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Quantitative trait analysis of fruit quality in cucumber: QTL detection, confirmation, and comparison with mating-design variation

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Abstract A cross within *C. sativus* var. *sativus* (GY14 × P1432860) and molecular markers were used to determine the number, magnitudes of effect, and overall variation described for genes conditioning the quantitatively inherited traits of length, diameter, seed-cavity size, color, L/D (length/diameter), and S/D (seed-cavity size/diameter). QTL effects were detected with MAPMAKER/QTL using 100 F_3 lines evaluated in a replicated field trial of two harvests over 2 years at one location. Multilocus models were constructed by fixing significant intervals and re-scanning using MAPMAKER/QTL. Marker inclusion in multilocus models was compared to an ANOVA "backward elimination" proce-dure. Generally the same loci were associated with QTLs among the two methods of model construction. Heritabilities of individual QTLs were confirmed by analysis of related backcrosses (67 BC_1P_1 lines and 68 BC_1P_2 lines). The majority of QTLs were confirmed in at least one backcross population. Pairs of backcrosses allowed overall additive variances and heritabilities to be calculated using a North Carolina Design III (NCIII design) and estimates were compared to overall variances attributable to markers. Heritability estimates using markers were comparable, but generally lower than additive variances estimated by co-variance relationships in the NCIII design. This suggests that neither the number nor the magnitude of QTL effects were overestimated. The utility of backcrosses to confirm individual QTLs and the overall variance described by QTLs is recommended to avoid false positives and over-estimation of effects. The number of QTLs, and/or the proportions of phenotypic variation described by markers and the mating design, agreed with previous reports of heritabilities employing similar germplasm.

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Introduction

The length, diameter, and color of cucumber (*C. sativus* L. var. *sativus*) fruit are economically important traits. Ideal pickling cucumbers have length-by-diameter (L/D) ratios of approximately 3.0, blocky shape, lightly colored skin, warts or tubercles, and an exocarp permeable to brining salt. Seed-cavity size and seed-cavity-by-diameter ratio (S/D) are important in reducing placental hollowness and carpel separation. A desirable slicing cucumber for the US market has an L/D ≥ 4.0, a slightly tapered shape, a dark uniform color, and a firm exocarp. Currently, fruit-quality traits important for both fresh-market and pickling cucumber include reducing seed-cavity size, seed-maturation rate, carpel separation, and placental hollowness.

The genetics of fruit quality may be simple or complex. Simply inherited traits include uniform skin color (*u*, Robinson et al. 1976) and the presence and color of spines (*B*, Shanmugasundaram et al. 1971; Lower and Edwards 1986; Strefeler and Wehner 1986). Length and L/D are reported to be under the control of at least four to five genes (Owens et al. 1985a; Lower and Edwards 1986). Genetic variances and heritabilities have been analyzed for length, L/D, fruit color, and seed-cavity size in NCI (Smith et al. 1978; Strefeler and Wehner 1986) and NCII (Owens et al. 1985b) mating designs. L/D had moderately high heritability (0.59) using full-sib populations (Smith et al. 1978). Length showed a relatively high narrow-sense heritability (0.79–0.82; Owens et al. 1985a). Color in the absence of *u* (light to dark green; 0.00–0.20) and seed-cavity size (0.01–0.02) exhibited low heritabilities (Smith et al. 1978; Strefeler and Wehner 1986). Environmental factors, e.g., temperatures (Lower and Edwards 1986), time of pollination (Tjiedens 1928), nutrients (Miller and Ries 1958) and plant density (Cantliffe and Phatak 1975), affect fruit shape and quality.

Genetic markers provide an understanding of quantitative inheritance by estimating location, magnitude of effect, parental contribution, gene action, and epistasis of quantitative trait loci (QTLs; Thoday 1961; Gelderman 1975). QTLs can be tested for stability across environments and genetic backgrounds (Paterson et al. 1991; Stuber et al. 1992). The overall proportion of quantitative trait variation described by QTLs can be estimated in multilocus models (Lincoln and Lander 1989; Knapp et al. 1992). The variance of individual QTLs can be estimated from genotypic effects and allele frequencies (Wright 1935; Falconer 1989) or with the expected mean squares described by markers (Knapp and Bridges 1990). While the genetic proportion of quantitative trait variation has predominantly been estimated with mating designs, few studies have compared marker-based and mating-design-based estimates of variation. The study herein combines approaches as the mapping population was incorporated into a North Carolina Design III (NCIII design). The integration of a mapping population into a mating design allowed a reference point for the magnitude of QTL-effect measures (e.g., are QTL-based variances higher or lower than mating-design-based variances?) and gave a more complete picture of quantitative inheritance than either estimate alone.

