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# Linkages among RFLP, RAPD, isozyme, disease-resistance, and morphological markers in narrow and wide crosses of cucumber

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**Abstract** A 58-point genetic map was constructed with RFLP, RAPD, isozyme, morphological, and disease-resistance markers spanning 766 cM on ten linkage groups for a cross within the cultivated cucumber (*Cucumis sativus* var. *sativus*). Relatively few DNA polymorphisms were detected, agreeing with previous studies documenting a narrow genetic base for cucumber. Most RFLPs within the cultivated cucumber appear to be changes at restriction-enzyme sites. Sixty-four percent of RAPD markers that fit expected ratios at $P< 0.001$ were unlinked, possibly due to poor amplification and the inefficiency of dominant markers to detect linkage in an $F_2$ family. A 70-point linkage map, spanning 480 cM on ten linkage groups, was constructed with RFLP, isozyme, morphological, and disease-resistance markers for a cross between the cultivated cucumber and the wild or feral *C. sativus* var. *hardwickii*. Unlinked markers and more linkage groups than chromosome pairs indicated that both maps were not saturated. Twenty-one markers doubly segregated in both families and regions of colinearity were identified.

**Key words** *Cucumis sativus*
*C. sativus* var. *hardwickii* · Genetic map

## Introduction

Cucumber (*Cucumis sativus* L. var. *sativus*) is known only in cultivation (Whitaker and Davis 1962) and may have a narrow genetic base as evidenced by limited isozyme variability (Dane 1983; Knerr et al. 1989), tolerance to inbreeding (Robinson et al. 1976), and low estimates of genetic variance (Smith et al. 1978). Nevertheless, 105 morphological and disease-resistance loci have been described (Pierce and Wehner 1990). Larger linkage studies have included 12 morphological loci (Whelan et al. 1975), 12 (Abul-Haya et al. 1975) and 15 (Fanourakis and Simon 1987) morphological and disease-resistance loci, and 14 isozyme loci (Knerr and Staub 1992). Although 44 morphological traits and disease resistances have been assigned to six linkage groups (Robinson et al. 1976; Pierce and Wehner 1990), a compilation of linkage groups is difficult because few of the same loci segregated in independent studies, coupled with name duplication and conflicting reports of linkage versus independent inheritance (Pierce and Wehner 1990). In this study, we report segregations and linkages among RFLP, RAPD, isozyme, disease-resistance, and morphological markers towards the development of a genetic map of cucumber.

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## Materials and methods

### Segregating populations

Two breeding lines and 14 USDA plant introductions (PIs) divergent for isozymes (Knerr et al. 1989) were surveyed for RFLPs using *Pst*I-genomic and cDNA clones (Dijkhuizen 1994). Maximum RFLPs were detected between Gy14 (chosen because of its superior horticultural characteristics) and PIs 432860 (*C. sativus* var. *sativus*; a monoecious long-fruited slicing cucumber from China) and 183967 (*C. sativus* var. *hardwickii*; a monoecious small-fruited wild or feral form). Gy14 was crossed as the female with 432860 and 183967. For each cross, a single $F_1$ plant and 101 (Gy14 × 432860) or 102 (Gy14 × 183967) $F_2$ plants were self-pollinated to generate $F_3$ lines. The sex of gynoecious and androecious $F_2$ individuals was converted with 3 mM of $Ag(S_2O_3)_3$ (Tolla and Peterson 1979) or etheral (Atsmon and Tabak 1979), respectively. For Gy14 × 183967, $F_2$ plants were propagated by cuttings to ensure adequate $F_3$ seed. Some $F_3$ lines were eliminated if fewer than 85 or 300 seeds were produced for Gy14 × 432860 and Gy14 × 183967, respectively. Leaf tissue from ten $F_3$ individuals from each line was bulked to determine $F_2$ genotypes, providing a 99% probability of correctly classifying heterozygotes (Hanson 1959).

### RFLP analysis

DNA was isolated from crude nuclear preparations (modified after Murray and Kennard 1984). Leaf tissue was minced and homoge-

nized in 4 °C extraction buffer (0.5 *M* hexylene glycol, 20 m*M* PIPES pH 7.0, and 10 m*M* $MgCl_2$), sieved through a 70-µm nylon mesh, and centrifuged at 500 g for 10 min. The nuclear pellet was resuspended in one part cold extraction buffer to one part lysis buffer (1.5 *M* NaCl, 2% CTAB, and 30 m*M* EDTA) and incubated at 65 °C for 2 h. DNA was subsequently extracted with phenol:chloroform:isoamyl alcohol (25:24:1) and chloroform:isoamyl alcohol (24:1), precipitated with ethanol, and dissolved in 10 m*M* TRIS (pH 7.6) and 1 m*M* EDTA.

