Theor Appl Genet (1992) 84:839–844

RFLP mapping of a major bruchid resistance gene in mungbean (*Vigna radiata*, L. Wilczek)

N. D. Young [1], L. Kumar [2], D. Menancio-Hautea [3], D. Danesh [1], N.S. Talekar [4], S. Shanmugasundarum [4], and D.-H. Kim [4]

[1] Department of Plant Pathology, 495 Borlaug Hall, University of Minnesota, St. Paul, Minnesota, USA
[2] Division of Biochemical Sciences, National Chemical Laboratory, Pune 411008, India
[3] Institute of Plant Breeding, University of the Philippines, Los Baños, Philippines
[4] Asian Vegetable Research and Development Center, P.O. Box 42, Shanhua, Taiwan 74199, Taiwan, Republic of China

Received February 10, 1992; Accepted February 26, 1992
Communicated by F. Salamini

Summary. Bruchids (genus *Callosobruchus*) are among the most destructive insect pests of mungbeans and other members of the genus, *Vigna*. Genetic resistance to bruchids was previously identified in a wild mungbean relative, TC1966. To analyze the underlying genetics, accelerate breeding, and provide a basis for map-based cloning of this gene, we have mapped the TC1966 bruchid resistance gene using restriction fragment length polymorphism (RFLP) markers. Fifty-eight F_2 progeny from a cross between TC1966 and a susceptible mungbean cultivar were analyzed with 153 RFLP markers. Resistance mapped to a single locus on linkage group VIII, approximately 3.6 centimorgans from the nearest RFLP marker. Because the genome of mungbean is relatively small (estimated to be between 470 and 560 million base pairs), this RFLP marker may be suitable as a starting point for chromosome walking. Based on RFLP analysis, an individual was also identified in the F_2 population that retained the bruchid resistance gene within a tightly linked double crossover. This individual will be valuable in developing resistant mungbean lines free of linkage drag.

Key words: *Callosobruchus* – DNA Markers – Insect – Legume – Restriction fragment length polymorphisms

Introduction

Restriction fragment length polymorphisms (RFLPs) are genetic markers based on cloned fragments of DNA. Using RFLPs, high density linkage maps can be constructed and used to locate genes of economic importance. Once a gene of interest is associated with a linked RFLP marker, selection for that gene can be based on the genotype of the RFLP rather than the phenotype itself. This can be extremely valuable if a phenotype is difficult or expensive to score. RFLPs can also be used to select against unwanted genomic segments, which is important if one of the parents in a breeding program is uncultivated and carries undesirable characters (Young and Tanksley 1989 b). Finally, RFLPs can act as starting points for map-based cloning, a strategy for cloning genes known only by their genetic map location, not their biochemical product (Young 1990).

In the past, RFLP research has focused on crops important in highly developed countries; crops such as maize (Beavis et al. 1991), wheat (Sharp et al. 1989), tomato (Tanksley et al. 1988), lettuce (Landry et al. 1987), barley (Huen et al. 1991), and soybean (Keim et al. 1990). There is now a growing awareness of the value of RFLP technology to "orphaned" crops grown primarily in developing countries. One of these crops is mungbean, *Vigna radiata*. Mungbean is a major pulse crop throughout Asia (AVRDC 1988) but yields are relatively low (300–800 kg/hectare) compared to other legumes. Clearly, the application of RFLP technology to mungbean has the potential to enhance yield and benefit farmers in less developed countries.

One of the most serious problems in mungbeans and other *Vigna* species are insect pests of the genus, *Callosobruchus* (*Coleoptera: Bruchidae*), particularly *C. chinesis* and *C. maculatus*. Members of this genus are often referred to simply as seed weevils or "bruchids". These insects attack stored seeds and can lead to nearly complete loss (Talekar 1988). In previous research, genetic resistance to bruchids was found in a wild mungbean relative, line TC1966 of the subspecies *sublobata* (Fuji et al. 1989). Unfortunately, TC1966 has many other

Correspondence to: N.D. Young

characteristics that make it highly undesirable for use as a cultivated line.

