Use of Isogenic Lines and Simultaneous Probing to Identify DNA Markers Tightly Linked to the *Tm-2a* Gene in Tomato

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Manuscript received February 17, 1988
Revised copy accepted June 22, 1988

ABSTRACT

The *Tm-2a* gene of tomato confers resistance to the viral pathogen, tobacco mosaic virus. Like many economically important plant genes, *Tm-2a* has been characterized phenotypically and by classical linkage analysis, yet nothing is known about its gene product. We report here the isolation of two DNA clones which are very tightly linked to the *Tm-2a* gene. These clones were identified by testing 122 genomic clones as hybridization probes against Southern blots consisting of DNA from pairs of nearly isogenic lines with or without the *Tm-2a* gene. Screening such a large number of clones in a short period of time was facilitated by co-labeling and simultaneous probing of sets of up to 10 random genomic clones. Tightly linked clones were distinguished by the fact that they exhibited one or more restriction fragment length polymorphisms between the nearly isogenic lines. Tight linkage of the clones with *Tm-2a* was verified in a segregating F_2 population. Both mapped to the same locus 0.4 ± 0.4 centimorgans away from *Tm-2a* and may provide starting points for a genomic "walk" to this gene. Due to the availability of isogenic lines in many plant species, the strategy outlined in this paper should be widely applicable for selecting DNA clones tightly linked to genes of interest.

OFTEN, the most interesting genes are the ones most difficult to clone. In tomato (*Lycopersicon esculentum*), over 200 genes controlling such important traits as disease resistance, floral and fruit development, and growth habit have been placed on a classical genetic linkage map (TANKSLEY, MUTSCHLER and RICK 1987). While the phenotypes and map positions of these genes are known, virtually nothing is known about their gene products. Since knowledge of a gene's mRNA or protein product is essential for most widely used cloning procedures (MANIATIS, FRITSCH and SAMBROOK 1982), there has been little progress to date in cloning these valuable genes.

An alternative gene cloning strategy has been proposed that is based on map position. The approach begins with the identification of DNA markers that are very tightly-linked to the target gene. These markers are then used to begin a "chromosome walk" to the gene of interest (STEINMENTZ *et al.* 1982) or to identify very large fragments of genomic DNA that contain the target gene (MICHIELS, BURMEISTER and LEHRACH 1987). Large genomic fragments are generated by rare-cutting restriction enzymes and separated by pulsed-field electrophoresis (SMITH *et al.* 1986; CARLE, FRANK and OLSEN 1986). In tomato, where transformation and regeneration are relatively straightforward (MCCORMICK *et al.* 1986), the final step in cloning a target gene by this strategy would consist of transforming wild-type plants with subclones derived from the chromosomal region near the target gene to identify the specific sequences that confer the desired phenotype.

The first step in this strategy, namely isolating tightly linked DNA markers, generally requires that hundreds or thousands of genomic clones be mapped with respect to the target gene by use of restriction fragment length polymorphism (RFLP) analysis of segregating populations. This process is extremely painstaking and slow. In this report, we describe a method that is applicable for rapidly identifying DNA markers that are very tightly linked to one of a large number of important plant genes. This procedure involves testing genomic clones as hybridization probes against SOUTHERN (1975) blots consisting of pairs of nearly isogenic lines (NILs) that differ in the presence or absence of the target gene and a small region of flanking DNA. In this manner, clones need not be mapped by segregation analysis to be localized near the target gene. Instead, these relatively rare clones can be quickly identified as RFLPs between the NILs. To speed up the screening process even more, several clones can be pooled together and tested against the NILs simultaneously, thereby significantly reducing the number of hybridizations that must be carried out to find markers located near the gene of interest.

In order to understand how NILs are useful in identifying tightly linked DNA markers, it is necessary to understand how most NILs have been developed, which is by introgression. Introgression consists of

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repeatedly backcrossing a line or species carrying a gene of interest to a cultivated line chosen because of its otherwise favorable properties. After each cross, progeny are selected on the basis of the phenotype of the target gene. After several generations, the genome of the selected individuals consists almost exclusively of that of the line into which the gene is being introgressed (the recurrent parent). However, a small segment of foreign DNA flanking the target gene persists. After 20 generations, for example, it is predicted that the target gene will be flanked by an introgressed segment extending approximately 5 cM in both directions (HANSON 1959; STAM and ZEVEN 1981). The products of introgression will therefore be a pair of NILs which are identical except for a region near the target gene. If the source of the gene and the recurrent parent are sufficiently polymorphic with respect to one another, the introgressed segment can be used as a target to determine whether a given genomic clone is located near the gene of interest. Clones that are located outside the segment will exhibit identical restriction fragment patterns between the NILs, while clones located inside the segment may exhibit one or more polymorphisms. It is extremely important that the source and recurrent lines be divergent with respect to one another at the DNA level. If not, most restriction enzymes will fail to uncover RFLPs and sequences in the introgressed segment might be missed.