DNA was digested with *Dra*I, *Eco*RI, *Eco*RV, or *Hin*dIII (BRL, Gaithersburg, Md. or Promega, Madison, Wis.) and 5 µg were electrophoresed through 0.7%-agarose gels in 1 × TBE (Sambrook et al. 1989) at 11 mA for 16 h. Gels were stained in ethidium bromide and photographed over ultraviolet light. DNA was transferred in 0.4 *N* NaOH and 0.6 *M* NaCl to Zetaprobe membranes (Biorad, Waverly, Mass.) and baked under vacuum at 80 °C for 2 h.

Partial-genomic libraries were constructed from DNA isolated as described above and purified by CsCl-gradient ultracentrifugation. *Pst*I-digested DNA of the cultivar 'Sable' was size-fractionated through sucrose gradients (Sambrook et al. 1989) and fragments between 0.5 and 2 kb were ligated into the plasmid pGEM-3Zf(+) (Promega) at a 10-to-1 insert-to-vector ratio. For a second partial-genomic library, *Eco*RI-digested DNA from the inbred WI2757 was similarly size-fractionated and fragments between 2 and 6 kb ligated into λgt10 (Promega) at a 35-to-1 vector-to-insert ratio.

Polyadenylated RNA was extracted from leaf tissue of the cultivar 'Addis', precipitated with LiCl, and purified by oligo-dT column chromatography (Sambrook et al. 1989). cDNA was synthesized using a kit (Promega), *Eco*RI linkers ligated, and cloned into dephosphorylated pUC13 (Pharmacia, Milwaukee, Wis.). cDNAs of greater than 0.5 kb were chosen as probes.

Plasmids carrying *Pst*I-genomic or cDNA clones were isolated by alkaline lysis (Sambrook et al. 1989) and inserts digested out; *Eco*RI-genomic clones were amplified by the polymerase chain reaction (PCR) (Dorfmann et al. 1989). Digests or PCR reactions were electrophoresed through 0.7% low-melting-point agarose gels and fragments were removed and radiolabeled by random-hexamer priming (Feinberg and Vogelstein 1983). Membranes were hybridized in 40% deionized formamide, 7% SDS, 250 m*M* $Na_2HPO_4$ (pH 7.2), and 1 m*M* EDTA overnight at 42C and washed in 2 × SSC at 22 °C for 5 min, 2 × SSC at 42 °C for 15 min, and twice in 0.2 × SSC at 60 °C for 15 min. Membranes were exposed to Kodak XAR5 film for 1–4 days with intensifying screens at –80 °C.

## RAPD analysis

Gy14 and 432860 were evaluated for RAPDs. Leaf tissue of $F_2$ individuals was preferred; however equal weights of tissue from ten $F_3$ plants were bulked when $F_2$ tissue was unavailable. DNA was isolated from freeze-dried tissue (0.7 *M* NaCl, 50 m*M* TRIS pH 8.0, 10 m*M* EDTA, 1% CTAB), extracted with phenol-chlorform and chloroform-isoamyl-alcohol, and purified via S-1000 (Pharmacia) column chromatography (Hewish and Shukla 1983). Primers were obtained from Operon (Alameda, Calif.; kits A through Z, AA, and AB; 20 primers per kit) and the University of British Columbia (Vancouver, B.C.; kits 201–700). Reactions were performed in 15-µl volumes in 0.5-ml polypropylene tubes containing 20 ng of DNA, 0.2 m*M* of primer, 1 unit of *Taq* polymerase (Promega), 1 m*M* of each dNTP, and the commercial buffer. Light mineral oil (Sigma) was placed over the reaction to prevent evaporation. Samples were manually moved through water baths for 30 s at 94 °C, 60 s at 36 °C, and 90 s at 72 °C for 40 cycles. PCR products were electrophoresed through 1.4% agarose gels with 0.5 g/ml of ethidium bromide in 1 × TAE (Sambrook et al. 1989) at 90 V for 3.5 h. RAPDs were designated by primer kit and number, e.g., OPA10 [Operon (OP) kit A, primer number 10] or BC200 [University of British Colombia (BC) primer number 200]. The sequences of all primers detecting polymorphisms were listed by Kennard (1993). Small-case letters (e.g., OPA14a and OPA14b) were used to designate fragments of decreasing size amplified from the same primer. Because RAPDs were almost always dominant, individuals lacking bands were reanalyzed to confirm their absence.