To analyze the genetics of bruchid resistance, speed the introduction of this gene into cultivated lines, and establish a basis for map-based cloning, we report here the mapping of bruchid resistance using RFLPs, including one DNA marker approximately 3.6 centimorgans (cM) away. RFLPs have also enabled us to identify an F_2 line that is best for breeding a bruchid-resistant mungbean line that will be free of linkage drag. Moreover, because of mungbean's small genome size, molecular cloning of the bruchid resistance gene using nearby RFLP markers as starting points in chromosome walking (Steinmetz et al. 1981) may soon be feasible.

Materials and methods

Mapping population

A cross was made between VC3890, a mungbean cultivar, and line TC1966. The cross was advanced to the F_2 generation and 58 individuals were grown in a greenhouse in St. Paul, Minnesota. Leaf material was harvested from each F_2 plant, as well as the parents, and used for DNA isolation. After leaf harvest, plants were allowed to recover and set seed. F_3 seed from each F_2 individual was sent to the Asian Vegetable Research and Development Center (AVRDC) in Taiwan to assay bruchid reaction.

DNA clones

As sources of putative RFLP markers, three different cloned genomic libraries were used. One library consisted of genomic soybean DNA digested with the methylation-sensitive restriction enzyme *Pst*I and inserted into the phagemid pBS+ (Stratagene). These clones were the generous gift of Dr. Randy Shoemaker, Iowa State University, Ames, Iowa. More than 90% of the soybean clones tested showed strong hybridization signals when probed onto mungbean DNA, indicating a high degree of sequence homology between soybean and mungbean.

Additional genomic libraries were construced using *Pst*I-digested mungbean or cowpea (*V. unguiculata*) DNA. To prepare these libraries, DNA was digested with *Pst*I, separated according to size by sucrose gradient centrifugation, and the fraction between 500 and 3,000 base pairs ligated into pUC18 by standard molecular methods (Sambrook et al. 1989).

Plant DNA extraction, restriction digestion, and blotting

DNA was harvested from mungbean leaves by the method of Dellaporte (1983). Using this method, approximately 100 µg of high quality, digestable, DNA was isolated from each gram of fresh leaves.

DNA from both parents was digested with six restriction enzymes, *Bst*NI, *Dra*I, *Eco*RI, *Eco*RV, *Hae*III, and *Hin*dIII. Digested DNA was then analyzed by 1% agarose gel electrophoresis and transferred to a Hybond N+ membrane (Amersham) by a method adapted from Southern (1975). The major difference was that the transfer took place in an alkaline solution of 0.1 N NaOH and 0.1 M NaCl. These blots, which are referred to as "Parental surveys," made it possible to test individual DNA clones against digested DNA of both parents, and thereby determine which restriction enzyme showed the clearest fragment length polymorphism and would be best for segregation analysis.

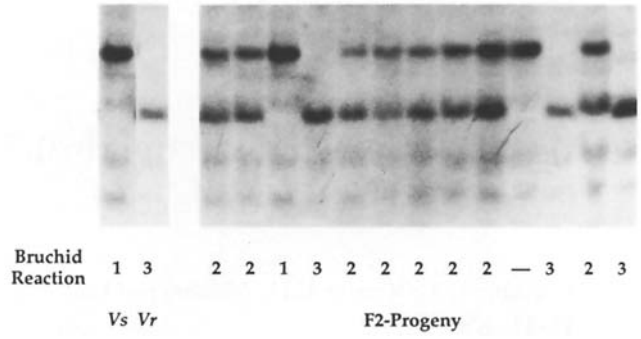

Fig. 1. F_2 segregation analysis of RFLP pR26 and reaction to bruchids. Fifty-eight F_2 progeny, derived from a cross between a resistant (TC1966, "*Vs*") and a susceptible (VC3890, "*Vr*") parent, were assayed for bruchid reaction and RFLP genotype. On the left, an autoradiograph showing the RFLP pattern for RFLP pR26 with the two parents is shown; on the right, the RFLP pattern for 13 F_2 progeny plants. Below the RFLP patterns, the inferred bruchid reaction is indicated by a numerical score. "*1*" indicates homozygous bruchid resistant; "*2*" indicates heterozygous; "*3*" indicates homozygous bruchid susceptible; "–" indicates that the bruchid reaction was uncertain. Note that among these 13 plants, there are no individuals with crossovers between pR26 and the bruchid resistance gene