In this paper we report the application of this technique in the isolation of two clones tightly linked to the *Tm-2a* gene of tomato (also known as $Tm2^2$). This gene is economically important, conferring resistance to the viral pathogen, tobacco mosaic virus (TMV), and has been extensively characterized in terms of classical genetics (HALL 1980). The source of the *Tm-2a* gene was *Lycopersicon peruvianum* (ALEXANDER 1971), which is highly divergent at the nucleotide level with respect to *L. esculentum* (J. C. MILLER, personal communication).

MATERIALS AND METHODS

Plant varieties: The following *L. esculentum* lines and related tomato species were used in these experiments: Craigella and Craigella-*Tm-2a* (S. A. BOWES, Glasshouse Crops Research Institute, Littlehamptom, United Kingdom), Vendor and Vendor-*Tm-2a* (M. MUTSCHLER, Cornell University, Ithaca, New York), VF36 and *Lycopersicon pennellii* (LA 716) (C. M. RICK, University of California, Davis, California), and *L. peruvianum* (PI 128650) (S. KRESOVIC, Northeast Regional P.I. Station, Geneva, New York).

DNA isolation: DNA was prepared from leaves as in MURRAY and THOMPSON (1980), as modified in BERNATZKY and TANKSLEY (1986).

Genomic libraries: In order to identify genomic clones located near the *Tm-2a* gene, a total of 122 clones from two different genomic libraries were examined. One library, described in detail elsewhere (ZAMIR and TANKSLEY 1988), consisted of tomato sequences prepared by shearing, homopolymer tailing, and insertion into pUC9. This library contained both single and multiple copy clones. The other library, described in detail in TANKSLEY *et al.* (1988), consisted of tomato genomic sequences digested to completion with *Pst*I and inserted into pUC9. This library consisted of over 90% single copy sequences because the C-methylation-sensitive enzyme, *Pst*I, preferentially selects single-copy sequences during digestion and subsequent cloning. Similar observations have been made in Arabidopsis (LEUTWEILER, HOUGH-EVANS and MEYEROWITZ 1984) and maize (BURR *et al.* 1988).

The clones contained in these two libraries represented anonymous sequences derived from sites all over the tomato genome. In a sense, these genomic clones can be thought of as "molecular *darts*" which could be rapidly tested for whether they were located in the polymorphic DNA "target" surrounding the *Tm-2a* gene.

Library screening: Screening consisted of plasmid isolation (WILLIMZIG 1985) and nick-translation (RIGBY *et al.* 1977), followed by SOUTHERN (1975) blot hybridization against a "*Tm-2a*-survey-filter." These filters were prepared by blotting DNA from pairs of NILs (the tomato variety, Vendor and its introgression derivative, Vendor-*Tm-2a*, or the variety Craigella and its introgression derivative, Craigella-*Tm-2a*) that had been digested with several different restriction enzymes and blotted onto GeneScreen Plus (New England Nuclear) (Figure 1). Positive clones were identified by the presence of an RFLP between the NILs. A total of 72 clones were analyzed from the *Pst*I-derived library and a total of 50 clones were analyzed from the sheared-DNA-library.

To speed the process of screening, five to eight clones were frequently pooled and tested on *Tm-2a*-survey-filters simultaneously (YOUNG, MILLER and TANKSLEY 1987), thereby determining whether any of the clones in the group was located in the target region. This technique required that most of the clones be single copy, so only clones from the *Pst*I-derived library could be screened in this way.

Mapping by F_2 segregation analysis: Newly identified RFLP markers were placed on an RFLP map of tomato consisting of approximately 300 loci located on all 12 chromosomes, by the procedure described in TANKSLEY *et al.* (1988). Distances between markers were determined by the method of ALLARD (1956). RFLP markers near the *Tm-2a* gene were also mapped in two segregating F_2 populations by the same procedure.

