Restriction fragment length polymorphism-mediated targeting of the *ml-o* resistance locus in barley (*Hordeum vulgare*)

(backcross lines/*Erysiphe graminis* f.sp. *hordei*/intravarietal crosses/linkage drag/plant resistance gene)

KARIN HINZE, RICHARD D. THOMPSON, ENRIQUE RITTER, FRANCESCO SALAMINI,
AND PAUL SCHULZE-LEFERT

Max-Planck-Institut für Züchtungsforschung, Abteilung für Züchtungsforschung und Ertragsphysiologie, D-5000 Cologne 30, Federal Republic of Germany

Communicated by J. Schell, December 5, 1990

ABSTRACT The *ml-o* locus in barley confers resistance to all known races of the fungus *Erysiphe graminis* f.sp. *hordei*. Since the molecular mechanisms underlying *ml-o*-mediated resistance are currently undefined, experiments have been initiated to isolate the gene by means of its map position. A collection of backcross lines containing *ml-o* alleles derived from six barley genotypes allowed us to identify a set of DNA markers very tightly linked to the resistance locus. These markers span an unexpectedly small segment of 8.6 centimorgans on chromosome 4 that includes the *ML-o* locus. Two of the markers cosegregate with the resistance locus on the basis of 44 homozygous resistant plants identified within a segregating F_2 population derived from an intravarietal cross. Colinearity of the resistance-linked markers was confirmed in an F_2 mapping population derived from a wide cross between *Hordeum vulgare* subsp. *vulgare* and *Hordeum vulgare* subsp. *spontaneum*. The two markers cosegregating with the resistance locus in the former cross define in the latter cross an interval of 2.4 centimorgans within which *ML-o* is most probably situated. The set of linked markers opens up the possibility of carrying out a bidirectional chromosomal walk or jump to the gene.

Erysiphe graminis f.sp. *hordei* is the causal agent of powdery mildew disease in barley. Several loci conferring resistance against powdery mildew infection have been identified and mapped through classical linkage analysis (1–3). Among these *ml-o* has two outstanding features: resistance alleles are recessive (most probably through the loss of function of the gene) and they mediate worldwide resistance to all known races of the fungus (4). A large collection of spontaneous and induced *ml-o* mutants in different barley genotypes have been described phenotypically and have been used in linkage studies (4, 5). The locus maps to the long arm of chromosome 4 (6). Despite an extensive genetic and physiological characterization of *ml-o* mutants (7–9) the molecular mechanisms underlying *ml-o*-mediated resistance are not yet understood.

In an attempt to identify the corresponding gene product(s) by a “reverse genetics” (10) approach, a strategy based on a search for very tightly linked DNA markers has been adopted. The identification of such markers usually requires a preexisting restriction fragment length polymorphism (RFLP) map built up from a very large number of probes. To bypass this time-consuming process, we used a collection of *ml-o* near-isogenic lines and the method of colabeling and simultaneous probing of sets of anonymous clones (11).

In this study we analyzed eight *ml-o* backcross lines (BC lines), produced through seven backcrosses of different *ml-o* mutants with cultivar Ingrid, which served as the recurrent parent. Importantly, the donor parents represent six different genetic backgrounds. The tight linkage among RFLP markers

identified in the *ml-o* BC line survey was assessed in the F_2 population of the wide cross *Hordeum vulgare* subsp. *spontaneum* $\times$ *Hordeum vulgare* subsp. *vulgare* cv. Aramir. Finally we measured distances of the markers relative to *ML-o* through analysis of the homozygous *ml-o* plants in an F_2 population derived from the intravarietal cross of *Hordeum vulgare* cv. Carlsberg II *ML-o* $\times$ *Hordeum vulgare* cv. G. Zweiz. *ml-o11*.

MATERIALS AND METHODS

Plant Material. The intravarietal cross was carried out between lines *Hordeum vulgare* subsp. *vulgare* cv. Carlsberg II *ML-o* and *Hordeum vulgare* subsp. *vulgare* cv. G. Zweiz. *ml-o11* (HOR 23937), the wide cross between lines *Hordeum vulgare* subsp. *spontaneum* and *Hordeum vulgare* subsp. *vulgare* cv. Aramir.

