Theor Appl Genet (1993) 87:243–249

THEORETICAL AND APPLIED GENETICS

Mapping oligogenic resistance to powdery mildew in mungbean with RFLPs

N. D. Young [1], D. Danesh [1], D. Menancio-Hautea [2], L. Kumar [3]

[1] Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA
[2] University of the Philippines at Los Baños, College Laguña, The Philippines
[3] National Chemical Laboratory, Pune 411008, India

Received: 20 November 1992 / Accepted: 1 March 1993

Abstract. We have used restriction fragment length polymorphisms (RFLPs) to map genes in mungbean (*Vigna radiata*) that confer partial resistance to the powdery mildew fungus, *Erysiphe polygoni*. DNA genotypes for 145 RFLP loci spanning 1570 centimorgans of the mungbean genome were assayed in a population of 58 F_2 plants. This population was derived from a cross between a moderately powdery mildew resistant ("VC3980A") and a susceptible ("TC1966") mungbean parent. F_3 lines derived from the F_2 plants were assayed in the field for powdery mildew response and the results were compared to the RFLP genotype data, thereby identifying loci associated with powdery mildew response. A total of three genomic regions were found to have an effect on powdery mildew response, together explaining 58% of the total variation. At 65 days after planting, two genomic regions were significantly associated with powdery mildew resistance. For both loci, the allele from "VC3890A" was associated with increased resistance. At 85 days, a third genomic region was also associated with powdery mildew response. For this locus, the allele from the susceptible parent ("TC1966") was the one associated with higher levels of powdery mildew resistance. These results indicate that putative partial resistance loci for powdery mildew in mungbean can be identified with DNA markers, even in a population of modest size analyzed at a single location in a single year.

Key words: Molecular markers – Polygenic – Quantitative trait loci (QTL)

Communicated by F. Salamini
Correspondence to: N. D. Young

Introduction

Plant disease resistance characters controlled by multiple genes have historically been difficult to study. The complex nature of host-parasite interactions, complicated further by several resistance loci, has made it extremely difficult to analyze oligogenic and polygenic disease resistance (Geiger and Heun 1989). Parlevliet (1976) investigated the genetics of latent period to leaf rust in barley (*Puccinia hordei*) and estimated the number of genes involved. Gene number and the effect of heterosis have been examined for complex resistance to wheat leaf rust (*Puccinia recondita* f. sp. *tritici*) (Lee and Shaner 1985), and the level of dominance involved in resistance to powdery mildew of barley (*Erysiphe graminis* f. sp. *hordei*) has been estimated (Jones et al. 1982). Heritability has also been estimated for several polygenic resistance characters (Johnson and Wilcoxson 1979; Lee and Shaner 1985; Randle et al. 1984). Nevertheless, these studies did not pinpoint specific genomic regions involved in resistance or characterize the effects of individual loci on disease response.

However, a new technique for identifying loci associated with complex genetic characters has recently been developed. The technique is based on DNA genetic markers, which include restriction fragment length polymorphisms (RFLPs) (Botstein et al. 1980) and random amplified polymorphic DNAs (RAPDs) (Williams et al. 1990). DNA genetic markers provide a large number of selection-neutral markers distributed throughout the genome. Using such DNA markers to monitor inheritance at high genetic resolution, scientists can potentially identify loci that individually control a portion of the variation in a complex phenotype. In the past, DNA genetic markers have been used primarily to study agronomic and morphological characters in plants. Examples in-

clude the use of RFLPs to determine the locations and effects of quantitative trait loci (QTL) related to fruit size, pH, and soluble solids in tomato (Paterson et al. 1988), hard seededness in soybean (Keim et al. 1990), seed size in mungbean and cowpea (Fatokun et al. 1992), heterosis in maize (Stuber et al. 1992), and morphological differences between maize and teosinte (Doebly et al. 1990).