## Isozyme analysis

Gy14, 432860, and 183967 were surveyed for 21 isozyme loci [*Ak-1, Ak-2, Fdp-1, Fdp-2, Gpi-1, Gr-1, G2dh, Idh, Mdh-1, Mdh-2, Mdh-3, Mpi-1, Mpi-2, Pgl, Pla, Pep-pap, Per, Pgm-1, Pgd-1, Pgd-2*, and *Skdh* (designated according to Knerr et al. 1989)] previously found to be polymorphic within *C. sativus* var. *sativus* (Staub et al. 1985; Knerr et al. 1989; Knerr and Staub 1992) using horizontal starch-gel electrophoresis. Sampling of cotyledonary tissue from 12 individuals of each $F_3$ line, enzyme extraction, and the preparation of starch gels and running buffer were all according to Knerr et al. (1989). Modified staining solutions of Staub et al. (1985), Allendorf et al. (1977), Brewer (1970), and Shaw and Prasad (1970) were used to visualize banding patterns. *Idh, Mpi-1, Pep-pap, Pgm-1,* and *Per* were polymorphic between Gy14 and 183967, and *Fdp-2, Mdh-3, Mpi-2,. Pgl,* and *Pgm-1* between Gy14 and 432860. *Fdp* is a monomeric and *Mpi* a dimeric system with two polymorphic loci each. *Mpi-1 (1)-95 and Fdp*-2 (1)-97 are new loci designated according to Knerr et al. (1989).

## Disease resistance and morphological traits

Twelve to fifteen progeny of each $F_3$ line from both segregating populations were evaluated for resistance to scab (caused by *Cladosporium cucumerinum* Ellis and Arth. culture MJH-661) and Gy14 × 183967 for resistance to downy mildew [caused by *Pseudoperonospora cubensis* (Berk. and Curt.) Rostow., isolate provided by J. Dodson, PetoSeed, Woodland, Calif.) using the seedling evaluations of Abul-Hayja (1975). Phenotypes were scored on a 0–9 scale; 0–3 were considered as resistant and 5–9 as susceptible (Abul-Hayja 1975). For downy mildew, linkage analysis was performed on an average of two replications and two inoculations; for scab, two replications and one inoculation were used.

Both populations segregated for *F* (gynoecy vs monoecy) (Pierce and Wehner 1990). For Gy14 × 432860, 20 $F_3$ individuals (five plants in two replications for 2 years) of 100 $F_3$ lines were rated at the West Madison Agricultural Experiment Station in 1991 and 1992. The first 15 main-stem nodes were categorized as all (100%=1), 12–14 (80–93%=2), 4–11 (26–73%=3), 1–3 (7–20%=4), or 0 (0%=5) pistillate. Means were calculated for each $F_3$ line over replications and years. A genotype was assigned on the basis of mean categories where *FF*=< 1.6, *Ff*=1.6< 3, and *ff*≥3. The position of the first pistillate node was also recorded (cotyledonary node=1) and genotypes as *FF* =< 4, *Ff*=4< 6, and *ff*≥6. For Gy14 × 183967, the percentage of the first ten flower-bearing nodes along the main stem were rated for 99 $F_2$ individuals in the greenhouse. Genotypes were assigned on the basis of percent female nodes where *F*–≥50% and *ff*≤50% pistillate.

Spine color, *B* (Pierce and Wehner 1990), was rated on 93 $F_3$ lines over 2 years in the field for the Gy14 × 183967 population. Genotypes were assigned to phenotypes of 2–3-week-old fruit according to *BB*=black, *Bb*=segregating, and *bb*=white.

## Segregation analysis and linkage detection

Chi-square goodness-of-fit tests were performed with LINKAGE-1 (Suiter et al. 1983). Polymorphisms with a deviation from expected segregation ratios of $P < 0.001$ were excluded from linkage analysis. Linkage and the orders of markers were estimated with MAPMAKER version 3.0 (Lander et al. 1987). Markers were initially associated with two-point comparisons of the *group* command threshold LOD 4.0 recombination 0.3. Markers were then placed in sequence via three-point analyses of the *orders* command. Scoring errors were detected by checking for double cross-overs. Final candidate orders were confirmed with multipoint analyses of the *compare* and *ripple* commands by observing windows of five contiguous loci. Markers that could not be confidently located were placed via the *try* command. Recombination frequencies were transformed to Kosambi distances.