Materials and methods

Plant materials and field experiment

GY14 (P1, gynoecious pickling breeding line) was crossed as the seed parent with USDA plant introduction (PI) 432860 (P2, long-fruited monoecious type) to generate the F_1. A single F_1 plant was selfed and F_2 individuals selfed to generate F_3 lines. We have previously described 59 RFLP, RAPD, isozyme, morphological, and disease-resistance loci comprising ten linkage groups spanning 748 cM (Kennard et al. 1994b). Segregating progenies for trait analyses were the same as those used in map construction. F_2 individuals in the mapping population were used as males in backcrosses to both parents. A total of 100 F_3 lines, 67 BC_1P_1, and 68BC_1P_2 were evaluated for fruit-quality traits. A total of 80 pairs of backcrosses to both parents (16 pairs of which were not evaluated for QTLs) were included in the NCIII design (Comstock and Robinson 1948). Four plots (two replications over 2 years with five plants/plot) for all lines, including parental and F_1s, were evaluated over two harvests at the University of Wisconsin West Madison Agricultural Experiment Station. Plots were experimental units and were planted in randomized complete blocks (0.30 m between plants and 1.80 m between rows). The F_3 lines were not interplanted with BC lines, but BC_1P_1 and BC_1P_2 populations were interplanted to maintain the NCIII design. To achieve equal size of replications (blocks), the BC populations were divided into two sets. Each set consisted of 80 BC lines (40 paired lines from a common F_2 parent) that were randomized in two replications over 2 years. To test for confounding plot-effects among F_3 and BC populations, 24 plots of P_1, P_2, and F_1 lines (two plots/block, six blocks, 2 years with five plants/plot) were interplanted as controls. Uniformity was maintained for irrigation, cultivation, and applications of insecticide (Sevin), fungicide (copper sulfate), and fertilizer (composted manure). Fruit were harvested from approximately 2-month-old plants and evaluated 10–20 days after anthesis. Fruit were over-sized for pickling but of appropriate size for slicing cucumber. Length was measured as the distance from the blossom to the stem end. The diameter was measured as the width through the midpoint between blossom and stem ends. For asymmetric fruit, diameter was measured through the midpoint of the enlarged section. Seed-cavity size was measured as the distance from the placental exocarp directly across the seed cavity to the endothelial transition zone (Esau 1977). Two traits derived from these measurements were length-by-diameter (L/D) and seed-cavity-size-by-diameter (S/D) ratios. Color was subjectively scored on a scale of light (1) to dark (5) green.

QTL detection and estimation of effects

F_3 and BC_1 line means were estimated by least-square means according to the model, line mean = population mean + mean year + mean harvest(year) + mean rep(harvest/year) + line mean, and were used for MAPMAKER/QTL analysis. Means, standard deviations, and tests of normality (Shapiro-Wilk Statistics) were performed with SAS(UNIVARIATE) (SAS Institute 1990). Phenotypic variances for QTL analysis were calculated by the MAPMAKER/QTL program on the basis of means alone (Lincoln and Lander 1989). Phenotypic variances among lines were also calculated to include interaction variances with expected mean squares ($\sigma_l^2 + \sigma_{ly}^2/y + \sigma_e^2/rhy$) from the model, line mean = population mean + mean year + mean harvest (year) + mean rep(harvest/year) + line mean + line × year mean + error, with the SAS(GLM) procedure. For pairs of traits, Pearson phenotypic correlations among traits were tested with the SAS(CORR) procedure.

Independent tests for QTL presence were performed with MAPMAKER/QTL (Lander and Botstein 1989) to obtain frequencies of significant tests and confirm intervals in multilocus models. Independent QTL tests were of the form: least-square line mean = population mean + additive effect + dominance effect + error. Putative QTLs were detected with LODs above 1.3 corresponding to a level of significance ($P < 0.05$) for independent tests. While false positives may occur with this threshold, we were interested in confirming QTLs across generations (F_3, BC_1P_1, BC_1P_2). LOD scores above 2.0 ($P < 0.01$ for independent tests) or 2.4 ($P < 0.05$ among all tests with the linkage map) are more appropriate in the absence of confirmation. Analyses to detect putative QTLs were also performed with SAS(GLM) of the model, least-square line mean = population mean + genotypic class effect + error, assuming errors were independent and equal among classes.

Multilocus models were constructed with MAPMAKER/QTL (Lincoln and Lander 1989) to determine the most likely number, positions, and magnitude of effects of QTLs on the basis of F_3-line means. MAPMAKER/QTL multilocus models were constructed by fixing a QTL and then re-scanning the genome for the greatest increase in LOD above 2.0 over the fixed QTL. Subsequent QTLs were fixed and the genome re-scanned until no further QTLs were identified (Stuber et al. 1992; Nodari et al. 1993).

One-way ANOVA multilocus models were compared to MAPMAKER/QTL multilocus models and used to estimate genetic-variance-attributable QTLs. The multilocus model was of the form: least square mean = population mean + genotyic class effect$_1$ + genotypic class effect$_2$ + ··· error. The data set was modified for the multifactor ANOVA. Genotypes consistent with flanking markers were assigned to missing data (heterozygotes were assigned in cross-over regions) to maintain set size. Single-marker loci were used to minimize data-set modification. As a comparison to the MAPMAKER/QTL "fixing and scanning" procedure, ANOVA multilocus models were constructed in a "backwards elimination" process. For a given trait, a model was constructed with all marker loci that showed significance in single-factor ANOVAs. Marker loci were then excluded one at a time, based on the criterion of least significant ($P < 0.05$) Type-III sum of squares (Kennard et al. 1994a). The MAPMAKER/QTL and ANOVA approaches (i.e., intervals vs single markers, maximum likelihood vs least-squares, linked vs unlinked markers, presence vs absence of recombination estimators, and different significance levels) allowed for confirmation of marker-locus inclusion. Multilocus models were also constructed to estimate genetic variance attributable to QTLs. One-way ANOVA models were constructed including the single marker locus nearest to the QTL as determined in MAPMAKER/QTL. The proportion of expected Type-I mean squares associated to marker effects was calculated as described below.

Estimates of marker variance from expected mean squares were contrasted to additive variances derived from effects estimated with MAPMAKER/QTL.

Additive effects are estimated as the mid-point of the homozygote class means i.e., the average effect in the absence of dominance (Falconer 1989). In our study, additive effects of intervals were calculated by MAPMAKER/QTL after inclusion into multilocus models (fixing and subsequently re-scanning). MAPMAKER/QTL multilocus models partition additive (weight) and dominance effects for each QTL, but only additive effects were reported. MAPMAKER/QTL allows models to be constrained to additive effects, but were not used in this study. Effects in our study are primarily additive because F_3 individuals were used.