All *ml-o* BC lines were a gift from James MacKey. They were generated through seven backcrosses with *H. vulgare* subsp. *vulgare* cv. Ingrid followed by at least six selfings. Table 1 summarizes appropriate information from all *ml-o* mutants that were used as donor parents in the backcross procedure.

Genomic Library. The source of probes utilized in this study is a genomic DNA library from *Hordeum vulgare* cv. Carlsberg II. In brief, genomic DNA was digested to completion with *Pst* I and size fractionated through ultracentrifugation on sucrose gradients. Size-selected DNA (0.5–3.0 kilobases) was inserted into plasmid vector pUC18 followed by transformation into *Escherichia coli* strain TG-2.

Library Screening. A primary screening procedure eliminated clones coding for repetitive or organellar sequences; plasmid DNA preparation (12) was followed by digestion with *Pst* I, separation of DNA fragments on 1% agarose gels, and transfer to nylon membranes (HybondN; Amersham). Those clones that gave rise to hybridization signals with labeled total genomic, or mitochondrial, or chloroplast barley DNA were discarded.

The *ml-o* BC-survey filters were prepared essentially as described by Gebhardt *et al.* (14). DNA restriction enzymes used for RFLP analysis were *Alu* I, *Hae* III, *Rsa* I, and *Taq* I, all of them recognizing a different 4-base pair (bp) target sequence.

Probe Labelings and Hybridizations. DNA inserts from barley plasmid clones were recovered and stored in agarose plugs after separation from vector DNA on 1% low-melting-point agarose gels. After melting of the insert containing agarose at 70°C, generally four to six insert DNAs (2–5 ng each) were mixed, labeled with 20 μ Ci of [α - 32 P]dCTP (3000 Ci/mmol; 1 Ci = 37 GBq) (15), and hybridized simultaneously to *ml-o* BC-line survey filters. All hybridizations were

Table 1. *ml-o* mutant alleles

Allele	Mutant	Mother variety	Mutagen
<i>ml-o1</i>	M66	Haisa	X-ray
<i>ml-o3</i>	M.C.20	Maltesia Heda	γ -Ray
<i>ml-o4</i>	SR 1	Foma	X-ray
<i>ml-o5</i>	Risø 5678	Carlsberg II	EMS
<i>ml-o8</i>	Risø 7372	Carlsberg II	EMS
<i>ml-o9</i>	SZ 5139b	Diamant	EMS
<i>ml-o10</i>	SR 7	Foma	γ -Ray
<i>ml-o11</i>	G. Zweiz.	G. Zweiz.	Spontaneous

EMS, ethylmethane sulfonate. G. Zweiz., Grannenlose Zweizeilige.

carried out in 10% dextran sulfate/1 M NaCl/1% sodium dodecyl sulfate/80 μ g of denatured salmon sperm DNA per ml at 65°C overnight.

Linkage Analysis. In the wide cross, maximum likelihood estimates of recombination frequency of all pairs of polymorphic loci defined by single restriction fragments were calculated as described in Ritter *et al.* (16). This calculation includes multipoint linkage and the EM algorithm for handling missing values. Since in the intravarietal cross only homozygous resistant *ml-o* plants were analyzed, maximum likelihood estimates of recombination frequencies were calculated within the recessive class of a 1:2:1 segregating F_2 population according to table 6, formula 16, of Allard (17): $p = (h + 2b)/2n$, where p = recombination frequency, n = number of homozygous recessive individuals in F_2 , h = number of heterozygous recombinant individuals in F_2 , and b = number of homozygous recombinant individuals in F_2 . The standard error within the recessive class was calculated according to Allard (17): $SD = \pm \sqrt{[p(1-p)]/2n}$.

Test for Resistance. Powdery mildew infections were carried out in the greenhouse with a random field population of *Erysiphe graminis* f.sp. *hordei* isolated and multiplied on *Hordeum vulgare* cv. Golden Promise. Four-hundred thirty-nine F_2 seedlings from the intravarietal cross were infected with powdery mildew conidia 10 days after sowing. Plants were grown at 18°C and scored 10 days after infection.

RESULTS

Identification of RFLPs Through *ml-o* BC Lines. Approximately 1100 anonymous genomic *Pst* I clones from barley detecting single/low-copy DNA sequences were tested by hybridization to *ml-o* BC-line survey filters (see *Materials and Methods*). Only five clones were identified that display RFLPs between DNA from the recurrent parent (cv. Ingrid) and DNAs from at least two of the eight tested *ml-o* BC lines.