## Results and discussion

### Frequencies of polymorphisms

Similar levels of polymorphisms (9% and 11%) between Gy14 and 432860 were detected for RAPDs and RFLPs (two to four restriction enzymes), respectively. Isozyme systems revealed 24% polymorphisms between Gy14 and 432860. This higher frequency may be because polymorphic isozymes had been previously characterized and parents were initially selected based on isozyme variability. For approximately the same number of hybridizations, twice as many RFLPs were detected between Gy14 and 183967 than with 432860 (Table 1). For Gy14 and 183967, 22% (*Pst*I-genomic), 35% (*Eco*RI-genomic), and 35% (cDNA) of clones detected RFLPs; for Gy14 and 432860, 10%, 13%, and 15% of the clones, respectively, detected RFLPs. Although *Eco*RI-genomic clones detected polymorphisms at approximately the same frequency as cDNAs, greater numbers of fragments were detected (2.6±2.0 for *Eco*RI-genomic, 1.8±1.1 for *Pst*I-genomic, and 1.7±1.1 for cDNA clones) making segregation analyses more difficult. For all classes of probes, *Eco*RI revealed more polymorphisms (14% and 20%) than *Hin*dIII (3% and 9%) for Gy14 × 432860 and Gy14 × 183967, respectively.

For Gy14 and 432860, 11% of probes that detected RFLPs were visualized with both *Eco*RI and *Hin*dIII as opposed to exclusively with one enzyme, suggesting that RFLPs within the cultivated cucumber may involve changes at restriction-enzyme sites. A greater percentage (24%) of polymorphisms were detected across enzymes in Gy14 × 183967, which may reflect more structural rearrangements between *C. sativus* var. *sativus* and *hardwickii*. Consistent with this observation, Neuhausen (1992) reported point mutations to be the basis of RFLPs within a related species, *Cucumis melo* L., whereas structural changes were involved between the species *C. melo* and *C. sativus*. The low frequency of RFLPs is consistent with the purported narrow genetic base within cucumber (Smith et al. 1978; Dane 1983; Knerr et al. 1989); this is comparatively less than among cultivars of other cross-pollinated species such as the Brassicas (Figdore et al. 1988) or maize (Helentjaris et al. 1986) and similar to self-pollinated species such as soybean (Apuya et al. 1988) or tomato (Helentjaris et al. 1986).

**Table 1** Number of DNA markers evaluated and those detecting polymorphisms, segregating normally, and exhibiting linkage

| Marker class | Number of markers | | | | | | |
|---|---|---|---|---|---|---|---|
| | Total Screened | Gy14 × 432860 | | | Gy14 × 183967 | | |
| | | Poly[a] | Seg[b] | Link[c] | Poly | Seg | Link |
| RFLP[d] | 617 | 66 | 32 | 30 | 156[e] | 64 | 61 |
| RAPD[f] | 1076 | 100 | 55 | 20 | – | – | – |

[a] Showed polymorphism with at least one major band among parents
[b] Provided segregation data with goodness-of-fit at $P>0.001$. Thirty-three RAPDs did not segregate; 12 showed unacceptable ($P< 0.001$) goodness-of-fit to expected ratios
[c] Exhibited linkage to at least one other marker with mapping criteria of LOD 4.0, recombination frequency 0.3
[d] Hybridized to *Eco*RI and *Hin*dIII or *Dra*I, *Eco*RI, *Eco*RV, and *Hin*dIII digests
[e] Of 156 polymorphic markers, 100 were chosen for segregation analysis
[f] RAPD markers were not evaluated in the GY14 × 183967 population

### Segregation analysis

Thirty-six of one-hundred RFLPs in Gy14 × 183967 either did not segregate (9) or gave poor signal-to-noise ratios (27); for Gy14 × 432860, 33 of 66 RFLPs and 33 of 100 RAPDs did not segregate (Table 1). Lack of segregation was due to heterogeneity within the PIs, heterozygosity of parents, and cytoplasmic clones. Although RFLP and RAPD markers were primarily codominant and dominant, respectively, we observed nine dominant RFLPs (CsC166, CsC366b, CsE031, CsE063, CsP064, CsP215, CsP266, CsP303, and CsP444) and five codominant RAPDs (OPA10, OPA14b, OPK14, OPN11, and OPP18a).

For Gy14 × 183967, 18 of 64 RFLPs (28%), one of five isozymes (20%), one of two morphological, and none of two disease resistances deviated significantly ($P < 0.05$) from expected segregation ratios. Eighteen of the twenty deviating marker loci were skewed towards the Gy14 alleles and may reflect unconscious selection against unadapted germplasm. The figure of 28% aberrantly-segregating loci is similar to that observed in interspecific crosses of lentil (25%, Havey and Muehlbauer 1989) and tomato (34%, Helentjaris et al. 1986).