Variance estimation from effects and mating design

Marker-based additive variance was estimated as the sum of variances attributable to additive effects. In an F_2 population where allele frequencies (p and q) are 0.5, dominance is negated, and the additive variance of a locus is $2pqa^2$ where a is the additive genotypic effect ($a = \alpha$, the average effect, in the absence of dominance; Falconer 1989). In our study, additive variances of QTLs were calculated with actual allele frequencies of the single marker locus closest to the QTL in the multilocus model. Dominance was disregarded in the calculation since deviation from 0.5 allele frequencies was slight, and F_3 line means were used to infer F_2 phenotypes (loss of 1/2 dominance effect). The variances associated with individual loci were then summed to estimate overall additive variance which should theoretically sum to the variances attributable to additive effects (Wright 1935). A marker-based heritability was calculated as the sum of additive variances divided by phenotypic variances $[\Sigma 2pqa^2/(\sigma_i^2 + \sigma_{iy}^2/y + \sigma_e^2/rhy)]$ where variance components are described as above. Marker-based genetic variance was calculated as a component of expected mean squares (Type 1) in the multilocus ANOVA model employing the nearest single marker locus to the QTL as determined by MAPMARKER/QTL. For each QTL in the multilocus model, the expected mean square was assumed to be $\sigma_e^2 + n\phi_Q^2$, where $\sigma_e^2 =$ is residual variance in the multilocus model, ϕ_Q^2 is the variance of the fixed effect of QTL genotypes, n is the weighted average of F_3 lines, and the number of degrees of freedom per QTL is 2 or 1 (co-dominant or dominant marker genotypes-1) (Knapp and Bridges 1990). Type-1 sums of squares were used to describe QTL variation in the same sequence as in the comparable MAPMAKER/QTL model.

Overall additive genetic variance of the F_2 mapping population was also estimated in a classic NCIII design. The sum of variances attributable to additive effects has been proposed to be directly related to the variance component associated to F_2 males in the NCIII design, $\sigma_f^2 = (1/8)\ \Sigma a_i^2 = 1/4\ \sigma_A^2$, (Comstock and Robinson 1952; Hallauer and Miranda 1988). Variance and co-variances in the NCIII design were calculated with SAS(GLM). Expected mean squares, additive variance components, and heritabilities from the 80 pairs of backcrosses were calculated according to Comstock and Robinson (1948). Additive genetic variance was calculated as expected mean squares associated to co-variances among pairs of half-sib families, $4\sigma_f^2$. Phenotypic variance was calculated as $4\sigma_f^2 + 4\sigma_{fy}^2/y + \sigma_{fp}^2 + \sigma_{fpy}^2 + \sigma^2/(rhy)$ where $\sigma_f^2 = F_2$ males variance, $\sigma_{fy}^2 = F_2 \times$ years variance, $\sigma_{fp}^2 = F_2 \times$ parents variance, $\sigma_{fpy}^2 = F_2 \times$ parents $\times$ years variance and $\sigma^2 =$ error variance. Narrow-sense heritabilities were calculated as the ratio of additive variance and phenotypic variances. Genotypic correlations were calculated for traits i and j by calculating cross-products associated to F_2 males (σ_{fij}^2) and dividing by the square of the product of the individual variances $[\sigma_{fij}^2/(\sigma_{fi}^2\sigma_{fj}^2)^{1/2}$; Mode and Robinson 1959].

Results and discussion

Parental and population distributions

Parental and F_1 lines were interplanted as controls among the F_3 and BC_1 lines to determine sources of environmental variation and compare block-to-block variation. All fruit-quality traits were significantly ($P < 0.001$) different among parents. The parental fruit represented extremes in phenotype for length (GY14 = 148.0 ± 17.8 mm, PI 432860 = 378.7 + 73.6 mm), diameter (GY14 = 63.0 + 8.7 mm, PI 432860 = 40.1 ± 11.8 mm), seed-cavity size (GY14 = 38.8 ± 5.5 mm, PI 432860 = 22.3 ± 6.3 mm), color (GY14 = 2.1 ± 0.5, PI 432860 = 4.3 ± 0.6), and L/D (GY14 = 2.36 ± 0.35, PI 432860 = 9.78 ± 1.88). S/D exhibited a less dramatic, but highly significant ($P < 0.001$), difference among parental lines (GY14 = 0.61 ± 0.04, PI 432860 = 0.54 ± 0.07). Significant environment and genotype-by-environment effects were found among parental lines. Length was significantly different ($P < 0.05$) over years; length, diameter, seed-cavity size, and color showed significant ($P < 0.05$) parent-by-year interactions. Variation over years may have been due to lower mean temperatures in 1992; fruit show lower L/Ds at cooler temperatures (Lower and Edwards 1986). No significant block-to-block variation was detected for any trait.

Significant effects for F_3 lines were observed for all traits (color, $P < 0.05$; all other traits, $P < 0.001$). F_3 line-by-year interactions were found for diameter, color, and L/D. Traits were predominantly normally distributed. Log transformations that normalized distributions did not alter the significance of the QTLs, and analyses were performed on non-transformed data.

Phenotypic correlations were significant ($P < 0.05$) among all pairwise comparisons of traits except S/D-by-color, which was marginally significant ($P = 0.057$). Genotypic correlations reflected phenotypic correlations in magnitude and sign, and were generally of greater magnitude for all comparisons except those associated with diameter. Since all traits except color are measures of fruit shape, they may be expected to be correlated.