As a representative example, the results obtained with one of the clones, bAN61, are shown in Fig. 1. The data produced when this probe was hybridized to DNA from each BC line are assigned to three groups: (i) BC lines *ml-o1*, *ml-o5*, and *ml-o8* display a different pattern compared to the recurrent parent cultivar Ingrid (Fig. 1a, lanes 1, 4, 5, and 9). The RFLP patterns obtained with each of these BC lines are also present in the DNAs from the respective donor parents (Fig. 1b, lanes 1 and 4). It is therefore concluded that in BC lines *ml-o1*, *ml-o5*, and *ml-o8*, clone bAN61 defines a locus of donor parental origin that is retained throughout the backcross procedure while selecting for *ml-o* resistance. (ii) DNAs from BC lines *ml-o3*, *ml-o4*, *ml-o9*, and *ml-o10* and from the recurrent parent produce a homomorphic fragment pattern (Fig. 1a, lanes 2, 3, 6, 7, and 9). A polymorphism, however, differentiates the recurrent and donor parental DNAs from these four BC lines (Fig. 1b, lanes 2, 3, 6, 7, and 9). Therefore the donor parental pattern was replaced by the one from the recurrent parent during the backcross procedure,

FIG. 1. Genomic Southern analysis of *ml-o* BC lines. The upper panel shows for each *ml-o* BC line analyzed and the recurrent parent (cultivar Ingrid) a graphical representation of barley chromosome 4. Introgressed fragments, including *ml-o* alleles derived from six different donor parental genetic backgrounds, are indicated for each BC line. BC lines *ml-o4/ml-o10* and *ml-o5/ml-o8* are derived from identical donor parental genetic backgrounds and are therefore denoted by an identical graphical underlay. The lower part displays the RFLP detected by probe bAN61 on genomic hybridization filters containing DNAs from each *ml-o* BC line (lanes 1–8 in a) and the respective donor parents (lanes 1–8 in b). Lane 9 represents the pattern detected with genomic DNA from the recurrent parent (RP).

indicating that locus *bAN61* in these four BC lines is outside the introgressed fragment containing *ml-o*. (iii) DNA from BC line *ml-o11* is homomorphic in comparison to the recurrent parent DNA (Fig. 1a, lanes 8 and 9). Since, however, probe bAN61 detects an identical fragment pattern in the DNA from the donor parent (Fig. 1b, lane 8), bAN61 is judged to be noninformative for BC line *ml-o11*.

A summary of the information obtained from the *ml-o* BC-line survey filters for each of the identified RFLP markers is given in Table 2. Additional characterization of each *ml-o* BC line was based on markers known to map distal to *ml-o* on chromosome 4 (K.H., unpublished results). With the exception of the cDNA coding for the β -amylase gene (18), none of the probes is informative for all BC lines.

Genetic Mapping of RFLP Markers Identified Through *ml-o* BC Lines. Location of the five RFLP markers identified in the *ml-o* BC-line survey on barley chromosome 4 was verified through a barley chromosome 4 addition line in wheat (ref. 19; data not shown). Furthermore, an F_2 population of 147 individuals of a wide cross between *Hordeum vulgare* subsp. *spontaneum* and *Hordeum vulgare* subsp. *vulgare* cv. Aramir was used to measure genetic distances between the respective loci (Fig. 2). Four of the five probes (bAP91, bAL88/2, bAO11, and bAN61) were found to be informative. They identify four polymorphic loci distributed within a total distance of only 8.6 cM. The fifth probe, bBE54, defines two loci (a and b) on the same linkage group (see also below). Locus *bBE54b*, also identified through the *ml-o* BC-line survey, was not informative in this wide cross-derived mapping population. The second locus, *bBE54a*, maps at a distance of 24.5 cM in a centromeric position with respect to *bAP91* and is not a marker or not informative for the BC lines tested (see also Table 2).