By contrast, the use of DNA markers to study disease resistance in plants has focused primarily on single locus resistance genes. Examples include: *Tm2* (resistance to tomato mosaic virus), *Pto* (*Pseudomonas syringe* pv. tomato), *Mi* (*Meloidogyne incognita*), and *I2* (*Fusarium oxysporum* f. sp. *lycopersici*) resistance genes in tomato (Martin et al. 1991; Messeguer et al. 1992; Sarfatti et al. 1989; Young et al. 1988), *Dm* (*Bremia lactucae*) resistance genes in lettuce (Michelmore et al. 1991), *Ml-o* (*E. graminis*) in barley (Hinze et al. 1991), leaf blast (*Magnaporthe grisea*) resistance in rice (Yu et al. 1991), and resistance to the insect pest, *Callosobruchus*, in mungbean (Young et al. 1992). There have been far fewer DNA marker studies examining complex forms of resistance in plants. A short communication about multiple loci affecting resistance to maize dwarf mosaic virus in maize has been published by (Romero-Severson et al. (1989). Otherwise, the use of DNA markers to characterize oligogenic or polygenic disease resistance has not been exploited widely.

Powdery mildew (*Erysiphe polygoni* R. Hedw. D. C.) is a major pathogen of mungbean (*Vigna radiata* (L.) Wilczek). Mungbeans are important throughout Asia where they are prized for drought tolerance, early maturity, and nitrogen-fixing capacity (Shanmugasundarum and McLean 1988). Mungbean is also valuable for molecular studies because of its relatively small genome size (estimated at 470 to 570 million base pairs) (Arumuganathan and Earle 1991; Murray et al. 1979). Several mungbean genotypes show partial resistance to powdery mildew, including one known as 'VC3890A'. In previous studies of crosses involving 'VC3890A' as the resistant parent, powdery mildew resistance behaved as if it were controlled by a few unlinked genetic loci influenced by environment (D. H. Kim, Asian Vegetable Research and Development Center (AVRDC), Shanhua, Taiwan, personal communication). One indication of this has been the difficulty in transferring adequate levels of powdery mildew resistance from 'VC3890A' to other cultivars by conventional breeding. In this report, the genetics of powdery mildew resistance in 'VC3890A' has been analyzed further through the use of RFLP genetic markers.

Materials and methods

Mapping population

A cross was made between 'VC3890A', a moderately powdery mildew resistant mungbean cultivar, and line 'TC1966', a wild mungbean susceptible to powdery mildew, and advanced to the F_2 generation at the AVRDC in Shanhua, Taiwan (F_2 seed provided by Dr. D-H. Kim). Fifty-eight F_2 individuals were grown in a greenhouse in St. Paul, Minnesota, and leaf material was harvested from each F_2 plant as well as from the parents for DNA isolation and RFLP analysis. Plants were allowed to recover and set F_3 seed, which was saved for planting the following summer to assay powdery mildew reaction for each F_2 line, as described below.

DNA clones

As sources of putative RFLP markers, three different cloned genomic libraries were used. One library consisted of soybean DNA digested with the methylation-sensitive restriction enzyme, *Pst*I, and inserted into the phagemid, pBS+ (Stratagene, La Jolla, Calif.). These clones were the generous gift of Dr. R. Shoemaker, Iowa State University, Ames, Iowa. Additional genomic libraries were constructed using *Pst*I-digested mungbean or cowpea (*V. unguiculata*) DNA ligated into pUC18, as described previously (Young et al. 1992).

Plant DNA extraction, restriction digestion, and blotting

Plant DNA was isolated by the method of Dellaporta et al. (1983) and analyzed as described in Young et al. (1992). Briefly, the best restriction enzyme for analyzing each DNA clone was determined by probing individual DNA clones against blots containing parental DNA digested with six different restriction enzymes (*Bst*NI, *Dra*I, *Eco*RI, *Eco*RV, *Hae*III, and *Hin*dIII). This indicated which restriction enzyme gave the clearest fragment length polymorphism for each DNA clone. Then, DNA samples from all 58 F_2 individuals were digested with the same restriction enzyme, and a blot containing these F_2 DNA samples was probed with the corresponding DNA clone.