RESULTS

Identification of tightly linked RFLPs: The majority of the random genomic clones tested by hybridization to *Tm-2a*-isoline-survey filters produced identical restriction fragment patterns with DNA from both Vendor and Vendor-*Tm-2a* (data not shown). However, two clones, pTG79 and pTG101 (both from the sheared library), exhibited very different patterns between the NILs (Figure 1). In the case of pTG79, the sequence was always present in DNA from Vendor-*Tm-2a*, but absent in Vendor alone. By contrast, clone pTG101 was present in both Vendor and Vendor-*Tm-2a*, but differed markedly in restriction fragment pattern. Although both of these clones were identified in the sheared-DNA-library, while no positive clones were found in the *Pst*I-derived library, statistical analysis does not indicate a significant dif-

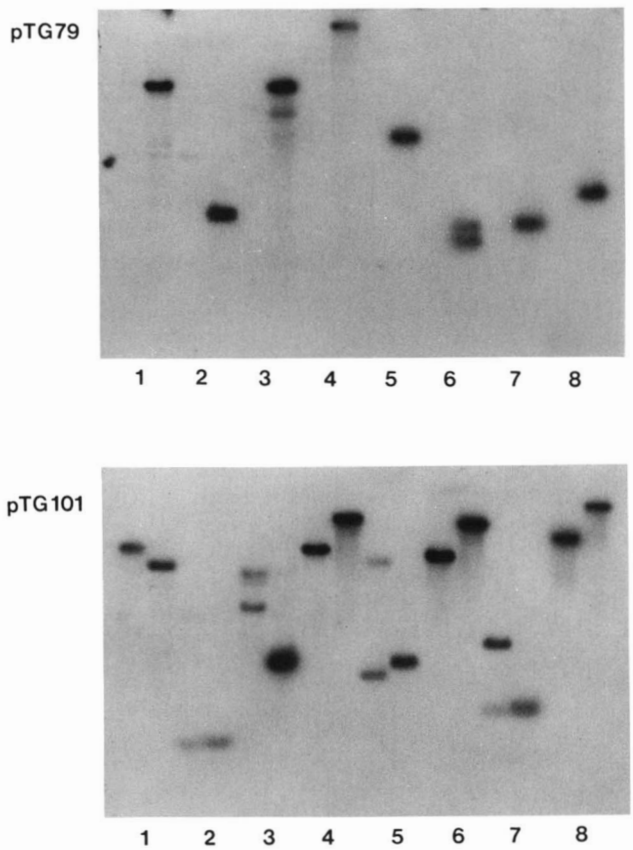

FIGURE 1.—Surveys of clones pTG79 and pTG101 with Vendor/Vendor-*Tm-2a*. A total of 122 tomato genomic clones were tested for the presence of RFLPs between the NILs, Vendor and Vendor-*Tm-2a*, by hybridization to *Tm-2a*-survey-filters, as described in the text. The two clones shown in this figure, pTG79 and pTG101, were the only ones that exhibited RFLPs between these NILs. Each *Tm-2a*-survey-filter consisted of eight pairs of DNA samples from Vendor (left lane of each pair) and Vendor-*Tm-2a* (right lane) in which 5 μg of DNA was digested with one of eight restriction enzymes, separated by 0.9% non-denaturing gel electrophoresis, and Southern blotted onto GeneScreen Plus. Restriction enzymes used for digestion were: 1, *Bst*NI; 2, *Dra*I; 3, *Eco*RI; 4, *Eco*RV; 5, *Hae*III; 6, *Hin*dIII; 7, *Taq*I; 8, *Xba*I. Note that there are two lanes associated with each restriction enzyme treatment on both *Tm-2a*-survey filters, but in the case of pTG79, no hybridization was observed with the DNA samples derived from Vendor alone.