Additional information with respect to map position within the linkage group was gained through the availability of a

Table 2. *ml-o* BC-line survey using barley chromosome 4 RFLP markers

<i>ml-o</i> BC line	RFLP probe						β -Amylase cDNA	bAL57	b4-104a
	bAN61	bBE54		bAP91	bAL88/2	bAO11			
		<i>a</i>	<i>b</i>						
<i>ml-o1</i>	+	NI	+	+	+	NI	—	NI	—
<i>ml-o3</i>	—	NI	+	+	+	+	—	NI	—
<i>ml-o4</i>	—	NI	NI	NI	NI	+	—	NI	—
<i>ml-o5</i>	+	NI	+	+	+	NI	—	NI	NI
<i>ml-o8</i>	+	NI	+	+	+	NI	—	NI	NI
<i>ml-o9</i>	—	NI	NI	+	+	NI	—	NI	—
<i>ml-o10</i>	—	NI	NI	NI	NI	+	—	NI	—
<i>ml-o11</i>	NI	—	NI	+	+	+	—	—	—

+, The donor parental polymorphism is still present in the BC line; —, the donor parental polymorphism is replaced through the recurrent parent polymorphism in the BC line; NI, noninformative probe: the donor and recurrent parent have an identical restriction fragment pattern.

cDNA coding for the β -amylase gene: this gene (*Bmy1* locus) is known to map in a telomeric position with respect to *ML-o* (20). Linkage was found between a polymorphism detected through the β -amylase cDNA probe and all RFLP loci identified in the *ml-o* BC-line analysis (Fig. 2). With respect to the position of this lateral marker, the chromosomal segment bordered by *bAO11* and *bAN61* can be located at a position on the long arm of chromosome 4, which approximates to the *ML-o* position based on multipoint tests using classical phenotypic markers (6, 21).

Genetic Distances of RFLP Markers to *ML-o*. We analyzed an F_2 population segregating for resistance to mildew conferred by an *ml-o* allele to assess the genetic distances between *ML-o* and the RFLP markers identified in the *ml-o* BC lines. A total of 439 F_2 plants from an intravarietal cross between *Hordeum vulgare* subsp. *vulgare* cv. Carlsberg II *ML-o* $\times$ cv. G. Zweiz. *ml-o11* were infected with *Erysiphe graminis* f.sp. *hordei* spores. Resistant and susceptible plants

segregated according to a ratio of 1:3.7 (expected, 1:3). This deviation from the expected ratio was probably due to misclassifications of resistant and susceptible plants: Plants that allowed the growth of very few fungal colonies were scored as susceptible in our bioassay. It has been reported, however, that *ml-o* resistance may frequently allow a very low level of colony formation on the infected leaves (22).

DNA from 44 clearly resistant plants (homozygous *ml-o ml-o*) was analyzed (Fig. 3). Loci *bAL88/2* and *bAO11* cosegregate with the allele *ml-o* since in all 44 plants only the respective RFLP fragments derived from the resistant parent G. Zweiz. *ml-o11* are present. Probes bAP91 and bBE54 display only in plant 35 a state of heterozygosity of the RFLP alleles derived from the two parents: these two markers therefore cosegregate and map at a distance of 1.1 cM relative to *ml-o*. Probe bAN61 detects four plants (nos. 8, 15, 30, and 35) that are heterozygous for *bAN61*. Since in the wide cross *bAN61* maps 5.4 cM centromeric from *bAP91* (Fig. 2), it was expected that plant 35—with a heterozygosity for *bAP91*—should also be heterozygous for *bAN61*. The results from this intravarietal cross are therefore consistent with the linear order and distances among loci *bAL88/2*, *bAP91*, and *bAN61* established by the wide cross summarized in Fig. 2.

"Linkage-Drag" Around *ML-o*. Based on the genetic distances among chromosome 4 loci defined by RFLP markers we estimate the maximum size of the introgressed fragment for each *ml-o* BC₇ line (Fig. 4). BC lines *ml-o3*, *ml-o4*, *ml-o9*, and *ml-o10* contain the smallest detectable introgressed fragment size bracketed by *bAN61* and *Bmy1*. Due to a lack of informative probes we cannot assess a centromeric border for BC lines *ml-o5* and *ml-o8*. It should be noted, however, that none of the remaining BC lines contains donor DNA extending into the other arm of chromosome 4 since marker *b4-104a*—according to map position—is still diagnostic for the *ml-o*-containing chromosome arm.