Thirty of thirty-two RFLPs (94%), 40 of 67 (60%) RAPDs, four of the five isozyme (80%), as well as the disease-resistance and the morphological markers fit expected ratios ($P>0.05$) for Gy14 × 432860. Twelve (OPA05, OPF07, OPI06, OPL03, OPM16a, OPW17, OPAB08, BC460b, BC503a, BC503b, BC542a, and BC542b) of the twenty-seven aberrant RAPDs deviated at $P < 0.001$ and were not used in linkage analysis. RAPDs did not show preferential segregation towards either parent. For the dominant RAPDs, nearly equivalent numbers were recessive (i.e., null) for each parent (24 for Gy14; 26 for 432860). However, of the 27 aberrantly-segregating RAPDs, 20 showed an overabundance of the absent class. Even though we repeated all amplifications for the absent class, aberrant segregation of RAPDs may be due to inefficient amplifications resulting in an excess of nulls. Our results illustrate the importance of establishing segregations prior to drawing any genetic or phylogenetic conclusions from RAPDs.

Segregations for previously characterized disease-resistance and morphological loci fit expected ratios ($P > 0.05$), except in Gy14 × 432860 where segregation of sex, as estimated by the percentage of pistillate nodes and by the position of the first node bearing a pistillate flower, showed relatively poor fits to expected ratios ($P=0.053$ and