QTL detection

QTLs were detected among F_3 lines with independent tests using MAPMAKER/QTL. Of 288 (six traits × 48 intervals) MAPMAKER/QTL tests, 113 (39.0%), 72 (25.0%), and 54 (18.7%) were significant at $P < 0.050$, $P < 0.010$, $P < 0.001$, respectively, where chi-squared values of 5.99, 9.21 and 11.10 = LODs of 1.3, 2.0 and 2.4 respectively, for each individual test. These probabilities are based on independent comparisons, and false positives may occur (Lander and Botstein 1989). A LOD of 2.4 has been proposed as the appropriate threshold of a single false positive at the 5% level with a map of intermediate density (15 cM), 12 chromosomes, and genomic length of 1000 cM. LODs at or above 1.3 ($P < 0.05$) are reported for each individual test because we wished to compare with ANOVAs at $P < 0.05$ and test the heritabilities of marginally significant QTLs. Comparable analysis with ANOVA indicated that of 348 (six traits × 58 markers showing linkage) one-way ANOVAs, 101

(29.0%), 52 (14.9%), and 24 (6.9%) were significant at $P < 0.050$, $P < 0.010$, $P < 0.001$, respectively. Fewer significant ANOVAs were detected with 35 unlinked RAPDs and three unlinked RFLPs; 26 (9.0%), six (2.6%), and none of 288 (6 × 38) tests were significant at $P < 0.050$, $P < 0.010$, $P < 0.001$, respectively. MAPMAKER/QTL detected more significant associations as compared to single-factor ANOVAs, perhaps because of the increased resolution with flanking markers or the use of recombination values within intervals. Fewer significant tests with RAPDs may be due to inefficient amplification events (Kennard et al. 1994b) making them appear unlinked.

Fewer significant tests were found in the related backcross lines than with F_3 lines. In the BC_1P_1 population 51 (17.7%), 23 (8.0%), 16 (6.0%) of 288 MAPMAKER/QTL tests were significant at LOD 1.3, 2.0, and 2.4 respectively. In the BC_1P_2 population 59 (20.5%), 33 (11.5%), 18 (6.3%) of the MAPMAKER/QTL tests were significant at LOD 1.3, 2.0, and 2.4 respectively. QTL detection rates were slightly lower at analogous P-values with ANOVA. In the BC_1P_1 population, 41 (11.8%), 16 (4.6%), and six (1.7%) of the 348 ANOVA tests were significant (P values < 0.050, 0.010, and 0.001, respectively). Similarly, in the BC_1P_2 population, 47 (13.5%), 19 (5.5%), and one (0.3%) were significant (P values < 0.050, 0.010, and 0.001 respectively). The lower number of significant tests were expected since fewer BC_1 lines (67 BC_1P_1 and 68 BC_1P_2) than F_3 lines (100) were used to confirm QTLs. In general, the magnitude of effects in the backcross populations were either smaller or nearly equivalent to those found in the F_3 population. A different magnitude of effects among backcross populations for a particular QTL may be the result of gene action. Both magnitudes of effect and variance should be one-half of an F_2 population (or mean F_3 lines; Hallauer and Miranda 1988). However, phenotypic variances in our backcross populations were generally greater than one-half of that among F_3 lines, perhaps due to scoring variable-sized fruit, larger measurements for a particular trait, or residual heterozygosity in either parental line. Large phenotypic variance, recessive gene action, or smaller sample size, could result in the detection of fewer QTLs in a given BC_1 population.

Rarely, a QTL was detected in one of the backcross populations while no corresponding effect on the same linkage group was detected in the F_3 populations. Five QTLs (LOD > 2.4) were found in the BC_1P_1 population, and six QTLs were found in the BC_1P_2 population. Of these, only one effect was detected that had no corresponding effect on the same linkage group in the F_3 population (i.e., diameter on linkage group A). QTL effects at LOD > 2 with no corresponding F_3 population effects were found at a similar rate (two of ten in both BC_1P_1 and BC_1P_2 populations). These population-specific effects may be due to spurious associations, gene action, maternal effects, or genetic background.

Putative QTLs for fruit quality were found predominantly on linkage groups A, B and DE. For independent tests (no fixing), 74.3% (84 of 113 > LOD 1.3) were found on these three linkage groups. Linkage groups A, B, and DE were significantly (LOD > 2.4) associated with length, seed-cavity size, and L/D. Putative QTLs were confirmed (LOD > 2.4) for length, seed-cavity size, and L/D on linkage group A and for length and L/D on linkage groups B and DE. Linkage groups F and K appear to have significant, but smaller effects, on fruit-quality. Significant effects for diameter (LOD 3.79), L/D (LOD 2.29) and S/D (LOD 2.83) were found on linkage group F; effects for length (LOD 2.04) and S/D (LOD 2.43) were found on linkage group K. Effects for length were confirmed in the BC_1P_1 population. Importantly, these effects with lower magnitude (< LOD 2.4) were found to be heritable, i.e., present in the backcross populations. Since most of these traits were genotypically and phenotypically correlated, we may have been measuring effects of the same genes.