Toward the telomeric end of the chromosome none of the BC lines retains the donor parental allele of the *Bmy1* locus. Therefore the size of the introgressed fragment telomeric from *ml-o* in all cases must be smaller than 17 cM (assuming *ml-o* to be located between *bAO11* and *bAL88/2*). In a BC₇ line according to Stam and Zeven (23) a replacement of a donor parental marker positioned at this distance to the selected marker locus is, however, expected to occur only with a probability of $p = 0.59$. If the same calculation is done for the marker couple *ml-o*–*bAN61*, which defines an interval centromeric from *ml-o*, replacement of the donor parental marker *bAN61* is expected to occur with $p = 0.39$. Since we find that at least in four of eight *ml-o* BC lines donor parental alleles of *bAN61* are replaced through the recurrent parent alleles, these data indicate that the linkage drag around *ML-o* is at least not larger than predicted from the theoretical estimates of Stam and Zeven.

FIG. 2. Linkage map of RFLP markers on barley chromosome 4. Estimated genetic distances between RFLP markers are indicated by numbers in centimorgans (cM) based on multipoint linkage analysis (16). The data were obtained from 147 individuals in a segregating F_2 population from the wide cross *Hordeum vulgare* subsp. *vulgare* $\times$ *Hordeum vulgare* subsp. *spontaneum*.

FIG. 3. Genetic distances of chromosome 4 RFLP markers to *Ml-o*. The right half shows the results of Southern hybridizations obtained by the indicated marker probes with genomic DNAs from 44 homozygous *ml-o*-resistant F_2 plants. Hybridization patterns obtained with DNAs from the resistant (P_1) and the susceptible (P_2) parent are shown in addition. The arrowheads mark patterns obtained with plant 35 indicating heterozygosity for *bAP91*, *bBE54b*, and *bAN61*. On the left, genetic distances of RFLP markers relative to *ml-o* are indicated by numbers in cM based on maximum likelihood estimates of recombination frequencies calculated within the recessive class of a 1:2:1 segregating F_2 population (see text and ref. 17).

DISCUSSION

Cloning by map position requires the identification of RFLP markers very tightly linked to the target gene. This demands a screening procedure for a very high number of genomic clones. The effectiveness of analyzing BC lines by simultaneous probing with sets of genomic clones has been demonstrated here and in a previous report (11). If such an approach is taken, our results strongly suggest the screening of a collection of BC lines produced from as many different genetic backgrounds as possible for the following reasons: (i) We frequently identified genomic clones detecting polymorphisms between the DNA from a single BC line and the recurrent parent. All of these clones, however, define loci not linked to *Ml-o* (by F_2 segregation criteria of the wide cross) and therefore identify *Ml-o* unlinked DNA segment(s) of donor parental origin retained during the backcross procedure. (ii) An analysis including 48 barley varieties revealed that in the barley genome restriction polymorphisms are detected only with an average frequency of 43% based on restriction enzymes identifying 6-bp target sequences (24). If only two barley lines are considered this predicts that in a pool of anonymous probes only half of the probes will be informative. Since many resistance genes in barley and other cereals were introgressed by intravarietal crosses,* this necessitates the inclusion of several genetic backgrounds in a BC-line survey to maximize the percentage of informative probes.

Each of the *ml-o* BC lines used in this study has been backcrossed seven times. According to Stam and Zeven (23), the size of the introgressed fragment that is retained in the marker chromosome should be 30 cM with a SD of 20 cM (assuming for chromosome 4 a total length of 150 cM). We therefore expected to identify 25–35 *Ml-o* linked markers within the pool of 1100 analyzed barley clones (calculated on the basis of a Poisson distribution and assuming a total length of 1.050 cM for the barley genome map). Since random

probes may detect more than one locus (e.g., *bBE54*), this number is more likely to be underestimation. There is, however, a clear discrepancy between the expected value and the number of *Ml-o* linked markers actually identified (a total of five). Also, the size of the introgressed fragment that is defined by these markers (8.6 cM) does not correspond to the prediction. We speculate that the low frequency of *Ml-o* linked markers and their confinement to a small chromosomal fragment around *Ml-o* are due to a nonrandom origin of cloned genomic *Pst* I fragments in the vicinity of this locus. Since all tested *Pst* I clones detect nonrepetitive low- or single-copy-number DNA sequences, the underrepresentation of such sequences could reflect an overrepresentation of repetitive DNA in the vicinity of the locus.