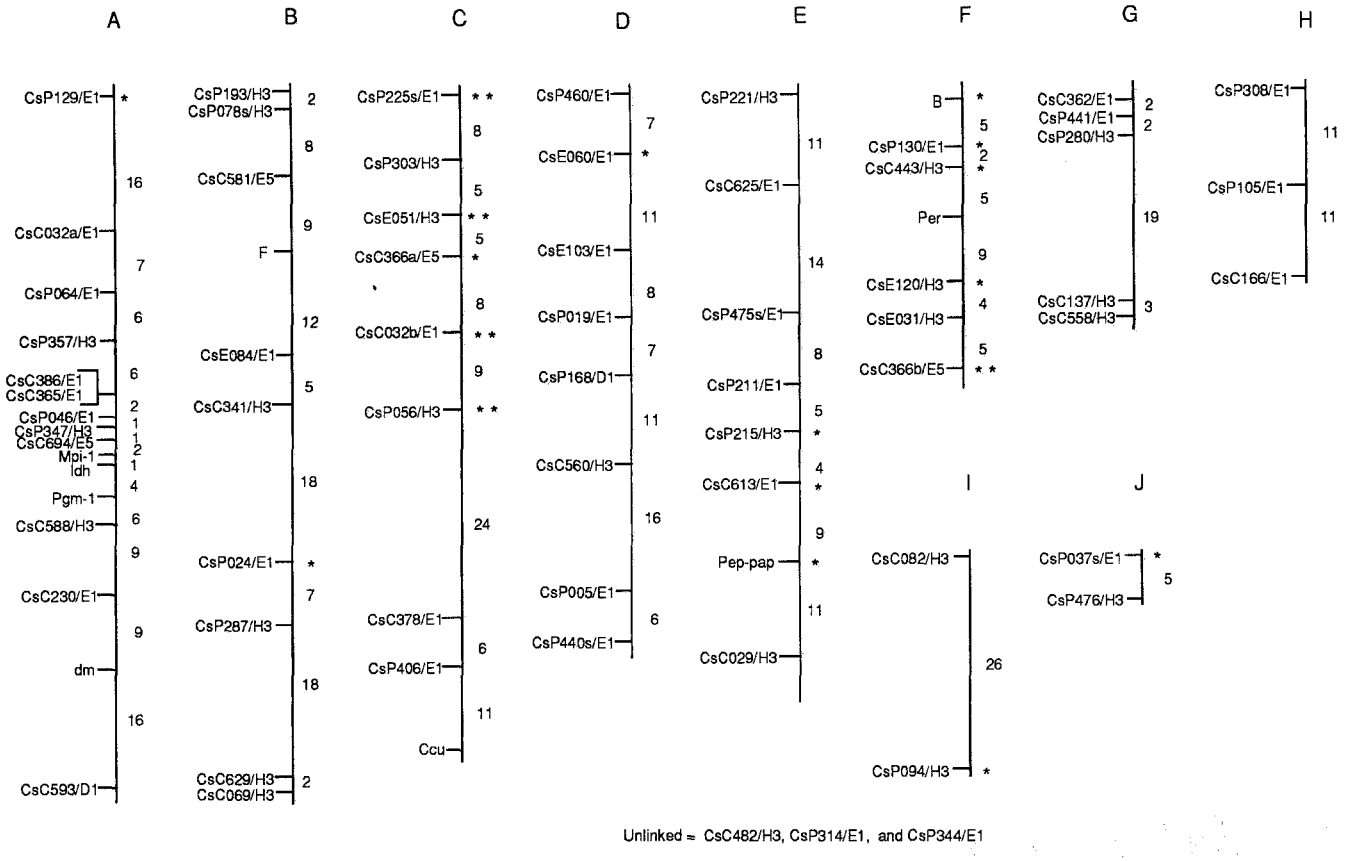


**Fig. 1** Linkage groups (designated by *upper-case letters*) detected in the cross Gy14 × 183967. Clones are designated as *CsC*=cDNA, *CsP=Pst*I-genomic, and *CsE=Eco*RI-genomic. *Lower-case a or b* represent two independently-segregating loci detected with one probe. *Lower-case s* denotes the slowest fragment digested out of the vector. Restriction enzymes designated as *D1*, *Dra*I; *E1*, *Eco*RI; *E5*, *Eco*RV; and *H3*, *Hin*dIII. Kosambi map distances are indicated on the right-side of each interval. Abberantly-segregating loci are designated as * or ** ($P$<0.05 or 0.01, respectively)

$P$=0.0375, respectively). This bias towards monoecy may be due to selection for adequate seed numbers among $F_3$ lines as the silver-treated gynoecious plants tended to produce fewer seed.

## Linkage analysis

We chose a LOD 4.0, corresponding to a 99.5% confidence level against false detection of spurious linkages (Lander and Botstein 1986), and a recombination frequency of 0.3 to construct linkage groups. Increasing the LOD threshold from 3.0 (95.0% confidence level) to 4.0 at the same recombination frequency eliminated linkage of 13 RAPDs.

Markers in Gy14 × 183967 were mostly codominant and formed ten linkage groups (Fig. 1). Seventy (61 RFLP, five isozyme, two morphological, and two disease-resistance markers) were linked to at least one other marker. The map is 480 cM in length with an average distance between loci of 8.0±5.4 cM. The greatest distance between any two loci is 26 cM. Eight linkage groups contain three or more markers and two linkage groups consisted of pairs. Three markers (4%) were unlinked (*CsC482*, *CsP314*, and *CsP344*). The cDNAs *CsC032* and *CsC366* detected five and three fragments, respectively, and different polymorphic fragments mapped to different regions of the genome [linkage groups A (*CsC032a*), C (*CsC032b* and *CsC366a*), and F (*CsC366b*)]. Because the cDNA clones were digested out of the plasmid vector and only one fragment was observed, it is unlikely that two independent cDNAs were cloned into the same plasmid. Duplicate polymorphic loci mapping to independent linkage groups appear to be rare in cucumber. The majority of aberrantly-segregating markers were clustered on linkage groups C, E, and F.

Markers in Gy14 × 432860 also formed ten linkage groups. Fifty-eight (31 RFLP, 20 RAPD, 5 isozyme, 1 morphological, and 1 disease-resistance) markers were linked to at least one other marker (Fig. 2). The map was 766 cM in length with an average distance between loci of 21±8 cM. The greatest distance between any two loci was 32 cM. Seven linkage groups contained five or more markers and three linkage groups consisted of pairs. Markers from two linkage groups (G and K) could be grouped (*Mpi-2* to *CsC482*) at less stringent mapping criteria (LOD 3.9, recombination frequency 0.30). Markers placed on linkage groups D (CsP019) and E (CsP215 and CsC613) in Gy14 × 183967 were placed together in Gy14 × 432860. Because these two groups (D and E) in Gy14 × 183967 could be joined (*CsP460* to *CsP221*) at less stringent criteria (LOD 2.4 and recombination frequency 0.33), we des-

