, Jeger MJ (eds) *Colletotrichum* biology, pathology and control. CAB International, Oxford, pp 134–167

Mahé A, Grisvard J, Dron M (1993) Two avirulent races of *Colletotrichum lindemuthianum* trigger a different time-course of plant defense reaction in bean. Mol Plant-Microbe Interact 6: 423–428

Maniatis T, Fritsch EF, Sambrook L (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

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

Martin GB, De Vicente MC, Tanksley SD (1993) High-Resolution linkage analysis and physical characterisation of the *Pto* bacterial resistance locus in tomato. Mol Plant-Microbe Interact 6: 26–34

Mastenbroek C (1960) A breeding program for resistance to anthracnose in dry shell haricot beans, based on a new gene. Euphytica 9: 177–258

Menezes JR, Dianese JC (1988) Race characterization of Brazilian isolates of *Colletotrichum lindemuthianum* and detection of resistance to anthracnose in *Phaseolus vulgaris*. Phytopathology 78: 630–655

Michelmore RW, Kesseli RV, Francis DM, Paran I, Fortin MG, Young CH (1992) Strategies for cloning plant disease resistance genes. In: Gurr S (ed) Molecular plant pathology, a practical approach, vol 2. IRC Press, Oxford, UK, pp 233–287

Muehlbauer GJ, Specht JE, Thomas-Compton MA, Staswick PE, Bernard RL (1988) Near-isogenic-lines. A potential resource in the integration of conventional and molecular marker linkage maps. Crop Sci 28: 729–735

Muehlbauer GJ, Staswick PE, Specht JE, Graef GL, Shoemaker RC, Keim P (1991) RFLP mapping using near-isogenic lines in soybean [*Glycine max* (L.) Merr.]. Theor Appl Genet 81: 189–198

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

Nodari RO, Tsai SM, Guzmàn P, Gilberston RL, Gepts P (1993b) Towards an integrated linkage map of common bean. III. Mapping genetic factors controlling host-bacteria interactions. Genetics 134: 341–350

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

Park SJ, Tu JC, Aylesworth JW (1987) Dresden field bean. Can J Plant Sci 67: 821–822

Pastor-Corrales MA, Tu JC (1989) Anthracnose in bean production problems in the tropics. In: Schwartz MP, Pastor-Corrales MA (eds). CIAT, Cali, Colombia, pp 77–105

Pryor T (1987) The origin and structure of fungal disease resistance genes in plants. Trends Genet 3: 157–161

Reed KC, Man DA (1985) Rapid transfer of DNA from agarose gels to nylon membranes. Nucleic Acids Res 13: 7207–7221

Stam P, Zeven AC (1981) The theoretical proportion of the donor genome in near-isogenic lines of self-fertilizers bred by back-crossing. Euphytica 30: 227–238

Tanksley SD, Young ND, Paterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding: new tools for an old science. Bio/Technology 7: 257–264

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

Williams JGK, Hanafey MK, Rafalski JA, Tingey SV (1993) Genetic analysis using Random Amplified Polymorphic DNA markers. Methods Enzymol 218: 705–740

Young ND, Tanksley SD (1989) RFLP analysis of the size of chromosomal segments retained around the *Tm-2* locus of tomato during back-cross breeding. Theor Appl Genet 77: 353–359