There are several reports describing colinearity of markers if wide- (including interspecific) and intravarietal-based linkage data are compared. Different distances between single marker couples have, however, been found frequently (25, 26). A comparison of our linkage data based on the wide- and intravarietal crosses (Figs. 2 and 3) indicates colinearity of *Ml-o* linked markers. In addition, we also find a good correspondence of marker-based distances in both crosses. The exception, however, is the marker couple *baO11*–*baL88/2*, forming a 2.4-cM interval only in the wide cross, whereas both markers cosegregate with *ml-o* in the intravarietal cross. The simplest explanation for this result is an inadequate sample size of homozygous *ml-o* plants in the intravarietal cross. Increasing the sample size should identify resistant plants in which a recombination has occurred between *ml-o*–*baL88/2* and/or *ml-o*–*baO11*.

Young and Tanksley (27) describe that backcross breeding was largely ineffective in reducing the size of linked DNA around the *Tm-2* gene, a resistance gene introgressed from *Lycopersicon peruvianum* into *Lycopersicon esculentum* (28). They describe a *Tm-2* BC line that retained, even after 11 backcrosses, one complete chromosome arm plus a portion of the other arm from the donor parent, indicating a strong linkage drag around *Tm-2*. Our results suggest that the linkage drag around *ml-o* may be lower than expected from the theoretical estimates described by Stam and Zeven (22).

*Vorträge für Pflanzenzüchtung, Proceedings of the Eucarpia Cereal Section Meeting, 1984, Weihenstephan, F.R.G.

FIG. 4. Graphical chromosome 4 genotypes of barley lines showing the estimated sizes of the introgressed segments flanking *Ml-o*. The top line denotes the relative position of RFLP markers on barley chromosome 4. Numbers indicate distances in cM. The *ml-o* locus (dotted line) is assumed to be situated in the 2.4-cM interval bracketed by markers *bAL88/2* and *bAO11*. Locus *Bmy1* maps proximal to the telomeric end of the chromosome, whereas markers *b4-104a* and *bBE54a* are oriented toward the centromere. The lower part shows a graphical representation of that part of chromosome 4 that flanks the *Ml-o* locus in each analyzed BC₇ line. White regions denote recurrent parent segments, black regions denote donor parental-derived segments, and darkly stippled regions indicate segments in which crossovers occurred.

A possible explanation for these two divergent findings could be the fact that recombination tends to be suppressed in genotypes heterozygous for a chromosomal segment originating from different species (29), an effect that is even amplified at centromere-proximal positions (30). Since *Tm-2* was introgressed through interspecific crosses and is located close to the centromere (31) whereas *ml-o* was introgressed through intravarietal crosses and maps distal from the centromere, we predict a significantly smaller linkage drag for *ml-o* introgression.

Although we cannot completely exclude a location of *Ml-o* neither centromeric from *bAL88/2* nor telomeric from *bAO11*, the available data indicate that the gene(s) is most likely to reside in the interval bracketed by markers *bAO11* and *bAL88/2*. This forms a rationale to start from the two mentioned markers a bidirectional walk or jump (32, 33) toward the *Ml-o* gene. The set of available x-ray-induced *ml-o* mutants will provide a powerful tool to assist long-range physical mapping on the basis of the two markers cosegregating with the resistance. In addition, a search for additional RFLP markers within the *bAO11*–*bAL88/2* interval and the molecular analysis of a larger population of resistant plants to find recombinants to the neighboring markers should parallel the long-range cloning approach.

We are grateful to Prof. James MacKey, Dr. S. Ceccarelli, and Dr. Mike D. Gale for their gifts of *ml-o* BC lines, F₁ kernels derived from the wide barley cross, and the β -amylase cDNA clone. Ingrid Herzer is gratefully acknowledged for her excellent technical assistance. We thank members of the Jeffery L. Dangel laboratory for comments and suggestions and J. L. Dangel and Thomas Debener for critical reading of the manuscript.