DNA from all 58 F_2 individuals were digested and blotted in a similar manner to produce "F_2 blots". Each F_2 blot contained DNA from all F_2 individuals digested with a single restriction enzyme (Fig. 1). In this way, each RFLP clone could be hybridized against F_2 DNA that had been digested with the appropriate restriction enzyme for that clone, based on the results of the corresponding parental survey.

DNA hybridizations

To prepare cloned DNA inserts for radiolabeling and nucleic acid hybridization, individual bacterial colonies were grown up overnight in suspension culture, centrifuged for 5 min at 2,000 g, rapidly frozen and thawed, and the lysed cell mixture centrifuged for 5 min at 2,000 g. Two microliters of the supernatant were then used in a polymerase chain reaction using oligonucleotide primers flanking the inserted sequence and thereby amplifying the sequence. Unincorporated nucleotides were removed either by ammonium acetate-precipitation or by brief centrifugation through a 1 ml column containing Sephadex G50 resin.

Approximately 50 ng of each amplified genomic sequence was radiolabeled by the random hexamer reaction of Feinberg and Vogelstein (1983). The radiolabeled product was then incubated with either a parental survey or an F_2 blot for 18–24 h at 60 °C in a hybridization solution of 5 × SSC, 0.1 M phosphate buffer, pH 7.5, 1 × Denhardt's solution (bovine serum albumin, Ficoll, polyvinylpyrrolidone, 0.02% each), 0.1% sodium dodecyl sulfate, and 5% dextran sulfate. After incubation, blots were washed three times for 15 min each at 60 °C; the first and second washes with 2 × SSC and the third wash with 1 × SSC. Each wash solution also contained 0.1% sodium dodecyl sulfate. After washing, blots were placed against Kodak X-AR film at −80 °C for 1–5 days to produce autoradiographs.

Bruchid assays

To determine the bruchid reaction of F_3 seed, ten to 16 seeds were placed in an Erlenmeyer flask and ten pairs of newly

emerged *C. chinesis* adults were confined in the flask for 1 week for oviposition. After 1 week, all insects were removed and the seeds were maintained at 30 °C for 4 weeks. After this time, the total number and number of bruchid-damaged seeds in each flask were counted. Seeds with holes were considered damaged.

Linkage mapping

A total of 300 genomic clones were analyzed by hybridization with parental surveys and, of these clones, 153 were subsequently probed against F_2 blots for segregation analysis. The result for each clone was coded into a numeric form and analyzed by two-way contingency table analysis with the computer program, Statview-II, and by the RFLP mapping program, Mapmaker (Lander et al. 1987). Linkage between RFLP markers (or between RFLP markers and bruchid resistance) was inferred if the probability of observing a chi-squared value was less than 0.001 or if the "LOD" score exceeded 3.0 (Lander et al. 1987). A LOD score is the log(10) of the ratio between the odds of one hypothesis (linked, in this case) versus an alternative hypothesis (unlinked, in this case). The order of markers was inferred from multipoint analysis in which the favored order of markers exceeded other possible orders by a LOD of 2.0 or greater.

To identify minor genes that might influence bruchid resistance (quantitative trait loci or "QTLs"), results were also analyzed using Statview-II and Mapmaker-QTL (Lander and Botstein 1989). A QTL was considered likely if the probability of observing an F-score (based on analysis of variance between an RFLP marker and the phenotype) was less than 0.01 (Statview-II), or if the LOD score for the presence of a QTL exceeded 2.5 (Mapmaker-QTL).