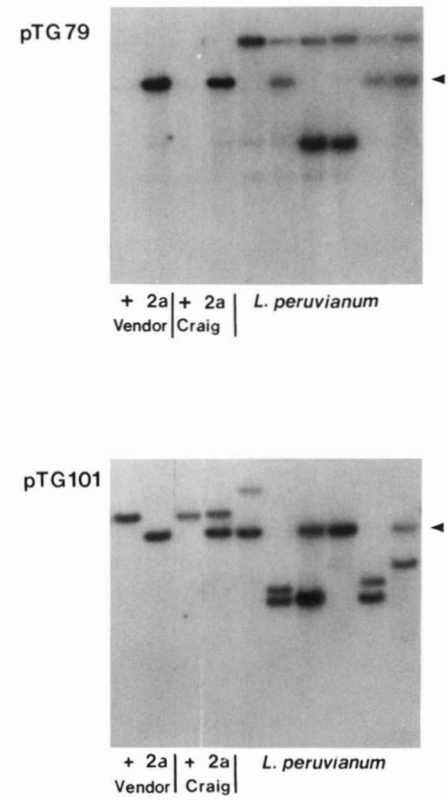

FIGURE 2.—Alleles of *TG79* and *TG101* in NILs of cultivated tomatoes and in *L. peruvianum*, P.I. 128650. DNA from two pairs of NILs, Vendor/Vendor-*Tm-2a* and Craigella/Craigella-*Tm-2a*, along with six individuals of Lycopersicon accession, P.I. 128650, were digested with *Bst*NI, fractionated, and Southern blotted onto GeneScreen Plus. These filters were probed with clones pTG79 and pTG101. The arrows mark alleles of *TG79* and *TG101* found in Vendor-*Tm-2a* and Craigella-*Tm-2a*, which were also observed among some of the individuals of P.I. 128650. Note that these alleles were not observed in Vendor or Craigella without the *Tm-2a* gene. Vendor, "+"; Vendor-*Tm-2a*, "2a"; Craigella, "+"; Craigella-*Tm-2a*, "2a."

ference between these two libraries as potential sources of DNA markers.

If the genetic loci recognized by clones pTG79 and pTG101 (namely, *TG79* and *TG101*) are derived from the segment of the *L. peruvianum* genome flanking the *Tm-2a* gene, then one prediction is that cultivated lines of tomato carrying the *Tm-2a* gene should contain alleles for *TG79* and *TG101* identical to those found in the *L. peruvianum* accession that provided the *Tm-2a* gene (*i.e.*, P.I. 128650). Two pairs of NILs, Vendor/Vendor-*Tm-2a* and Craigella/Craigella-*Tm-2a*, were examined in parallel with six individuals from P.I. 128650 by hybridization to clones pTG79 and pTG101. The results (Figure 2) demonstrated that P.I. 128650, while polymorphic at both loci, exhibited *TG79* and *TG101* alleles that were identical in restriction fragment pattern to those found in Vendor-*Tm-2a* and Craigella-*Tm-2a*. Thus, *TG79* and *TG101* appear to be located in the introgressed segment.

Genetic mapping of *TG79* and *TG101*: A linkage map consisting of over 300 RFLP markers already exists in tomato (TANKSLEY *et al.* 1988). Clones pTG79 and pTG101 were placed on this linkage map by segregation analysis (BERNATZKY and TANKSLEY 1986) and found to reside on chromosome *9* in a region inhabited by several previously identified RFLP markers. Earlier studies of the *Tm-2a* gene have demonstrated that this gene is located on the long arm of chromosome *9* very close to the centromere (SCHROEDER, PROVVIDENTI and ROBINSON 1967). An RFLP map of the portion of chromosome *9* near the *Tm-2a* gene is shown in Figure 3A.

Size of the introgressed segment: Knowledge of the RFLP markers that flank the *Tm-2a* gene makes it possible to analyze the size of the introgressed segment in different lines of tomato carrying the *Tm-2a* gene. This can be accomplished by RFLP analysis

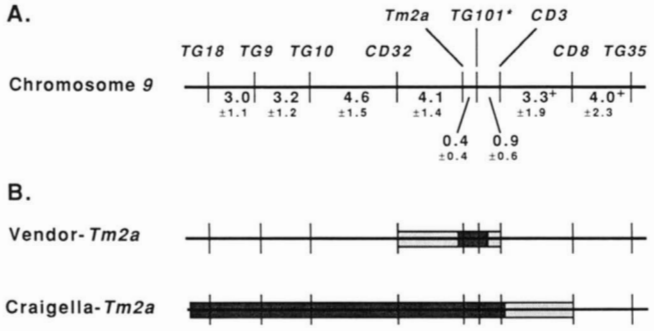

FIGURE 3.—A, RFLP map of chromosome *9* of tomato, showing region near the *Tm-2a* gene. A recombinational linkage map of chromosome *9* was developed by segregation analysis of RFLPs of *TG79, TG101* and other nearby DNA markers. All distances are given as cM (mean ± standard error) and were derived from the Craigella × Craigella-*Tm-2a* cross described in the text, except for the distances between *CD3, CD8* and *TG35* (denoted by a "+"), which were calculated from a VF-36 × *L. pennelli* cross described earlier (TANKSLEY *et al.* 1988). The star next to *TG101* denotes that RFLP markers, *TG79, TG3* and *PC6*, map to the same locus (*i.e.*, no recombinants in the population tested). B, Size of the introgressed segment from *L. peruvianum* in two independent introgression lines (Vendor-*Tm-2a* and Craigella-*Tm-2a*). Analysis of RFLP markers flanking *TG79* and *TG101* provided the information on the size of the introgressed segment around the *Tm-2a* gene. The dark cross-hatched regions represent the chromosomal segments derived from *L. peruvianum*; the lightly cross-hatched regions represent segments in which recombinational events have occurred; the plain line represents segments derived from *L. esculentum*.

of markers that are progressively further away from the gene.