Multilocus models and estimation of effects

The multilocus MAPMAKER/QTL model for length (fixing and scanning for the greatest increase over LOD 2.0) included intervals on linkage groups A (*OPRO4-Pgm-1*), B (*CsPO59-CsP471*), DE(*CsP287-OPW16*), K (*OPA10-CsC611*), and F (*CsC443-CsP266*) (Table 1). All intervals were detected (LOD > 2.0) in the original likelihood plot (no QTL fixing) except that on linkage F (LOD = 1.68). The model described 62.7% of the phenotypic variation for length and effects ranged from 9.4 to 19.6 mm (increase or decrease of an effect is relative to 432860). Heritability of four QTL intervals [(*OPRO4-Pgm-1*), (*CsPO59-CsP471*), (*CsP287-OPW16*), (*OPA10-CsC611*)] was confirmed in the BC_1P_1 population while only two [(*CsPO59-CsP471*), (*CsP287-OPW16*)] were confirmed in BC_1P_2. The ANOVA multilocus model (backwards elimination with a P value of 0.05) included markers (*Pgm-1*, *CsPO59*, *OPW16*, *OPA10*, and *CsC443*) from the same five intervals as in MAPMAKER/QTL, except that one additional unlinked marker was included (*BC652*).

The multilocus MAPMAKER/QTL model for diameter included intervals on linkage groups B (*CsP560-F*), DE (*CsC308-CsP073*), and F (*CsE120-CsE031*), and described 46.9% of the phenotypic variation. Effects of intervals ranged from a decrease of 1.52 to 2.48 mm. Only one interval, (*CsC308-CsP073*), was marginally confirmed (LOD = 1.44) in just one of the backcross populations, (BC_1P_2). The ANOVA multilocus model included markers on linkage groups A (*Pgm-1*) and DE (*CsP059*, *CsP471s*, and *CsC308*).

The multilocus MAPMAKER/QTL model for L/D included intervals on linkage groups A (*OPR04-Pgm-1*), B (*CsP287-OPW16*), DE (*CsP059-CsP471s*), F (*CsP443-CsP266*), and K (*OPA10-CsC611*), and described 64.3% of the phenotypic variation. All intervals were the same

Table 1 Effects of QTLs for fruit-quality traits detected with F_3 families using MAPMAKER/QTL, confirmation of effects in BC_1 families, and derived genetic and additive variance of QTLs

Trait	Locus		Additive effect			Variance due to QTL	
	Linkage group[a]	Interval of locus[b]	F_3^c	$BC_1P_1^d$	$BC_1P_2^e$	ϕ_Q^2 (Genetic)[f]	$2pqa^2$ (Additive)[g]
Length	A	*OPR04*-**Pgm-1**	14.53	9.93***	–ns–	204.69	105.53
	DE	**CsP059**-*CsP471s*	19.63	7.85***	18.06**	143.75	192.37
	B	*CsP287*-**OPW 16**	12.33	12.16***	20.00***	149.19	75.72
	K	**OPA10**-*CsC611*	9.37	8.93**	–ns–	54.97	43.69
	F	**CsC443**-*CsP266*	13.27	–ns–	–ns–	159.46	87.73
Diameter							
	DE	**CsC308**-*CsP073*	−2.15	–ns–	−2.03*	3.10	2.30
	F	**CsE120**-*CsE031*	−2.48	–ns–	–ns–	2.14	2.95
	A	*OPR04*-**Pgm-1**	−1.52	–ns–	–ns–	1.57	1.15
Seed-cavity size							
	DE	**CsC308**-*CsP073*	−1.91	–ns–	−1.47*	1.97	1.82
	A	*OPR04*-**Pgm-1**	−1.52	–ns–	−1.85***	1.61	1.15
	B	***F*-CsP024**	−1.15	ns–	–ns–	0.28	0.65
Color							
	K	*OPA10*-**Cs611**	0.69	0.54***	0.17*	0.30	0.24
	L	*OPT18*-**OPAB14b**	0.35	0.23***	0.20**	0.02	0.06
L/D							
	DE	**CsP059**-*CsP471s*	0.622	0.149**	0.592**	0.221	0.192
	B	*CsP287*-**OPW 16**	0.381	0.196***	0.589*	0.113	0.072
	A	*OPR04*-**Pgm-1**	0.433	0.214***	−1.372**	0.151	0.092
	F	**Cs443**-*CsP266*	0.410	–ns–	–ns–	0.107	0.084
S/D							
	F	**CsE120**-*CsE031*	0.0200	–ns–	–ns–	0.00007	0.00019

[a] Linkage group of QTL as described by Kennard et al. (1994 b)
[b] Intervals included in multilocus MAPMAKER/QTL model. Marker-locus nearest to QTL used to estimate ϕ_Q^2 in bold
[c] Additive effect (mm) estimated in multilocus MAPMAKER/QTL from F_3 families, −/+ with respect to genotype of PI 432860
[d] Additive effect (mm) estimated in MAPMAKER/QTL individual locus-by-locus tests from backcross families to GY-14 using F_2 mapping genotypes, −/+ with respect to genotype of 432860; *, **, ***, indicate significance levels at LOD 1.3 ($P < 0.05$), 2.0 ($P < 0.01$), and 2.4 ($P < 0.01$), respectively
[e] Additive effect (mm) estimated in MAPMAKER/QTL individual locus-by-locus tests from backcross families to PI 432860 using F_2 mapping genotypes, −/+ with respect to genotype of 432860; *, **, ***, indicate significance levels at LOD 1.3 ($P < 0.05$), 2.0 ($P < 0.01$), and 2.4 ($P < 0.001$), respectively
[f] Variation of QTL effects derived from expected mean squares of multilocus models (see text)
[g] Variation of QTL derived from $2pqa^2$, where a is estimated in the multilocus model in MAPMAKER/QTL, and p and q are estimated from the marker genotype nearest to QTL (in bold)

as in the multilocus model for length except for that on linkage group K. Length and diameter were inversely correlated and controlled by genes in same regions of the genome. QTLs for length showed more significant effects since the F_3 variation is greater for length. Confirmation of QTL heritability was provided for three of four intervals in the MAPMAKER/QTL mode [(*OPR04-Pgm-1*), (*CsP287-OPW16*), and (*CsP059-CsP471s*)] with at least one backcross population. However for one interval (*OPRO4-Pgm-1*), the genotypic class ranking of a significant (LOD 2.06) confirming effect in the BC_1P_2 population was reversed (GY14 > PI 432860). This result is surprising and may due to dominance and/or strong genetic background effects. The ANOVA multilocus model for L/D included markers (*Pgm-1*, *OPW16*, *CsP471s*, *CsP019*, *CsP073*, *CsE120*, *BC503a*, *BC652*) on the same linkage groups as in the MAPMAKER/QTL model.