DNA hybridizations

Cloned DNA inserts were amplified by the polymerase chain reaction (Saiki et al. 1990) for use in radiolabeling reactions and nucleic acid hybridization as described in Young et al. (1992).

Construction of a mungbean RFLP linkage map

A total of 251 genomic clones were analyzed by hybridization with parental DNA, and of these clones, 141 were subsequently analyzed for segregation analysis among the F_2 progeny from the 'VC3890A' × 'TC1966' cross. The results of the segregation data were used to construct a linkage map with the mapping program, MAPMAKER-II (Lander et al. 1987). A minimum LOD score of 3.0 was used as a basis for declaring linkage between two RFLP markers, and a LOD score of 2.0 was used to establish the final order and distance between markers. Further details on the construction of the mungbean RFLP linkage map can be found in (Menancio-Hautea et al. (1993a, b)).

Powdery mildew disease assay

The powdery mildew response for each F_2-derived line was analyzed by testing F_3 plants in the field in St. Paul, Minnesota. For each F_2 line, duplicate rows of six F_3 plants were laid out in a completely randomized design during the summer of 1991. Duplicate rows of the parents were planted in the same field. Mungbeans were planted at the beginning of June with a spacing between plants of 25 cm and between rows of 75 cm. Cowpea plants, infected with a powdery mildew isolate originally derived from a single colony growing on a mungbean plant in the greenhouse, were used to initiate an artificial epidemic. This was accomplished by planting seeds of California Blackeye No. 5 cowpea around the mungbean plots (approximately 800 cowpea