Utilizing this approach, the sizes of the introgressed segment in Vendor-*Tm-2a* and Craigella-*Tm-2a* were estimated. Linkage analysis showed that several RFLP markers, *TG18, TG9, TG10* and *CD32A*, flank *TG79* and *TG101* on one side, while *CD3, CD8* and *TG35* flank *TG79* and *TG101* on the other side (Figure 3A). *Tm-2a*-survey-filters were prepared for both sets of NILs and the filters were analyzed with these flanking RFLP markers. The results of this analysis showed that the introgressed segment in Vendor-*Tm-2a* includes both *TG79* and *TG101*, since both markers were polymorphic between the NILs. None of the other RFLP markers were polymorphic between this pair of NILs. By contrast, *TG18, TG9, TG10, TG79, TG101, CD32A* and *CD3* were all polymorphic between Craigella and Craigella-*Tm-2a*, while *CD8* and *TG35* were not. *Tm-2a*-survey data for the locus, *CD32A*, are shown in Figure 4. These results demonstrate that the introgressed segment in Craigella-*Tm-2a* extends beyond the most distal RFLP marker of the short arm of chromosome *9* and at least 2 cM further along the long arm than Vendor-*Tm-2a* (Figure 3B). The breeding program introducing *Tm-2a* into Vendor consisted of 19 backcross generations, while the program leading to Craigella-*Tm-2a* consisted of only 11 backcross generations, so these results are not unexpected.

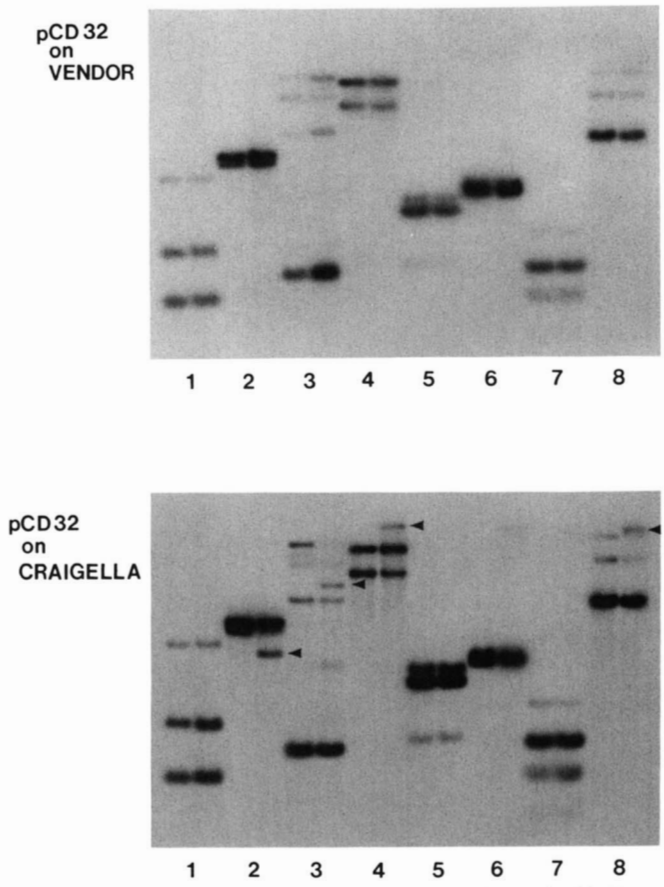

FIGURE 4.—*Tm-2a*-survey-filters probed with clone pCD32. Two survey filters, one consisting of DNA from Vendor and Vendor-*Tm-2a*, and another consisting of DNA from Craigella and Craigella-*Tm-2a*, were probed with the RFLP marker, pCD32. Restriction enzymes used for digestion were: 1, *Bst*NI; 2, *Dra*I; 3, *Eco*RI; 4, *Eco*RV; 5, *Hae*III; 6, *Hin*dIII; 7, *Taq*I; 8, *Xba*I. The arrows mark RFLPs present in the Craigella/Craigella-*Tm-2a* pair not observed in the Vendor/Vendor-*Tm-2a* pair of NILs. Similar filters were analyzed with all of the RFLP markers residing on chromosome *9* and the results are summarized in Figure 3B.