The multilocus MAPMAKER/QTL model for seed-cavity size included intervals from linkage group A (*OPR04-Pgm-1*), B (*F-CsP024*), and DE (*CsC308-CsP073*), and described 41.2% of the phenotypic variation. Effects ranged from 1.15 to 1.91 mm with the MAPMAKER/QTL model. Some of the same intervals or markers significant for diameter were found significant for seed-cavity size; these traits were phenotypically (0.327) correlated and may be controlled by some of the same genes. Heritability for two of three intervals [(*OPR04-Pgm-1*) and (*F-CsP024*)] was confirmed in the BC_1P_2 population. The ANOVA multilocus model for seed-cavity size included markers on linkage groups B (*CsC560* and *OPE13a*) and DE (*CsC308*).

The multilocus MAPMAKER/QTL model for S/D included only one interval (*CsE120-CsE031*) on linkage group F describing 12.0% of the phenotypic variation and was not confirmed in either backcross population. The effect in the MAPMAKER/QTL model was 0.0007. The ANOVA multilocus model included intervals from linkage groups A (*Pgm-1*), DE (*CsC308*), and unlinked markers (*BC652*, *BC503*, *CsC477*). The inconsistency among MAPMAKER/QTL and ANOVA may be due the small measured effects; this trait was only slightly different between the parents.

The multilocus MAPMAKER/QTL model for color included two intervals; one on linkage group K (*OPA10-CsC611*) and one on linkage group L (*OPT10-*

OPAB14b). Uniform fruit color has been described as a single locus *u* (Robinson et al. 1976). Although darkness of color was scored (as opposed to a uniform vs stipled color), our quantitative analysis detected a single interval (*OPA10-CsC611*) describing 42.0% (ignoring *OPT10-OPAB14b*) of the phenotypic variation. Effects ranged from 0.348 to 0.688. The locus described herein appears dominant for dark green color; however *u* is described as recessive for uniform (dark green versus stipled) color. Both QTL intervals (*OPA10-CsC611*) and (*OPT10-OPAB14b*) were confirmed in both backcrosses. Only the marker associated with the major locus (*CsC611*) was incorporated into the multilocus ANOVA model.

MAPMAKER/QTL and ANOVA tests closely reflected each other for both independent tests and multilocus models. For all independent tests, a QTL found with MAPMAKER/QTL (LOD > 2.0) with the F_3 lines was also detected on the same linkage group with SAS(GLM). With BC_1 lines, there were two instances where QTLs were found with MAPMAKER/QTL (LOD > 2.0) with no corresponding SAS(GLM) effect. This may reflect the increased sensitivity with MAPMAKER/QTL which employs flanking markers and accounts for recombination within intervals. Multilocus models were comparable among methods despite complexity and different modes of construction. Indeed for length, diameter, color and L/D, markers and intervals were included from the same linkage groups (excluding unlinked markers included in ANOVA analyses). ANOVA models generally included single markers from the same intervals included in MAPMAKER/QTL models. Consistency among methods lends support to the models presented.

Variance derived from QTLs and NCIII design

Variances attributable to loci or intervals included in MAPMAKER/QTL models were calculated in two ways, as an effect from expected mean squares ϕ_Q^2 (approximating the genetic variance of a QTL) and as deviations of additive effects (approximating the additive variance of a QTL; Table 1). Expected mean squares were calculated to check if MAPMAKER/QTL additive-effect variances were reasonable. Genetic variance should theoretically be greater or equivalent to additive variance depending on the degree of dominance. As expected, estimates were generally greater for genetic variance components than as deviations of additive effects (Table 1). Of the 18 QTLs included in multilocus models, five were lower in magnitude as effects from expected mean squares (genetic variance) than as deviations of additive effects (additive variance). The disparity may indicate the differences among computational methods with variance components in SAS(GLM) and effect estimation with MAPMAKER/QTL (intervals vs single markers, least-squares vs maximum likelihood, presence vs absence of recombination estimators, and effects of flanking markers). For the remaining 13 QTLs, the average proportion of additive variance was 70.6% (standard error = 12.8%) of the genetic variance.

Heritabilities in the NCIII design were calculated for comparison of marker-based estimates as a reference to other studies and to test the significance of variance components. Expected mean squares associated to F_2 males (parents of BC_1 line pairs) were significant for all traits except diameter. NCIII-design heritabilities ranged from 0.29 for diameter to 0.77 for color (Table 2). Heritabilities for length (0.49) and L/D (0.68) were mo-

Table 2 Comparison of MAPMAKER/QTL model, marker-based additive genetic variation, and NCIII mating-design additive genetic variation