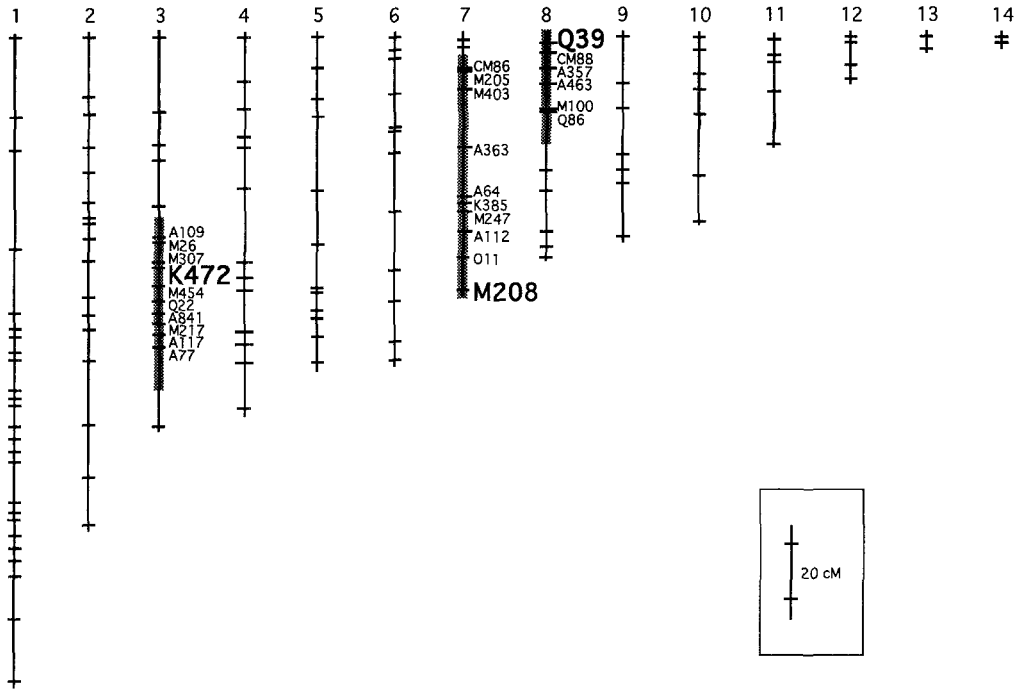

Fig. 1. RFLP map of mungbean showing locations of putative powdery mildew resistance loci. The 14 coherent linkage group of the mungbean RFLP linkage map are shown as *long vertical lines*, and RFLP markers are shown as *short horizontal lines* transecting the linkage groups. RFLPs unlinked to any other are not shown. A scale indicating genetic distance (measured in centimorgans) is located in the *lower right corner*. Locations of putative powdery mildew resistance loci are *highlighted in gray*, extending over contiguous RFLP loci showing an association significant at $P<0.01$. The RFLP markers in each region associated with powdery mildew response (without their two letter prefixes) are also shown. The markers showing the highest association with powdery mildew response are shown in *larger type*

plants in total) 2 weeks before planting the mungbeans. Upon emergence and then 3 weeks later, cowpea plants were inoculated with powdery mildew by shaking infected cowpea leaves over the young cowpea seedlings.

At 65 and 85 days after planting the mungbeans, each row of F_3 plants was scored as a group for powdery mildew response on a scale of 1 (no visible mycelial growth), 2 (0–25% foliage area covered by fungus), 3 (25–50% foliage area covered), 4 (50–75% foliage covered), and 5 (75–100% foliage covered). Every scoring was repeated twice, and any row showing conflicts between readings was re-evaluated a third time. For each F_2 line at each time point, readings from the duplicate F_3 rows were averaged to estimate disease response.

Mapping quantitative resistance loci

To identify genomic regions associated with powdery mildew resistance, RFLP results for each F_2 plant were compared to the mean powdery mildew reaction in corresponding F_3 row plots. This analysis was performed primarily with Statview-II (Abacus Concepts, Berkeley, Calif.) software on a Macintosh II-cx computer. An association between an RFLP marker and powdery mildew response was considered significant if the probability of observing an r-squared value, based on linear regression, was less than 0.002. This significance level, based on the reasoning of Lander and Botstein (1989), was selected to insure that a minimum number of false positive loci would be declared experiment-wide. To confirm the results of the regression analysis, data were also analyzed using the nonparametric test, Spearmann's rank correlation coefficient, as well as the program, Mapmaker-QTL (Lander and Botstein 1989).

Results

Current status of mungbean RFLP linkage map

The 141 DNA clones analyzed in this study mapped to 145 RFLP loci on 14 coherent linkage groups (plus six unlinked loci) spanning a total of 1570 centimorgans (cM) (Fig. 1). A substantial portion of the mungbean genome has been mapped with RFLPs, but a few gaps remain. For this reason, it is possible that some genetic loci associated with powdery mildew resistance may have been missed in our RFLP analysis. Further details on the current mungbean RFLP map can be found elsewhere (Menancio-Hautea et al. 1993a, b).

Disease response among F_2 lines

In the experimental plots inoculated with powdery mildew, 'VC3890A' rows showed moderate resistance at both 65 and 85 days after sowing (disease score = 2.0). 'TC1966' rows were highly susceptible at both scorings (disease score = 5.0). Duplicate rows of F_3 progenies derived from individual F_2 plants were examined for powdery mildew disease reaction at the same time. The correlation between duplicate row plots was high for each time point ($r=0.556$ at 65 days, $P=0.0001$, and $r=0.674$ at 85 days, $P=0.0001$) and between the means

