

Comparative analysis of marker-assisted and phenotypic selection for yield components in cucumber

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Abstract Theoretical studies suggest that marker-assisted selection (MAS) has case-specific advantages over phenotypic selection (PHE) for selection of quantitative traits. However, few studies have been conducted that empirically compare these selection methods in the context of a plant breeding program. For direct comparison of the effectiveness of MAS and PHE, four cucumber (*Cucumis sativus* L.; $2n = 2x = 14$) inbred lines were intermated and then maternal bulks were used to create four base populations for recurrent mass selection. Each of these populations then underwent three cycles of PHE (open-field evaluations), MAS (genotyping at 18 marker loci), and random mating without selection. Both MAS and PHE were practiced for

yield indirectly by selecting for four yield-component traits that are quantitatively inherited with 2–6 quantitative trait loci per trait. These traits were multiple lateral branching, gynoeious sex expression (gynoeicy), earliness, and fruit length to diameter ratio. Both MAS and PHE were useful for multi-trait improvement, but their effectiveness depended upon the traits and populations under selection. Both MAS and PHE provided improvements in all traits under selection in at least one population, except for earliness, which did not respond to MAS. The populations with maternal parents that were inferior for a trait responded favorably to both MAS and PHE, while those with maternal parents of superior trait values either did not change or decreased during selection. Generally, PHE was most effective for gynoeicy, earliness, and fruit length to diameter ratio, while MAS was most effective for multiple lateral branching and provided the only increase in yield (fruit per plant).

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Introduction

Theoretical-based simulation studies suggest that the effectiveness of marker-assisted selection (MAS) for polygenic traits can be greater than traditional trait-based selection (Lande and Thompson 1990; Zhang and Smith 1992; Edwards and Page 1994; Gimelfarb and Lande 1994a; Gimelfarb and Lande 1994b). In general, these studies agree that MAS efficiency is enhanced when markers are tightly linked (<5.0 cM) to quantitative trait loci (QTL), selection is performed in early generations prior to recombination between markers and QTL, large populations are used, and selection is practiced on traits with low heritability. However, the underlying assumptions of simulation studies may not be upheld in practice which may reduce

their applicability to empirical studies (van Berloo and Stam 2001). In practice, MAS has been effective for the introgression of simple traits or a small number of genes in several crop species including disease resistance in common bean (*Phaseolus vulgaris* L.; de Oliveira et al. 2005), disease resistance and grain protein in wheat (*Triticum aestivum* L.; Kuchel et al. 2007), and root traits in rice (*Oryza sativa* L.; Steele et al. 2006). However, MAS appears less effective for complex traits such as yield (Francia et al. 2005; Collard and Mackill 2008; Xu and Crouch 2008). Although MAS has been successfully reported in commercial breeding programs, details of these successes are limited and implementation of MAS in public breeding programs has been slow (as reviewed by Xu and Crouch 2008). Empirical comparisons of MAS to phenotypic selection (PHE) are scarce and often conflicting (Zhang et al. 2006; Moreau et al. 2004; Davies et al. 2006) suggesting the need for a direct comparison of the effectiveness of MAS and PHE for complex traits.

Yield is a complex trait that has been a focus of cucumber (*Cucumis sativus* L.; $2n = 2x = 14$) breeding for over 50 years (Lower and Edwards 1986; Wehner 1989; Wehner et al. 1989). Although the yield of US processing cucumber increased steadily from 1950 to 1980, it has reached a plateau since the early 1980s (Shetty and Wehner 2002). Selecting directly for yield is difficult which is partially due to its low narrow-sense heritability (0.07–0.25) and the dramatic influence of the environment on trait expression (as reviewed by Wehner 1989). The most effective breeding approach for yield improvement in cucumber may be selection for traits directly related to yield (Wehner 1989; Cramer and Wehner 1998; Cramer and Wehner 2000b).

Four important yield components in cucumber are earliness, gynoecious sex expression (gynoecy), fruit length to diameter ratio, and multiple lateral branching (Cramer and Wehner 2000a; Fazio et al. 2003a). Earliness, gynoecy, and multiple lateral branching have been shown to be positively correlated with the number of fruit per plant (Cramer and Wehner 2000a; Cramer and Wehner 2000b; Fazio 2001), and length to diameter ratio is an important determinant of marketable fruit yield (Serquen et al. 1997a). Each of these traits is under the control of two to six major genes with relatively large effects. The narrow-sense heritabilities (h^2) of each trait range from 0.14 to 0.48 depending on trait and environment (Serquen et al. 1997b; Fazio et al. 2003b). The negative correlations that exist between these yield components (e.g., gynoecy with multiple lateral branching and earliness with length to diameter ratio) make the simultaneous improvement of these traits a challenge.