Trait	MAPMAKER/QTL multilocus model of F_3 Lines			Total phenotypic and marker-based additive variation		Total phenotypic and NCIII design additive variation	
	Line phenotypic variance[a]	Number of loci[b]	% Model variance[c]	Total phenotypic variance[d]	Total additive variance $\Sigma 2pqa^2$ (h^2)[e]	Total phenotypic variance[f]	Total additive variance σ_A^2:NCIII (h^2)[g]
Length	829.4	5	62.7	1010.5	505.0 (0.50)	1584.9	772.4 (0.49)
Diameter	16.0	3	46.9	30.6	6.4 (0.21)	23.9	6.8 (0.29)
Seed cavity-size	10.2	3	41.2	13.0	3.6 (0.28)	11.0	7.3 (0.67)
Color	0.49	2	63.9	1.01	0.30 (0.30)	0.46	0.35 (0.77)
L/D	0.81	4	64.3	0.86	0.44 (0.51)	1.42	0.97 (0.68)
S/D	0.0009	1	12.0	0.0013	0.0002 (0.15)	0.0012	0.0005 (0.42)

[a] Phenotypic variance of least-square means of F_3 families, [Sum (F_3 family mean − population mean)^2/(number of F_3 families − 1)]

[b] The number of intervals included in the MAPMAKER/QTL multilocus model

[c] Overall (%) variance of multilocus model (fixing and scanning for LOD 2 increase) as calculated with MAPMAKER/QTL

[d] Phenotypic variance of least-square means of F_3 families including interaction-variance components $[\sigma^2/(rhy) + \sigma_{ly}^2/y + \sigma_l^2]$ where σ^2, σ_{ly}^2, and σ_l^2 are variance components associated with error, F_3 × years, and F_3s, respectively, and r = number of reps, h = number of harvests, y = number of years

[e] Additive variance estimated as the sum of squared additive effects ($\Sigma 2pqa^2$) of QTLs in the multilocus model (Table 1), where pq = allele frequencies estimated via marker data, and a = additive effects estimated in multilocus models in MAPMAKER/QTL, h = narrow-sense heritability = total additive variance/total phenotypic variance

[f] Phenotypic variance in NCIII mating design $[\sigma^2/(rhy) + \sigma_{fpy}^2/y + \sigma_{fp}^2 + 4\sigma_{fy}^2/y + 4\sigma_f^2]$ where σ^2, σ_{fpy}^2, σ_{fp}^2, σ_{fy}^2, and σ_f^2 are variance components associated with error, F_2 × parents × years, F_2 × parents, F_2 × years, F_2s, and error respectively, and r = number of reps, h = number of harvests, and y = number of years

[g] Additive variance estimated as 4 variance component of the F_2 male parent in backcross pairs ($4\sigma_f^2$) in the NCIII design, h = narrow-sense heritability = total additive variance/total phenotypic variance

derately high and comparable to those reported with similarly wide crosses, 0.79–0.82 for length (Owens et al. 1985a) and 0.59 for L/D (Smith et al. 1978). Color heritability (0.77) was greater than for previous reports estimated from crosses within fresh-market cucumber (0.00; Strefeler and Wehner 1986) and pickling cucumber (0.25; Smith et al. 1978). Heritabilities were also greater for seed-cavity size (0.67) than previous estimates (0.01) from within fresh-market cucumber (Strefeler and Wehner 1986).

Marker-based variation has been reported as R^2 values and proportions of sums of squares (Edwards et al. 1987; Keim et al. 1990, Beavis et al. 1991; Stuber et al. 1992) or as variance explained in MAPMAKER/QTL (Paterson et al. 1991; Stuber et al. 1992; Nodari et al. 1993). We report multilocus variance as calculated in MAPMAKER/QTL (Table 2), which is similar to a SAS(GLM) R^2 in the absence of recombination between marker and QTL (Lincoln and Lander 1989). In our study, multilocus models described greater than 60% of among-line variation for length, color, and L/D; greater than 40% of among-line variation for diameter and seed-cavity size; and 12% of the among-line variation for S/D. The per cent variation compares to other marker studies of traits with moderately high heritability [plant height (28–73%) and grain yield (59%) in maize, (Edwards et al. 1987; Beavis et al. 1991; Stuber et al 1992; hard seededness in soybean (57%), Keim et al. 1990; soluble solids (44%) and pH (34%) in tomato, Paterson et al. 1991; and bacterial blight resistance (75%) and nodule number (52%) in common bean, Nodari et al. 1993]. Heritability estimates derived from additive effects of markers were generally lower than R^2 values. These two descriptions of variation include different parameters. In the R^2 value, dominance effects were included in the genetic variation, and interaction components were excluded in the phenotypic variation. Therefore, a marker-based heritability estimate may give a more realistic picture of the utility of a marker as a selection tool.

Variances derived from additive effects of QTLs were compared to overall additive variance estimated in the mating design. The variance component associated to co-variance of half-sibs should be directly related to the sum variances of the genes; $4\sigma_m^2 = \Sigma 2pqa^2$ (Comstock and Robinson 1952; where σ_m^2 is the expected mean square associated with male parents in NCIII, p and q are allelic frequencies as determined by our markers, and a is the additive effect as determined by MAPMAKER/QTL). In our study, marker-based estimates showed lower magnitudes of additive variance than the co-variance estimate for all six traits (Table 2). The average proportion of marker-based additive variance was 73.4% (standard error 26.8%) of the NCIII estimate. This may be expected if marker saturation was incomplete. Proportionally lower additive variation may also be a result of overall lower phenotypic variation of the F_3 population (i.e., length and L/D).