Results and discussion

RFLP linkage map of mungbean

The 153 RFLP markers now on the mungbean map span 14 linkage groups and 1,295 cM, with an average distance between adjacent markers of 9.3 cM. Six additional RFLPs are linked to only one other marker and ten RFLPs are unlinked to any other. Since there are known to be 11 chromosomes in mungbean by cytogenetic analysis (Joseph and Bouwkamp 1978), additional RFLPs will need to be added to unify the linkage map. Expected Mendelian segregation rations (1:2:1 or 3:1) were observed for 85% of the RFLPs ($P > 0.01$) by chi-squared analysis. Further details on the RFLP map for mungbean are found in Menancio-Hautea et al. (1992).

Inheritance of bruchid resistance

Previous studies on bruchid resistance from TC1966 have indicated that a single dominant gene controls this trait (Fujii et al. 1989). Therefore, F_3 samples with no damaged seeds were inferred to be derived from an F_2 parent homozygous for bruchid resistance, while samples with all seeds damaged were inferred to be derived from an F_2 parent homozygous for bruchid susceptibility. Samples that contained between 20% and 80% damaged seeds were inferred to be heterozygotes (Table I). While this insured that false positives and disease escapes were excluded, it also meant that a few lines were not included.

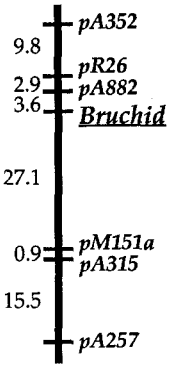

Fig. 2. Linkage map around the bruchid resistance gene (*Bruc*). The RFLP markers that make up linkage group VIII of mungbean are shown, along with the apparent map location of the bruchid resistance gene. Marker order and distances were determined using MAPMAKER (Lander et al. 1988). Distances are given in centimorgans

Table 1. Distribution of bruchid reactions in F_2 lines

Percent infested seeds in F_3 progeny	Number of F_2 lines	Inferred genotype of F_2 line[a]	Percent of all F_2 lines classed by genotype[b]
0	7	Homo.-resistant	16.0
1–19	4	–	–
20–80	26	Heterozygous	59.0
81–99	7	–	–
100	11	Homo.-susceptible	25.0

[a] Inferred bruchid resistance genotype for F_2 lines based on F_3 progeny tests

[b] Percent of all F_2 lines that were definitively grouped according to resistance genotype

Of the 58 F_2 lines in the experiment, 55 produced seed and were tested for bruchid reaction. Of these 55 lines, 44 yielded results that could be confidently classed according to bruchid resistance phenotype (Table I). Treated in this manner, the segregation of bruchid resistance was in agreement with single-gene inheritance, giving a chi-squared value of 2.182 ($P = 0.3359$) when tested against a 1:2:1 ratio.

Linkage mapping of bruchid resistance using RFLPs

In two-point linkage analysis between each RFLP marker and a bruchid resistance phenotype, six RFLPs showed significant associations based on two-way contingency table analysis. All of these RFLPs map to linkage group VIII and of these markers pA882 and pR26 showed the most significant linkage.

In multipoint linkage analysis, bruchid resistance was located to a single locus between marker pA882 and pA315 (Fig. 2). Marker pA882 is closest to the bruchid resistance gene, approximately 3.6 cM away. Marker pR26 is located on the same side of the bruchid resistance gene, approximately 2.9 cM further away. RFLP marker pA315 is located 27 cM away from the bruchid resistance gene on the other side and is very closely linked to a second RFLP, pM151, which is less than one cM further away.

QTL mapping of bruchid resistance

To identify other possible genes with minor effects on bruchid resistance, F_3 data in the form of the proportion of damaged seeds were analyzed by Statview-II and Mapmaker-QTL. In this way, loci with subtle effects on bruchid resistance could potentially be uncovered. However, throughout the entire RFLP map, only the region on linkage group VIII was found to be significant by this analysis, with the putative bruchid resistance locus mapping to same position identified by multipoint linkage analysis. The LOD value for the presence of a resistance gene at this locus was 15.3 and the proportion of the variation in the phenotype that could be attributed to the locus was 87.5%.