Genetic distance between *TG79, TG101* and the *Tm-2a* gene: In order to determine the genetic distance between *Tm-2a, TG79, TG101* and the other nearby RFLP markers, an F_2 population segregating for *Tm-2a* resistance was analyzed. This F_2 population was produced by crossing the cultivated variety, Craigella, with Craigella-*Tm-2a*. All F_2 individuals were scored for their reaction to TMV strain U1 (SIEGEL and WILDMAN, 1954). It was possible to determine all three *Tm-2a* genotypes, since heterozygotes could be identified by distinctive necrotic and dwarfing symptoms at elevated temperatures (CIRULLI and ALEXANDER 1969). The genotypes of all plants with respect to *TG79* and *TG101*, as well as nearby markers, *TG18, TG9, TG10, CD32A, TG3, PC6* and *CD3* were also determined (Figure 5). Since *TG79* is not present in *L. esculentum*, this locus could not be scored as a codominant marker. Instead, it was necessary to utilize differences in the intensity of the hybridization signal

TABLE 1

Frequency of genotypes of RFLP markers in the Craigella × Craigella-*Tm-2a* F_2 population

Genotype	Locus									
	TG18	*TG9*	*TG10*	*CD32A*	*Tm-2a*	*TG101*	*TG79*	*PC6*	*TG3*	*CD3*
e/e[a]	7	7	10	12	15	15	15	13	15	15
e/p[a]	29	32	32	37	34	37	36	38	34	36
p/p[a]	82	80	68	70	61	65	65	64	61	65
χ^2	125.8[b]	115.0[b]	80.4[b]	73.6[b]	54.5[b]	61.1[b]	60.0[b]	58.5[b]	54.5[b]	59.8[b]

[a] *e/e* = *esculentum/esculentum*; *e/p* = *esculentum/peruvianum*; *p/p* = *peruvianum/peruvianum*.
[b] Denotes significantly different from expected (1:2:1) at $P < 0.001$.

(when present) by a "within-lane" comparison method similar to that employed by YOUNG, MILLER and TANKSLEY (1987) in order to distinguish between homozygotes and heterozygotes.

A significant deviation from the expected 1:2:1 segregation ratio was observed for all loci examined (Table 1). This was probably due to the presence of the gene, *Gp* or gamete promoter, in these lines (PELHAM 1968). This gene is known to be linked to *Tm-2a* in Craigella-*Tm-2a* and its phenotype is to promote gametes that carry the *Gp* allele.

Among a total of 140 F_2 progeny examined, no recombinants were recovered between *TG79* and *TG101* and only one recombinant was recovered between these markers and the *Tm-2a* gene. It is therefore impossible to determine the gene order of these three loci, although *TG79* and *TG101* must be on the same side of the *Tm-2a* gene. The recombinational distance between *TG79* and *TG101* and the *Tm-2a* gene, based on the results of this segregating population, is 0.4 ± 0.4 cM. Previously identified RFLP markers, *PC6* and *TG3*, were also found to reside at the same locus as *TG79* and *TG101*. Graphical representations of the recombinant individuals near the *Tm-2a* gene in this F_2-population are shown in Figure 6.

A second segregating F_2-population was also examined (data not shown). This population, which consisted of 72 individuals, was derived from a cross between a line of VF36 carrying the *Tm-2a* gene and the TMV-sensitive tomato relative, *L. pennellii* (LA716). In this cross, no recombinants were recovered between *TG79*, *TG101* and *Tm-2a*. This result confirms the conclusion that *TG79* and *TG101* are very tightly linked to the *Tm-2a* gene.

DISCUSSION

The results presented in this paper demonstrate the feasibility of rapidly identifying DNA markers that are very tightly linked to specific plant genes. The technique, which is based on the ability to screen for RFLPs between NILs whose genomes are identical except for a small target segment around the specific gene, should be easily applied to many other plant systems. The only prerequisites are (1) the availability of an appropriate set of NILs derived from sufficiently polymorphic parents to provide a target (many such NILs already exist, see ZEVEN and VAN HARTEN, 1979) and (2) a single (or low copy) genomic library, to provide a source of DNA clones ("molecular darts") for testing. Moreover, co-labeling and simultaneous probing of test clones (YOUNG, MILLER and TANKSLEY 1987) are extremely valuable in speeding up the screening procedure.