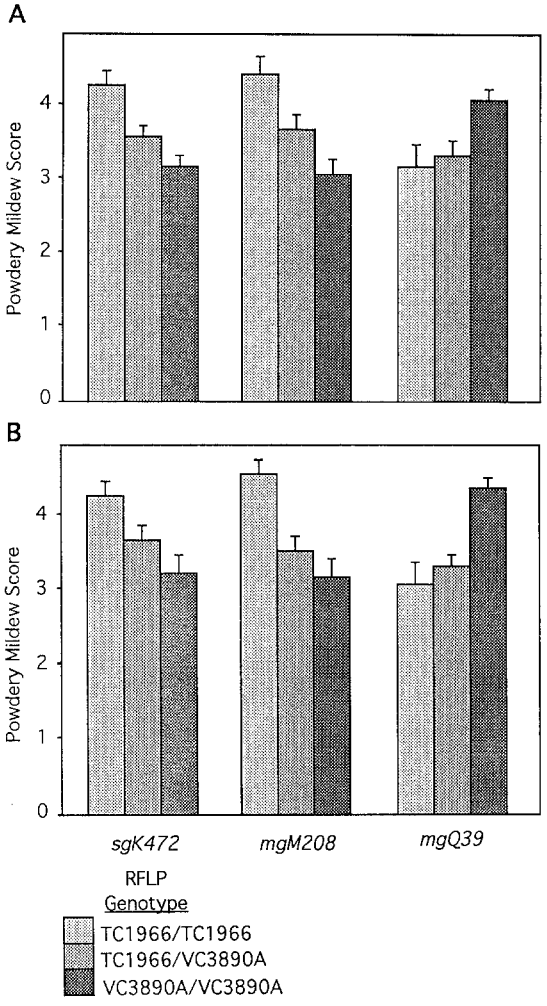

Fig. 2A, B. Comparison of disease score means for different RFLP genotypes at markers, sgK472, mgM208, and mgQ39. Average disease scores for the three different genotypic classes (VC3890A/VC3890A, VC3890A/TC1966, and TC1966/TC1966) at each RFLP marker 65 (**A**) and 85 (**B**) days after planting are shown. For each marker/genotype combination, the standard error of the mean is also indicated. Note that mgQ39 was not found to be significantly associated with powdery mildew response at 65 days, but is included for comparison with other data

of the two time points ($r=0.728$, $P=0.0001$). At 65 days, the distribution of powdery mildew disease response was symmetric, but by 85 days, the distribution had become distinctly skewed toward susceptibility (data not shown).

RFLPs associated with powdery mildew resistance loci at 65 days

At 65 days after planting, two unlinked loci with significant effects on powdery mildew resistance were observed (Figs. 1 and 2A, Table 1). On the basis of regression analysis, these loci together explained 51% of the variation in response to powdery mildew. Both loci were significant at the $P<0.001$ level. Analysis of the data with

Table 1. RFLP markers associated with powdery mildew response

Marker	Linkage group	65 days[a]	85 days
sgK472	3	0.242[b] 0.0002[c]	0.174 0.0017
mgM208	7	0.270 0.0003	0.225 0.0011
mgQ39	8	–[d] –	0.284 0.0001

[a] Days after planting
[b] R-squared values based on regression analysis
[c] Probability of observing the r-squared value
[d] Not significant at $P<0.002$

Spearmann's rank correlation coefficient and Mapmaker-QTL gave essentially the same results as regression, so only the results from the regression analysis will be described.

One marker showing a strong association with powdery mildew response was sgK472, located on linkage group three of mungbean (Figs. 1 and 2A, Table 1). This marker had an r-squared value of 0.242 and $P=0.0002$. Homozygotes for the 'VC3890A' allele at sgK472 were nearly 1.2 disease score units less than homozygotes for the 'TC1966' allele. This translated to a phenotypic difference in which 'TC1966' homozygotes for sgK472 were highly diseased on average compared to VC3890A homozygotes, which showed only intermediate powdery mildew symptoms on average. Heterozygotes at this locus had a mean powdery mildew disease response slightly more resistant than the midpoint between the two homozygous classes (Fig. 2A) – indicating both additive and dominant gene action for this putative resistance locus.

The second RFLP marker showing a highly significant association with powdery mildew response was mgM208, located on linkage group seven (Figs. 1 and 2A, Table 1). In this case, the r-squared value was 0.270 and $P=0.0003$. Homozygotes for the 'VC3890A' allele were almost 1.4 disease score units lower than homozygotes for 'TC1966'. Heterozygotes were nearly equal in disease score to the midpoint between the homozygous classes, suggesting that the putative powdery mildew resistance gene near mgM208 behaved in a predominantly additive manner.