In a two-factor analysis of variance between bruchid resistance, pA882, and all remaining RFLP markers on the map, a single region was found to show a significant interaction with pA882. This region was unlinked to the major bruchid resistance locus on linkage group VIII, but was instead located on linkage group III. The region encompassed three RFLP markers, with pM167 showing the greatest effect. The F value for the interaction term was 4.486 and the probability was 0.0047. However, because of the relatively small population size, and consequently the low power of two-factor analysis of variance, it was difficult to decide whether a gene with a meaningful effect on bruchid resistance actually resided in this region of the mungbean genome.

RFLP marker-based selection

Mapping the bruchid resistance gene with RFLPs made it possible to pinpoint those F_2 lines that have a crossover very near the gene. Ideally, one or more lines could be identified with crossovers (or a double crossover) very near the bruchid resistance gene using RFLPs. This line could then be further backcrossed to VC3890 in order to obtain a line carrying the bruchid resistance gene, but with a minimum of TC1966 germplasm flanking the gene (minimum linkage drag).

Knowing the location of the bruchid resistance gene also made it possible to select directly against TC1966 genomic segments unlinked to the resistance gene. Simultaneously selecting against TC1966 germplasm both linked and unlinked to the bruchid resistance gene using RFLPs could accelerate the process of developing a bruchid-resistant mungbean that is agronomically comparable to VC3890.

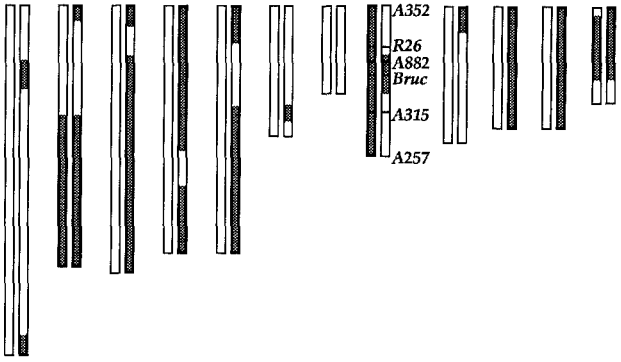

Fig. 3. Graphical genotype (Young and Tanksley 1989a) for F_2 plant no. 19. Based on the RFLP genotype for all 153 markers throughout the mungbean genome, the graphical genotype for all 58 F_2 plants was determined. *Dark regions* represent genomic segments from TC1966; *light regions* represent genomic segments from VC3890. Among the F_2 individuals, plant no. 19 was found to be best for subsequent breeding based on RFLP genotype. Note that this plant contains a double crossover surrounding the bruchid resistance gene and RFLP marker pA882. Note also that F_2 plant no. 19 also contains far-less DNA from TC1966 (approximately 34%) based on RFLP genotype than the F_2 population at large (approximately 45%). This is another desirable feature from the standpoint of plant breeding

On the basis of these criteria, F_2 line number 19 (Fig. 3) was found to be best for further breeding. Significantly, based on RFLP results, this line contained a double-crossover around the bruchid resistance gene. Line 19 was homozygous for bruchid resistance and had also retained the TC1966 allele for pA882. However, markers pR26 and pM315 both showed the allelic form of VC3890, indicating crossovers between pM315 and bruchid resistance on one side and between pA882 and pR26 on the other. Moreover, F_2 line 19 was found to contain only 34% TC1966 germplasm based on RFLPs throughout the genome, compared to 45% for the F_2 population at large. Because of these desirable properties, a double crossover around the bruchid resistance gene and a relatively large proportion of the genome already free of TC1966 germplasm, our results predict that F_2 line 19 would be best for subsequent breeding. We are now testing this prediction.