While the primary goal in tagging the *Tm-2a* gene was to provide DNA clones to act as starting points for "chromosome walking" and physical mapping experiments, the segregating F_2 populations analyzed in these studies illustrate a very powerful application of gene tagging to classical plant breeding. Among the F_2 progeny analyzed in this study, some of the individuals with recombinational events between *Tm-2a* and its flanking RFLP markers represent novel genotypes with potentially superior properties. In certain of these recombinant individuals, the genome still includes introgressed *L. peruvianum* DNA in the region very near the *Tm-2a* gene, but more distal portions of the introgressed segment have been replaced with DNA from *L. esculentum* (Figure 6). Undesirable properties, such as fruit size depression (LATTEROT 1971), due to this "linkage-drag" (ZEVEN, KNOTT and JOHNSON 1983), have been associated with the introgressed segment from *L. peruvianum*. The recombinant individuals, identified in the population by RFLP mapping in the introgressed region near *Tm-2a*, potentially combine the desirable property of *Tm-2a* resistance without the negative side effects due to linkage drag. Of course, foreign DNA can also be removed through traditional breeding by additional backcrossing, but to achieve significant progress, many generations are required (HANSON 1959; STAM and ZEVEN 1981). By contrast, identifying recombinant individuals with tightly linked RFLP markers allows much of the introgressed segment to be removed in only one or two generations.

In order to utilize the tightly linked molecular markers, *TG79* and *TG101*, to clone the *Tm-2a* gene, it is necessary that they be very close to one another

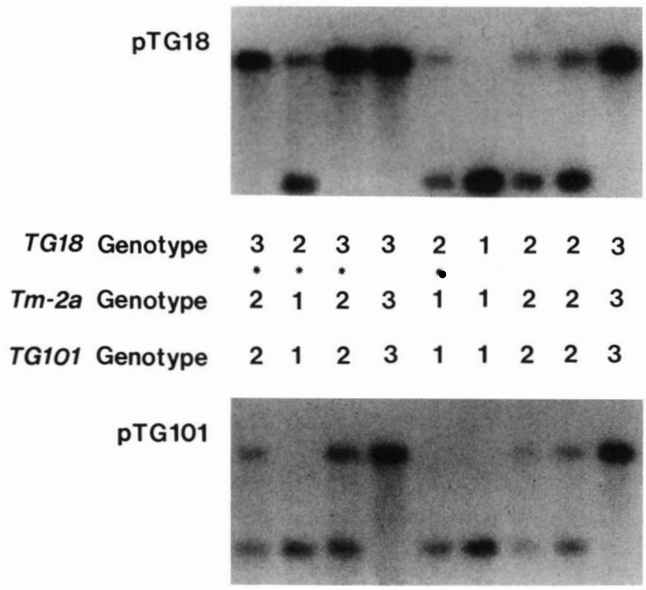

FIGURE 5.—Mapping RFLP clones relative to the *Tm-2a* gene. In order to determine the genetic distance between RFLP markers and the *Tm-2a* gene, 140 F_2 progeny from a cross between Craigella and Craigella-*Tm-2a* were analyzed for reaction to TMV and by Southern blot analysis of the RFLP markers known to reside on chromosome *9*. The results obtained with *TG101* and *TG18* are shown here in parallel with the inferred *Tm-2a* genotype for nine of the F_2 individuals. Recombinant individuals are noted by a "*." Other RFLP markers on chromosome *9* were analyzed in a similar manner. Genotypes; 1, *esculentum/esculentum*; 2, *esculentum/peruvianum*; 3, *peruvianum/peruvianum*.