In addition to the two loci described above, two other loci were associated with powdery mildew response, but did not reach the stringent cutoff level used in this analysis. One of these regions was located on linkage group four near RFLP sgA515 (r-squared = 0.164; $P=0.0027$), the other was located on linkage group eight near RFLP mgQ39 (r-squared = 0.135; $P=0.0067$).

RFLPs associated with powdery mildew resistance loci at 85 days

Both genomic regions found to be significant at 65 days (sgK472 and mgM208) were also found to be significant 20 days later (Fig. 2B, Table 1). Nevertheless, the significance for both regions dropped off during this time interval. At 85 days, sgK472 showed an r-squared value of 0.174, $P=0.0017$, while mgM208 showed an r-squared value of 0.225, $P=0.0011$.

A third RFLP marker associated with powdery mildew response at 85 days was mgQ39 (Figs. 1 and 2B, Table 1). This locus had just missed being classed as significant at 65 days, but by 85 days it was highly significant with an r-squared value of 0.284 and $P=0.0001$. In contrast to the other putative resistance loci described above, the allele from 'TC1966' at mgQ39 was the one associated with higher level of resistance to powdery mildew (Fig. 2B). Homozygotes for the 'TC1966' allele at this marker were nearly 1.3 disease score units lower than 'VC3890A' homozygotes – despite the fact that 'TC1966' is itself more susceptible to powdery mildew disease. Also, in contrast to the significant loci described above, heterozygotes were nearly as resistant to powdery mildew as 'TC1966' homozygotes, suggesting that this putative resistance locus is mostly dominant in effect. Together with the other two putative resistance loci, 58% of the total variation in powdery mildew response could be explained by all three RFLP markers at 85 days.

A fourth genomic region on linkage group nine at RFLP sgA882 was also associated with powdery mildew response at 85 days, but did not reach the cutoff for significance. This RFLP showed an r-squared value of 0.173, $P=0.0022$. Like mgQ39, the allele from TC1966 was the one associated with resistance to powdery mildew in the case of sgA882.

Discussion

DNA marker technology enables scientists to characterize complex genetic traits in greater detail than ever before. Before the availability of high density DNA genetic maps, it was extremely difficult to monitor the inheritance of specific segments throughout a genome in detail. This made it difficult to characterize relationships between a specific genetic locus and a multigenic character. With the advent of high density RFLP maps, mapping quantitative trait loci has become routine, and characters that are under the control of multiple genes can now be dissected into individual components and examined for their individual effects.

Parameters and limitations in RFLP mapping of resistance loci

Our results indicate at least three genetic loci with major individual effects on powdery mildew disease response exist in mungbean. The population size used in this study (58 F_2 lines) is relatively small for mapping genes underlying complex characters, so loci with minor effects were probably not uncovered. However, even with a population of this size, loci with a major effects on a powdery mildew resistance should easily have been discerned (Lander and Botstein 1989). The putative resistance loci described in this paper ranged in effect from 17% to more than 28% of the total variation in disease response. That there are few loci of intermediate effect involved in powdery mildew resistance in mungbean is in accord with previous studies of oligogenic resistance against other biotrophic fungi, where gene numbers from two (Lee and Shaner 1985) to eight (Parlevliet 1976) have been described.

In all cases where a putative resistance locus was identified, several contiguous RFLP markers were found to have a significant correlation to the disease response (Fig. 1). This reflects the uncertainty that is inherent in QTL mapping – namely, that the region of the genome containing a disease resistance locus can be identified, but the precise location of the QTL on the genetic map is often uncertain. Moreover, there was always the possibility that multiple resistance loci were located near one another on the chromosome and appeared as a single significant genomic region in the statistical analysis.