The ratio of additive variance to total phenotypic variance was estimated as the narrow-sense heritability for fruit-quality traits. Heritabilities were calculated from both MAPMAKER/QTL effects and co-variances in the NCIII mating design. Heritabilities calculated in the NCIII design ranged from 0.29 for diameter to 0.68 for L/D. In contrast, marker-based estimates ranged from 0.15 for S/D to 0.51 for L/D. In our study, marker-based heritabilities were lower for five of the six traits. Rankings of heritabilities by trait were not consistent among methods. For length, diameter, and L/D, marker-based estimates were 73 to 103% of the NCIII design estimate; for seed-cavity, color, and S/D, estimates ranged between 35 to 42% of the NCIII design estimate. Again, this lack of consistency may be due to incomplete marker saturation, as effects for seed-cavity, color, and S/D may have gone undetected or were under-estimated. Thus, no evidence is provided that QTL effects were over-estimated in this study. The result indicates that markers accounted for a large fraction, but not all, of the additive variance estimated by co-variance among relatives.

Since phenotypic variances were different among populations, comparison of heritabilities may be more appropriate than additive variance alone. Phenotypic variance in NCIII was greater for length and L/D, but lower for diameter, seed-cavity size, color, and S/D. Total phenotypic variances of F_3 families were higher than those calculated in the MAPMAKER/QTL because interaction and error variances are included (Hallauer and Miranda 1988; see Materials and methods). The variance components associated with F_2 parents and F_2 parents × years in the NCIII design (σ_f^2 and σ_{fy}^2) were multiplied by four to reflect that of the F_2 population. Phenotypic differences among means of BC_1 pairs and F_3 populations may be due to genetic background, maternal effects, sampling, or residual heterozygosity in parental lines.

The two heritability estimates may indicate adequacy of marker-based descriptions of variation. For length, marker-based heritability was 0.50 and mating-design heritability was 0.49. The moderately high heritability was reflected by individual QTLs, four of five were confirmed in BC lines. The agreement between these estimates suggests that the major QTLs for length have been identified, since a similar magnitude of variation was described. Using a cross with similar germplasm, five genes were estimated to condition fruit length (Owens et al. 1982). For diameter, marker-based heritability was 0.21 and the non-significnat mating-design heritability was 0.28. While similar estimates indicate that the majority of additive variation was described by markers, lower heritabilities were reflected in the lack of confirmation of QTLs in BC_1 lines. Only one of three was marginally confirmed in the multilocus model. Calculating R^2 alone for this trait (46.9%) may provide a misleading estimate of the heritability of diameter. While a high degree of genetically correlated information may be described (R^2), a large fraction of this

variation may not be heritable. For the traits seed cavity-size and S/D, a much higher heritability was calculated with the mating design (0.67 and 0.42 respectively) than with markers (0.28 and 0.15 respectively). The large discrepencies among estimates suggest undetected or under-estimated loci for these traits. Differences among MAPMAKER/QTL and ANOVA models for seed-cavity and S/D may indicate exclusion of QTLs with marginal effects. Evidence for exclusion of real QTLs for S/D comes from confirmed independent tests of loci that were below the threshold of inclusion in the multilocus model. For seed-cavity and S/D, smaller differences in magnitude among parents may have contributed to the lack of QTL detection. A large difference among heritability estimates was found for color as well (mating design 0.77 vs markers 0.30). However, this discrepancy may largely be attributable to different phenotypic variances, and is most likely related to a very high line × year variance component found predominantly in the F_3 population. Heritability estimates for L/D were somewhat similar (mating design 0.68 vs markers 0.51) suggesting that the majority of QTLs have been found. The moderately high heritabilites allowed for confirmation for three of four QTLs, predominantly the same ones describing length. Still, the greater variation described in NCIII may indicate undetected QTLs.

Differences among marker-based and NCIII mating-design variances and heritabilities may be expected because both models require assumptions. Variation described by markers may be under-estimated due to incomplete marker saturation, recombination between a marker and QTL, QTLs of minor effect not being detected, and elimination of multiple linked QTLs. Other factors that may impact differentially among marker-based and mating-design estimates, include sampling (not all F_3 lines had corresponding BC lines in NCIII and vice versa), epistasis, and maternal effects. Given these shortfalls toward an accurate comparison, marker-based and mating-design methods allow for cross-reference of genetic and phenotypic description of variation.

For all traits, no serious overestimate of heritability was described by markers. This may be due to the methods employed in model construction. We chose a MAPMAKER/QTL multilocus model construction threshold on the basis of previous reports (fixing and scanning at LOD 2). "Backwards elimination" ANOVA thresholds were chosen for similar detection rates. The strategies and thresholds used herein may not be optimized for multilocus model QTL inclusion or exclusion. However, no indication was provided that thresholds were too liberal. Higher marker-based heritabilities would suggest that the number of QTLs or effects were over-estimated. While we cannot directly test the magnitudes of effects in this study, the constructed models describe reasonable magnitudes of variation in relation to the mating-design estimates.

The integration of the mapping population into a mating design offers benefits over QTL analysis of a single mapping population. Individual QTL can be confirmed for heritability among related progeny (without further genotyping). Lack of QTL confirmation may reflect low overall heritability as determined in the mating design. The BC_1 families used to confirm QTLs can be used to estimate co-variance relationships. Genetic variances in the NCIII mating design provide a reference point for additive genetic variance and heritabilities. Thus, the mating design estimates can support, or indicate disparities of, markers as descriptors of genetic variation. Numbers and/or effects of QTLs can be over- or under-estimated in complicated multilocus models. Overall additive variance estimates can indicate whether a large extent of heritable variation has been described by QTLs. This may be important for QTL analysis of narrow-based genetic crops, such as cucumber, where saturated maps are difficult to construct. Comparisons of marker-based and mating-design variances over many traits may indicate a high or low trend for either estimate. Even with a single trait, estimates with both markers and co-variance among relatives gives a more complete picture of heritable variation than either estimate alone.

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