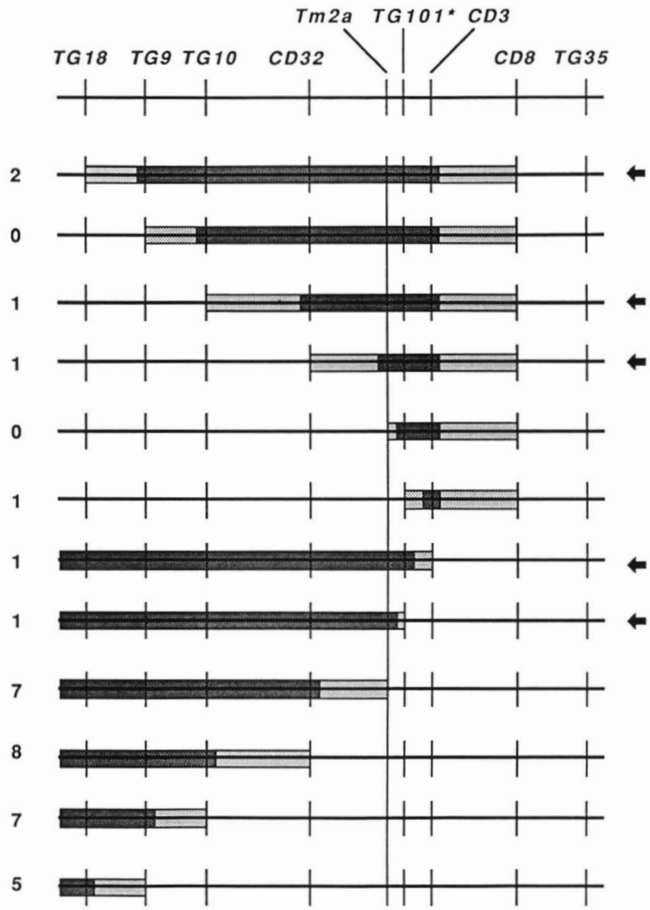

FIGURE 6.—Recombinants from the Craigella × Craigella-*Tm-2a* F_2 progeny. Genotypic data for the RFLP markers near *Tm-2a* were used to deduce a graphical genotype for this portion of chromosome *9* for each of the recombinants in the F_2 population. The dark cross-hatched areas represent chromosomal segments derived from *L. peruvianum*; the lightly cross-hatched areas represent regions in which crossovers took place; the plain line represents regions derived from *L. esculentum*. The total number of recombinants of each haplotype class is shown on the left. The arrows denote haplotypes in which the *L. peruvianum* segment has been retained at the *Tm-2a* locus, while flanking segments on one or the other side of *Tm-2a* have been replaced by segments from *L. esculentum*. The star next to *TG101* denotes that RFLP markers *TG79*, *TG3* and *PC6* map to the same locus.

in terms of physical as well as genetic distance. *TG79* and *TG101* are located approximately 0.4 cM from the *Tm-2a* gene and, at first glance, this seems quite close. In tomato, with a genome of approximately 1400 cM (based on the current RFLP map) and 7.15×10^8 bp per haploid genome (GALBRAITH *et al.* 1983), there should be approximately 5.1×10^5 bp per cM. Thus, *TG79* and *TG101* could be as close as 2×10^5 bp from the *Tm-2a* gene. However, recombination is known to be suppressed in interspecies crosses (RICK 1969) and this is exacerbated in lines carrying the *Gp* gene. Moreover, *Tm-2a* is located near the centromere of chromosome *9* (SCHROEDER, PROVVIDENTI and ROBINSON 1967) and recombination is known to be suppressed in pericentric regions of chromosomes (RICK 1971). *TG79* and *TG101* (and the other tightly linked RFLPs, *TG3* and *PC6*) may therefore be much more distant (in a physical sense) from one another or from the *Tm-2a* gene than expected from genetic linkage data.

To be of value in cloning the *Tm-2a* gene, it is also necessary to determine the gene order of *TG79* and *TG101* relative to *Tm-2a*, so that attempts to move along the genome proceed in the correct direction. In the crosses described here, no recombinants were obtained between *TG79* and *TG101* (or with the other very tightly linked RFLP markers, *TG3* and *PC6*). It is not possible, therefore, to determine the order of these loci in this study. Larger F_2 populations could be examined in order to find recombinants in the region very near *Tm-2a*, thereby providing the necessary information to prepare a high resolution linkage map of the RFLP markers near *Tm-2a*. However, information about gene order, as well as physical distance, can be obtained directly from techniques such as field inversion gel electrophoresis, which separate very large nucleic acid molecules.

We have begun to analyze the region around *Tm-2a* by this procedure in order to physically map this region. Preliminary results indicate that *PC6* and *TG3* reside on the same 6×10^5 bp *Sal*I fragment, while the other nearby RFLP markers are all located on different large DNA fragments (M. W. GANAL, N. D.

YOUNG and S. D. TANKSLEY, unpublished data). It seems likely, therefore, that physical mapping around the *Tm-2a* gene will be possible and the relation between genetic and physical distance in this region determined. Depending on the physical distance between the tightly linked RFLP marker and the *Tm-2a* gene, as well as the interspersion pattern of repetitive DNA in this region, it may soon be possible to begin to "walk" (STEINMETZ *et al.* 1982) or "jump" (POUSTKA *et al.* 1986) or to isolate large DNA clones in this region from a genomic library of tomato in yeast artificial chromosomes (BURKE, CARLE and OLSON 1987).

We would like to thank MARIE-CARMEN VICENTE and NORA LAPITAN for technical assistance. We would also like to thank MARK SORRELLS and JAMES PRINCE for reading the manuscript. This work was supported by grants from the U.S. Department of Agriculture (85-CRCR-1-1609), the National Science Foundation (DCB-8517773) and the Cornell Biotechnology Program.

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Communicating editor: M. T. CLEGG