Comparison to other insect resistance genes

There have been few previous RFLP mapping studies of insect resistance genes in plants. One report described RFLPs near genes controlling the production of 2-tridecanone in tomato, a trait related to insect resistance (Nienhuis et al. 1987). In this case, the trait was complex and polygenic. Three QTLs with moderate effects on the

character were identified and they accounted for 38% of 2-tridecanone production. Another ongoing RFLP mapping project involves the brown planthopper of rice (S. Tanksley, Cornell University, personal communication). Otherwise, most previous plant RFLP mapping studies have focused on genes for resistance to non-insect pests, such as viruses (Young et al. 1988), bacteria (Martin et al. 1991), fungi (Michelmore et al. 1991), and nematodes (Messeguer et al. 1991). This is surprising considering the large number of insect resistance genes, including single gene resistances, that have previously been described in major crop plants using classical techniques (e.g., Gallun and Patterson 1977; Lakshminarayana and Khush 1977).

Map-based cloning of the bruchid resistance gene

An attractive feature of genome analysis in mungbean is its small genome size. While the genomes of many higher plants are nearly 1 billion base pairs or more (Bennett and Smith 1976), mungbean is estimated to have a genome between 470 (Murray et al. 1979) and 560 (Arumuganathan and Earle 1991) million base pairs, making it the smallest of all grain and vegetable legumes. Mungbean is also known to be relatively low in repetitive DNA sequences (Murray et al. 1979) and has a rapid life cycle of only 50 days. These features make it highly desirable for map-based gene cloning.

To clone the bruchid resistance gene using RFLPs, DNA markers near the gene must be close enough to carry out chromosome walking. Dividing the total genome size (in base pairs) by the number of centimorgans in the mungbean genome indicates that A882 is approximately 1.2 million base pairs away from the bruchid resistance gene. However, the current estimated size of the mungbean genome (1,295 cM) is probably an underestimate, so this method probably overestimates the distance between A882 and bruchid resistance. Moreover, the relation between crossover frequency and physical distance is known to vary greatly, even within the genome of a single species (Ganal et al. 1989).

While it might be possible to chromosome walk over a distance of 1 million base pairs or more, a better strategy is to increase the density of RFLP markers on the mungbean genome, and thereby significantly shorten the distance that must be traversed. This can be accomplished either by targeting the region around the bruchid resistance gene with RFLPs using bulked DNA from homozygous individuals (Michelmore et al. 1991) or by increasing the total number of markers throughout the mungbean genome. At the same time, crossovers very near the bruchid resistance gene will be essential for orienting the chromosome walk.

Cloning the bruchid resistance gene from mungbean could potentially be important to other legumes. For example, cowpea (*V. unguiculata*), which is a major source of vegetable protein in Africa and South America, is extremely susceptible to the same bruchid species (Singh and Allen 1979). Because the insect pests are identical between mungbean and cowpea and have a similar site of attack, a cloned bruchid resistance gene from mungbean might be effective if transformed into cowpea.

Acknowledgements. We thank Dr. Christian Fatokun, Gary Foley, and Quynh An Ngyuen for their technical assistance. This research was supported, in part, by a grant from the Rockefeller Foundation. This paper is published as a contribution of the series of the Minnesota Agricultural Experiment Station on research conducted under Project 015, supported by G.A.R. funds.

References

AVRDC (1988) Mungbean: Proceedings 2nd Symposium. Asian Vegetable Research and Development Center, Shanhua, Taiwan

Arumuganathan K, Earle E (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218

Beavis WD, Grant D (1991) A linkage map based on information from four F_2 populations of maize (*Zea mays* L.). Theor Appl Genet 82:636–644

Bennett MD, Smith JP (1976) Nuclear DNA amounts in angiosperms. Phil Trans R Soc Lond Ser B 274:227–273

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

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction fragments to high specific activity. Anal Biochem 132:6–13