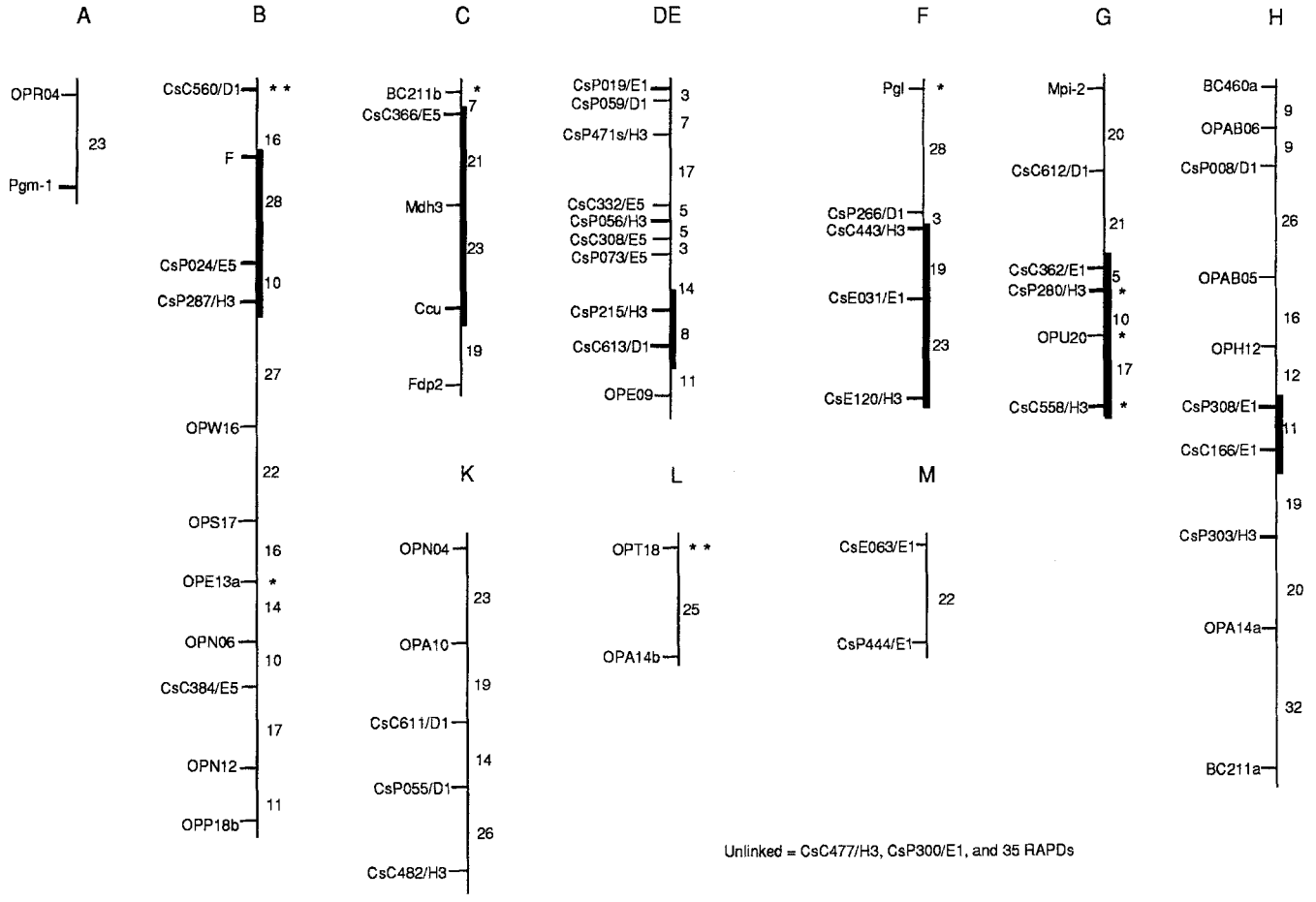


**Fig. 2** Markers, enzymes, linkage groups, and Kosambi distances detected in the cross Gy14 × 432860 (designated as in Fig. 1; see Materials and methods for naming of RAPD markers). Probes detecting polymorphic loci also segregating in Gy14 × 183967 are shown in *bold type*. Conserved linkages with respect to Gy14 × 183967 are illustrated with *bold lines*

ignated the linkage group as DE in Gy14 × 432860 (Fig. 2). Three of the seven linked markers showing distorted segregations were clustered on linkage group G.

Two RFLPs (6%) and 35 RAPDs (64%) were unlinked in Gy14 × 432860 (Table 1). The unlinked RAPDs were detected with primers BC204, BC210, BC254*, BC262, BC263, BC340**, BC460c, BC549*, BC555, BC584*, BC652, BC688, BC697, OPC09, OPD13**, OPE13b**, OPE16**, OPK14, OPL19*, OPM14, OPM16, OPN08a, OPN08b**, OPN11, OPP18a, OPQ03, OPQ05, OPS19, OPT08, OPU07, OPU09**, OPX03, OPY14a, OPZ06, OPZ07 (* and ** represent goodness-of-fit to expected ratios of $P < 0.05$ and 0.01, respectively). RAPDs may be disproportionately unlinked in this population because dominant markers are less informative in an $F_2$ population (Allard 1956). However, the greater overall map length of the Gy14 × 432860 population and the excess of nulls for RAPD markers may be the result of random inefficient amplifications. Our results indicate that, under the conditions employed, RAPDs must be considered dubious until acceptable fits to expected Mendelian ratios are demonstrated and linkages are repeatable.