- Jensen, J. P. & Jørgensen, J. H. (1981) *Tidsskr. Planteavl* **85**, 303.
- Schwarzbach, E. & Fischbeck, G. (1981) *Z. Pflanzenzücht.* **87**, 309–318.
- Wiberg, A. (1974) *Hereditas* **77**, 89–148.
- Jørgensen, J. H. (1976) in *Barley Genetics III*, ed. Gaul, H. (Karl Thieme, Munich), pp. 446–463.
- Jørgensen, J. H. & Mortensen, K. (1977) *Phytopathology* **67**, 678–685.
- Jensen, J. (1987) in *Barley Genetics V*, eds. Yasuda, S. & Kouishi, T. (Sanyo, Okayama, Japan), pp. 189–199.
- Jørgensen, J. H. (1983) *Induced Mutations for Disease Resistance in Crop Plants II* (International Atomic Energy Agency, Vienna), pp. 73–87.
- Aist, J. R., Gold, R. E., Bayles, C. J., Morrison, G. H., Chandra, S. & Israel, H. W. (1988) *Physiol. Mol. Plant Pathol.* **33**, 17–32.
- Skou, J. P., Jørgensen, J. H. & Lilholt, U. (1984) *Phytopathol. Z.* **109**, 147–168.
- Orkin, S. H. (1986) *Cell* **47**, 845–850.
- Young, N. D., Zamir, D., Ganai, M. W. & Tanksley, S. D. (1988) *Genetics* **120**, 579–585.
- Birnboim, H. C. & Doly, J. (1979) *Nucleic Acids Res.* **7**, 1513–1523.
- Young, N. D., Miller, J. C. & Tanksley, S. D. (1987) *Nucleic Acids Res.* **15**, 9339–9348.
- Gebhardt, C., Ritter, E., Debener, T., Schachtschabel, U., Walke-meier, B., Uhrig, H. & Salamini, F. (1989) *Theor. Appl. Genet.* **78**, 65–75.
- Feinberg, A. P. & Vogelstein, B. (1984) *Anal. Biochem.* **137**, 266–267.
- Ritter, E., Gebhardt, C. & Salamini, F. (1990) *Genetics* **125**, 645–654.
- Allard, R. W. (1956) *Hilgardia* **24**, 235–278.
- Kreis, M., Williamson, M. S., Shewry, P. R., Sharp, P. & Gale, M. (1988) *Genet. Res.* **51**, 13–16.
- Islam, A. K., Shepherd, K. W. & Sharrow, D. H. (1981) *Heredity* **46**, 161–174.
- Nielsen, G., Johansen, H. & Jensen, J. (1983) *Barley Genet. Newslett.* **13**, 55–57.
- Jørgensen, J. H. (1977) *Induced Mutations Against Plant Diseases* (International Atomic Energy Agency, Vienna), pp. 533–549.
- Kjaer, B., Jensen, H. P., Jensen, J. & Jørgensen, J. H. (1990) *Euphytica* **46**, 185–193.
- Stam, P. & Zeven, A. C. (1981) *Euphytica* **30**, 227–238.
- Graner, A., Siedler, H., Jahoor, A., Herrman, R. G. & Wenzel, G. (1990) *Theor. Appl. Genet.* **80**, 826–832.
- Kinzer, S. M., Schwager, S. J. & Mutschler, M. A. (1990) *Theor. Appl. Genet.* **79**, 489–496.
- Paterson, A. H., Lander, E. S., Hewitt, J. D., Peterson, S., Lincoln, S. E. & Tanksley, S. D. (1988) *Nature (London)* **335**, 721–726.
- Young, N. D. & Tanksley, S. D. (1989) *Theor. Appl. Genet.* **77**, 353–359.
- Alexander, L. J. (1963) *Phytopathology* **61**, 611–617.
- Rick, C. (1969) *Genetics* **62**, 753–768.
- Rick, C. (1972) *Biol. Zbl.* **91**, 209–220.
- Ganai, M. W., Young, N. D. & Tanksley, S. D. (1989) *Mol. Gen. Genet.* **215**, 395–400.
- Steinmetz, M., Minard, K., Harvath, S., McNicholas, J., Srelinger, J., Wake, C., Long, E., Mach, B. & Hood, L. (1982) *Nature (London)* **300**, 35–42.
- Poustka, A., Pohl, T., Barlow, D. P., Zehetner, G., Craig, A., Michiels, F., Ehrlich, E., Frischauf, A.-M. & Lehrach, H. (1986) *Cold Spring Harbor Symp. Quant. Biol.* **51**, 131–139.