The use of MAS in cucumber breeding has potential for increasing the efficiency and effectiveness of selection for yield components through line and population improvement. Moderately saturated linkage maps have been devel-

oped for cucumber and genomic regions have been identified that have proven useful for selection of yield components by MAS during backcross breeding (Fazio et al. 2003a; Fan et al. 2006). These studies utilized yield-associated QTL identified initially by Serquen et al. (1997b) and then by Fazio et al. (2003b) in separate mapping populations derived from a cross between lines Gy-7 (synom. G421) and H-19. Fazio et al. (2003b) confirmed the marker-QTL linkages of a single trait, multiple lateral branching, and demonstrated that selection with these markers increased the number of branches equal to phenotypic selection during two cycles of backcrossing. Fan et al. (2006) evaluated the effectiveness of MAS for multiple yield components by backcrossing after three cycles of PHE by recurrent selection. Markers utilized for MAS were linked to QTL for earliness (LOD ≥ 4.1), gynoecy (LOD ≥ 3.0), length to diameter ratio (LOD ≥ 4.2), and multiple lateral branching (LOD ≥ 3.0). Selection by PHE improved multiple lateral branching and length to diameter ratio and MAS continued improvement of these traits as well as gynoecy. These two studies indicate that MAS is effective for selecting yield components in cucumber by backcross breeding typical of breeding line development. However, the efficacy of MAS for yield components in cucumber population improvement typical of breeding programs has not yet been established.

Given the potential utility of MAS, a study was designed to increase cucumber yield by simultaneous selection of multiple yield components employing MAS and PHE, and to directly compare these methods for response to recurrent selection. In order to test their efficacy, both methods used the same selection scheme, which was designed to overcome previously documented negative correlations between yield components. Four populations were created by intermating four inbred lines, and then each population underwent three cycles of recurrent selection by PHE and MAS, as well as random mating without selection (RAN). This study will allow for the development of appropriate breeding strategies for the use of PHE and MAS in cucumber.

Materials and methods

Germplasm and population development

Four inbred lines were chosen as parents from the US Department of Agriculture (USDA) cucumber breeding program, Madison, WI, to develop four separate populations (Table 1; Fig. 1). Lines 6996A and 6995C were drawn from a recombinant inbred line (RIL) population (Gy-7 \times H-19, F₉; Staub et al. 2002). Line 6823B originated from a cross between the RIL parent H-19 and a USDA

Table 1 Mean values of yield component traits of checks and inbred lines used in this study

Inbred line/Check	Population ^a	EAR ^b	GYN ^c	L:D ^d	MLB ^e	Yield ^f	Leaf type ^g
6632E	1	2.47 ^h	99.1	2.57	2.1	2.04	Standard
6823B	2	0.76	5.7	3.51	4.5	2.11	Little
6996A	3	1.84	99.2	2.71	0.9	1.50	Standard
6995C	4	0.43	10.6	3.01	3.1	1.60	Standard
Gy-7 (check)	Line	2.13	99.8	2.74	1.0	1.70	Standard
H-19 (check)	Line	0.42	6.1	3.03	5.7	1.84	Little
‘Vlasset’ ⁱ (check)	Hybrid	1.98	77.9	2.70	3.0	2.28	Standard

^a Entries are inbred parents of populations 1–4 (described in “Materials and methods”) or an inbred line or hybrid used as a check

^b Earliness (number of fruits per plant in first harvest)

^c Gynoecy (percent female flowers in the first ten nodes)

^d Fruit length to diameter ratio (mean length to diameter ratio of 5–10 randomly selected fruit averaged over three harvests)

^e Multiple lateral branching (number of lateral branches at least three internodes long on the mainstem in the first ten nodes)

^f Yield (mean number of fruits per plant over four harvests)

^g Leaf type classified as Standard (>40 cm²) or Little leaf (30–40 cm²; Staub et al. 1992)

^h Means are from the replicated trial described in “Materials and methods”

ⁱ Commercial cultivar from Seminis Vegetable Seeds, Inc, Oxnard, CA

elite processing line whose progeny were then selected for H-19 attributes. Line 6632E is morphologically similar to the RIL parent Gy-7, but does not have either parent in its pedigree (Staub and Crubaugb 2001). These lines were specifically chosen because their complementary phenotypes (Table 1) provided the basis for selection of earliness, gynoecy, multiple lateral branching, and length to diameter ratio.

The four parental inbreds were intermated to create four distinct populations that subsequently underwent selection (Fig. 1). Crosses were made in a greenhouse in Madison, WI, in 2000 by pollinating female flowers of each inbred with bulked pollen from the other three lines. The resulting seeds were bulked by maternal parent to create four populations (i.e., Pop. 1–4; Table 1; Fig. 1) which had not undergone selection and were designated as cycle 0 (e.g., Pop. 1 C0). Each of these populations subsequently underwent PHE, MAS, and RAN for three cycles (C1–C3). All selection and mating was performed within each of the four populations, independent of the other three populations (i.e., intrapopulation improvement only). PHE was performed based on phenotype alone (i.e., without marker information), and MAS was applied without regards to phenotypic information (i.e., marker information only).