Meiotic analysis revealed an average of 2.12 chiasmata per chromosome pair (Ramachandran and Seshadadri 1986) corresponding to 742 cM (2.12 chiasmata/chromosome, seven chromosomes, and 50 cM/chiasmata) in cucumber. In Gy14 × 432860, 58 markers spanned 766 cM, approximating to this prediction; in Gy14 × 183967, 70 markers spanned 480 cM. However in both crosses, the ten linkage groups and unlinked RFLPs indicate we have not reached saturation. The shorter map length of Gy14 × 183967 may be due to lower recombination frequencies in a wider cross. Comparison of recombination among conserved linkages indicates no clear-cut trend. Of the six conserved regions, three showed greater, and three less, recombination among marker loci.

Twenty-one markers segregated in both populations (doubly segregating) and were used to assess colinearity and to assemble unresolved linkages (Fig. 2). Linkages of 15 of 21 markers were conserved (*F*, *CsP024*, and *CsP287* on B; *CsC366* and *Ccu* on C; *CsP215* and *CsC613* on DE; *CsC443*, *CsE031*, and *CsE120* on F; *CsC362*, *CsP280*, and *CsC558* on G; and *CsP308* and *CsC166* on H). Four doubly-segregating markers showed linkage to different conserved linkage groups (*CsC560* on B, *CsP019* and *CsP056* on DE, and *CsP303* in H in Gy14 × 432860), one was unlinked to any other doubly-segregating marker (*Pgm-1* on A), and one was unlinked to any other marker

in Gy14 × 183967 (*CsC482*). The conserved linkage groups maintained the same order of markers, except in linkage group F where the order of *CsE031* and *CsE120* was reversed. Of the four markers linked to different conserved regions, three contained multiple bands that potentially detected different segregating loci (*CsC560* on B, *CsP019* and *CsP056* on DE, and *CsP303* in H). These probes may detect duplicated loci that were mapped exclusively in only one population. However, for *CsP019*, a single pair of codominant bands was observed in both populations further supporting linkage of groups D and E in the Gy14 × 183967. Other evidence for linkage comes from two-point data, as markers among these linkages are the first to be grouped when mapping thresholds were relaxed (LOD 2.4 recombination frequency 0.33). Further, the most likely orientation (*CsP460* to *CsP221*) is consistent with the order in the conserved linkage.

Differences among cultivated and wild accessions are due, in part, to selection for desirable horticultural characteristics. Selection for common alleles among the cultivated accessions would reduce the frequency of genetic markers linked to those traits. While we have not achieved saturation with either population, we found it surprising that linkage group A from Gy14 × 432860 comprised only two loci and was the most saturated group in Gy14 × 183967. Although only one locus cosegregated in both populations, this discrepency could be explained by fixation of this linkage group in the cultivated cucumber.

While we did not confirm any previously-reported linkages, gynoecious sex expression (*F*), black spine (*B*), and resistances to downy mildew (*dm*) and scab (*Ccu*) segregated independently, as expected (Pierce and Wehner 1990). Using these markers, we can assign our linkage groups to the composite map of Pierce and Wehner (1990). Our linkage group A corresponds to IV (based on *dm*), B to I (based on *F*), C to V (based on *Ccu*), and F to III (based on *B*). We chose not to use numbers to designate our linkage groups because they have not been assigned to chromosomes.

Isozymes segregating in our study that have previously been evaluated for linkage include *Pgm-1*, *Idh-1*, *Pep-pap*, *Mdh-2*, *Per*, and *Mpi-2*. A previously reported linkage (5 cM) between *Pgm-1* and *Idh-1* (Knerr et al. 1992) was confirmed in the Gy14 × 183967 population (4 cM). *Pep-pap* and *Mdh-2* have been reported to be linked at 35 cM. Although this linkage could not be directly confirmed (*Pep-pap* was scored only in Gy14 × 183967 and *Mdh-2* was scored only in Gy14 × 432860), flanking loci in both populations placed the isozymes on different linkage groups.

Although we characterized 137 new markers (80 RFLPs, 55 RAPDs, and two isozymes) and exceeded the coverage of previously-reported linkages (Abul-Hayja et al. 1975; Fanourakis and Simon 1987; Whelan et al. 1975; Pierce and Wehner 1990; Knerr and Staub 1992), we have not yet resolved the seven linkage groups of cucumber. Future mapping efforts in cultivated cucumber must recognize the narrow genetic background and will require techniques that detect small structural or single-base-pair changes, e.g., use of restriction enzymes recognizing 4-bp sequences, denaturing gradient gel electrophoresis (Dweikat et al. 1993), or restriction-enzyme digestion of PCR products (Rafalski and Tingey 1993). Backcross families or recombinant inbred lines are more efficient to detect linkage for dominant markers such as RAPDs (Allard 1956; Reiter et al. 1992). The construction of a detailed genetic map within cultivated cucumber will require these and other novel strategies.

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