Details on the putative powdery mildew resistance loci

For two of the putative resistance loci identified in this study, the allele from the resistant parent ('VC3890A') conferred higher levels of resistance, as expected. Nonetheless, for one putative resistance locus, the allele from the more susceptible parent ('TC1966') actually conferred higher levels of resistance among the progeny. This third locus was also observed to be more important later in the infection process, while the other two loci appeared to be relatively less significant at the later time point. This may suggest that different genes play a role in response to powdery mildew at different times after infection. It may also reflect environmental conditions around the time of disease scorings. In the case of the 65 day reading, there had been no rain for 10 days preceding the scoring, but it had rained several times in the days leading up to the 85 day reading.

Together, the three loci uncovered in this study explained nearly 58% of the total variation in powdery mildew response. This is actually a large proposition of the variation when one considers that it represents total variation, which includes environmental effects and experimental error in addition to variation controlled by genotype (equivalent, in this analysis, to the RFLP score).

Still, the results in this paper describe the genetics of powdery mildew resistance in only a single environment

and a single year, and it will be important to determine whether the putative resistance loci described here are effective in different environments and against different isolates of *Erysiphe polygoni*. Indeed, powdery mildew assays with sibling material from the 'VC3890A' × 'TC1966' cross have recently been carried out by collaborators at the AVRDC in Taiwan. Their preliminary results indicate that only two of the three genomic regions described in the current study (sgK472 and mgM208) potentially play a role in Taiwan, and the associations were significant only at $P=0.01$ (D.H. Kim and G. Hartman, AVRDC, Shanhua, Taiwan, personal communication). Moreover, an additional locus (mgQ43 on linkage group two) that was without effect in Minnesota was found to be significant at $P=0.001$ in Taiwan. Whether these discrepancies are due to environment, experimental method, or *Erysiphe polygoni* isolate is still uncertain.

Because a more thorough study of oligogenic resistance to powdery mildew is warranted, we are collaborating with the scientists at AVRDC to develop recombinant inbred lines from the 'VC3890A' × 'TC1966' cross. These lines, which are derived by single seed descent of individual F_2 plants to the F_9 generation or later, provide a set of inbreds that are each genetically homogeneous and unlimited in supply. Seed from these lines can be tested in multiple environments over several years and, in this way, variations due to testing small groups of F_3 plants in a single environment and year would be eliminated. With recombinant inbreds, it will be possible to estimate the effect of environment, experimental technique, and powdery mildew isolate on quantitative resistance genes segregating in this cross.

RFLPs, marker-assisted selection, and genetic dissection

Determining the locations of putative disease resistance loci has the potential to accelerate the breeding of powdery mildew resistant mungbeans. Now that genomic regions associated with powdery mildew resistance are known, selection for powdery mildew resistant plants can be carried out by genotype, instead of by phenotype alone. Complex disease resistance can be difficult to breed for at the phenotypic level. Now, rather than selecting mungbean lines solely on the basis of powdery mildew response, plants in a population can be selected by virtue of carrying the desirable alleles at the RFLP loci uncovered in this study. This should be particularly significant for the putative resistance loci in which the allele for resistance comes from the susceptible parent, since selection often assumes that resistance genes only come from the resistant parent.

In addition to playing a role in marker-assisted breeding, mapping partial resistance loci with RFLP markers should make it possible to create lines in which the individual effects of each putative resistance locus can be examined in detail (Paterson et al. 1990). In particular, RFLP markers can be used to develop lines that are homozygous for all of the putative resistance loci except one. The remaining resistance locus would then be expected to segregate as a Mendelian factor, and its individual effect could be examined without variation due to the other putative resistance loci.

Acknowledgements. We thank Dr. C. Fatokun, G. Foley, and J. Reily for help and advice and Dr. D. H. Kim and G. Hartman of the Asian Vegetable Research and Development Center for their valuable collaboration. This research was supported, in part, by a grant from the Rockefeller Foundation and is published as contribution no. 20,243 in the series of the Minnesota Agricultural Experiment Station.