Fujii K, Ishimoto M, Kitamura K (1989) Patterns of resistance to bean weevils (Bruchidae) in *Vigna radiata – sublobata* complex inform the breeding of new resistant varieties. Appl Entomol Zool 24:126–132

Gallun RL, Patterson FL (1977) Monosomic analysis of wheat for resistance to hessian-fly. J Hered 68:223–226

Ganal MW, Young ND, Tanksley SD (1989) Pulsed-field gel electrophoresis and physical mapping of large DNA fragments in the *Tm-2a* region of chromosome 9 in tomato. Mol Gen Genet 215:395–400

Huen M, Kennedy AE, Anderson JA, Lapitan NLV, Sorrells ME, Tanksley SD (1991) Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome 34:437–447

Joseph LS, Bouwkamp JC (1978) Karyomorphology of several species of *Phaseolus* and *Vigna*. Cytologia 43:595–600

Keim P, Diers BW, Olson TC, Shoemaker RC (1990) RFLP mapping in soybean: association between marker loci and variation in quantitative traits. Genetics 126:735–742

Lakshminarayana A, Khush GS (1977) New genes for resistance to the brown planthopper in rice. Crop Sci 17:96–100

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLPs linkage maps. Genetics 121:185–199

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER; an interactive computer program for constructing genetic linkage maps of experimental and natural populations. Genomics 1:174

Landry BS, Kesseli RV, Farrara B, Michelmore RW (1987) A genetic map of lettuce (*Lactuca sativa* L.) with restriction

fragment length polymorphism, isozyme, disease resistance and morphological markers. Genetics 116:331–337

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

Menancio-Hautea D, Kumar L, Danesh D, Young N (1992) A genome map for mungbean [Vigna radiata (L.) Wilczek] based on DNA genetic markers. In: Stephen O'Brien (ed) Genetic Maps – 1992 (6th edition). Cold Spring Harbor Laboratory Press (in press)

Messeguer R, Ganal M, de Vicente MC, Young ND, Bolkan H, Tanksley SD (1991) High resolution RFLP map around the root knot nematode resistance gene (*Mi*) in tomato. Theor Appl Genet 82:529–536

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregation analysis: a rapid method to detect markers in specific genome regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Murray MG, Palmer JD, Cuellar RE, Thompson WF (1979) Deoxyribonucleic acid sequence organization in the mungbean genome. Biochemistry 18:5259–5264

Nienhuis J, Helentjaris T, Slocum M, Ruggero B, Schaefer A (1987) Restriction fragment length polymorphism analysis of loci associated with insect resistance in tomato. Crop Sci 27:797–803

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning* (2nd edn.). Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Sharp PJ, Chao S, Desai S, Gale MD (1989) The isolation, characterization and application in the Triticeae of a set of wheat RFLP probes identifying each homeologous chromosome arm. Theor Appl Genet 78:342–348

Singh SR, Allen DJ (1979) Cowpea pests and diseases. International Institute of Tropical Agriculture, Ibadan, Nigeria

Southern E (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517

Steinmetz M, Minard K, Harvath S, McNicholas J, Srenlinger J, Wake C, Long E, Mach B, Hood L (1981) A molecular map of the immune response region of the major histocompatibility complex of the mouse. Nature 300:35–42

Talekar NS (1988) Biology, damage and control of bruchid pests of mungbean. In: Mungbean: Proceedings of the Second International Symposium, Asian Vegetable Research and Development Center, Shanhua, Taiwan, pp 329–342

Tanksley SD, Miller JC, Paterson AH, Bernatzky R (1988) Molecular mapping of plant chromosomes. In: Gustafson JF, Appels R (eds) Chromosome structure and function. Plenum Press, New York, pp 157–173

Young ND (1990) Potential applications of map-based cloning to plant pathology. Physiol Mol Pl Path 37:81–94

Young ND, Tanksley SD (1989a) Restriction fragment length polymorphism maps and the concept of graphical genotypes. Theor Appl Genet 77:95–101

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

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 120:579–585