References

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

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

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

Doebly J, Stec A, Wendel J, Edwards M (1990) Genetic and morphological analysis of a maize-teosinte F_2 populations: implications for the origin of maize. Proc Natl Acad Sci USA 87:9888–9892

Fatokun CA, Menancio-Hautea D, Danesh D, Young ND (1992) Evidence for orthologous seed weight genes in cowpea and mungbean based on RFLPs. Genetics: 132:841–846

Geiger HH, Heun M (1989) Genetics of quantitative resistance to fungal diseases. Annu Rev Phytopathol 27:317–341

Hinze K, Thompson RD, Ritter E, Salamini F, Schulze-Lefert P (1991) Restriction fragment length polymorphism-mediated targeting of the *ml-o* resistance locus in barley (*Hordeum vulgare*). Proc Natl Acad Sci USA 88:3691–3695

Johnson DA, Wilcoxson RD (1979) Inheritance of slow rusting of barley infected with *Puccinia hordei* and selection of latent period and number of uredia. Phytopathology 69:145–151

Jones IT, Sethar H, Davies I (1982) Genetics of partial resistance to barley mildew. Barley Genet 4:449–457

Keim P, Diers BW, Shoemaker RC (1990) Genetic analysis of soybean hard seededness with molecular markers. Theor Appl Genet 79:465–469

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP 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

Lee TS, Shaner GE (1985) Oligogenic inheritance of length of latent period in six slow leaf-rusting wheat cultivars. Phytopathology 75:636–643

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, Fatokun C, Kumar L, Danesh D, Young ND (1993a) Comparative genome analysis of mungbean (*Vigna radiata* L. Wilczek) and cowpea (*V. unguiculata* L. Walpers) using RFLP mapping data. Theor Appl Genet/In press)

Menancio-Hautea D, Fatokun C, Kumar L, Danesh D, Young ND (1993b) RFLP linkage map for mungbean (*Vigna radiata*). In: Stephen O'Brien (ed) *Genetic maps* – 1992, 6th edn. Cold Spring Harbor Press, Cold Spring Harbor, N.Y. pp 6259–6261

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

Parlevliet JE (1976) Partial resistance of barley to leaf rust, *Puccinia hordei*. III. The inheritance of the host plant effect on latent period in four cultivars. Euphytica 25:241–248

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721–726

Paterson AH, DeVerna JW, Lanini B, Tanksley SD (1990) Fine mapping of quantitative trait loci using selected overlapping recombinant chromosomes, in an interspecies cross of tomato. Genetics 124:735–742

Randle WM, Davis DW, Groth JV (1984) Improvement and genetic control of partial resistance in sweet corn. J Am Soc Hortic Sci 109:777–781

Romero-Severson J, Lotzer J, Brown C, Murray M (1989) Use of RFLPs in analysis of quantitative trait loci in maize. In: Helentjaris T, Burr B (eds) *Development and application of molecular markers to problems in plants*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. pp 97–102

Saiki RK, Gelfand DH, Stoffel S, Scharf S, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491

Sarfatti M, Katan J, Fluhr R, Zamir D (1989) An RFLP marker in tomato linked to the *Fusarium oxysporum* resistance gene *I2*. Theor Appl Genet 78:22–26

Shanmugasundarum S, McLean BT (eds) (1988) Mungbean. Proc 2nd Int Symp Asian Vegetable Research and Development Center, Shanhua, Taiwan

Stuber CW, Lincoln SE, Wolff DW, Helentjaris T, Lander ES (1992) Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics 132:823–839

Williams J, Kubelik A, Livak K, Rafalski J, Tingey S (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

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

Young ND, Kumar L, Menancio-Hautea D, Danesh D, Talekar NS, Shanmugasundarum S, Kim DH (1992) RFLP mapping of a major bruchid resistance gene in mungbean (*Vigna radiata* L., Wilczek). Theor Appl Genet: 84:839–844

Yu ZH, Mackill DJ, Bonman JM, Tanksley SD (1991) Tagging genes for blast resistance in rice via linkage to RFLP markers. Theor Appl Genet 81:471–476