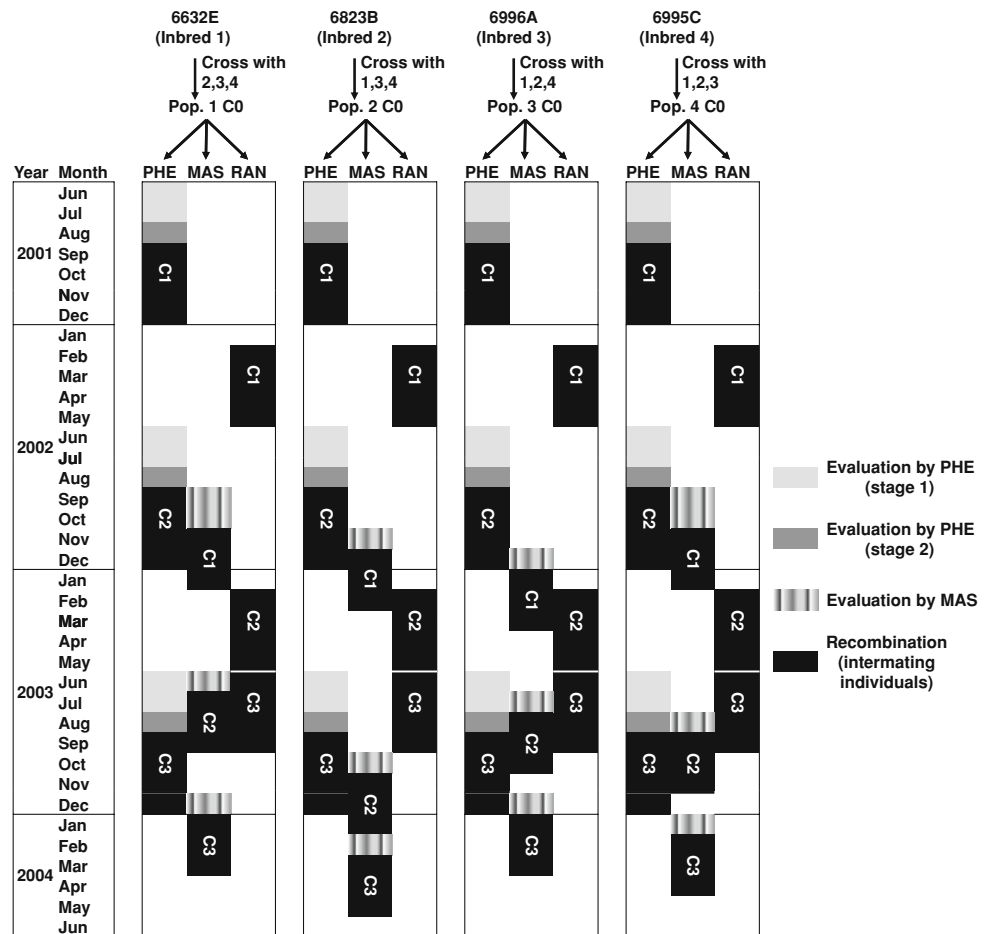
Selection scheme

Phenotypic selection for earliness, gynoecy, multiple lateral branching, length to diameter ratio, and standard-leaf type (leaf area > 40 cm²; Staub et al. 1992) was practiced under open-field conditions at the University of Wisconsin Experiment Station, Hancock, WI (UWESH) in soil classified as

Plainfield loamy sand (Typic Udipsamment; sandy, mixed, mesic). Data were taken on individual plants, where leaf type was classified as standard (*LL*) or little leaf (*ll* = 30–40 cm²; Staub et al. 1992). Earliness was assessed as the number of days from planting to anthesis of the first female flower. Sex expression was measured as the percentage of the first ten flowering nodes bearing female flowers (nodes with both male and female flowers were classified as male) where 100% was designated gynoecious, 50–90% was considered predominantly female (PF), and less than 50% was classified as monoecious. Fruit length to diameter ratio was estimated by visual inspection of at least four immature fruit (USDA grade size 3A–3B; 3.0–5.0 cm in diameter). Multiple lateral branching was recorded at or after anthesis as the number of lateral branches (at least three internodes in length) in the first ten nodes of the mainstem.

Phenotypic selection was accomplished in two stages within each cycle of selection (Fig. 1) using minimum trait thresholds for the first stage, and index selection for the second stage. For Stage 1, a total of 400 C0, 600 C1, or 600 C2 plants from each population were evaluated in 2001, 2002, and 2003, respectively. Individual plants were first evaluated for leaf type, earliness, gynoecy, and multiple lateral branching, since these are the first traits to be expressed developmentally in cucumber. Only individuals that met pre-established thresholds of standard-sized leaves, earliness as <48 days to the first female flower, gynoecy as >50% female flowers, and multiple lateral branching as >3 branches were evaluated for fruit length to diameter ratio. Those plants with a length to diameter ratio above the threshold (2.8) were designated selections at Stage 1. A subjective index was employed at Stage 2 of PHE where

Fig. 1 Timeline and schematic illustrating the procedure used to compare the effects of phenotypic selection (PHE), selection by marker (MAS), and random mating (RAN) for three cycles (C1–C3) on cucumber yield-related traits in four cucumber populations (Pop. 1–4 C0). Four cucumber inbred lines (6632E, 6823B, 6996A, and 6995C) were intermated and the seed bulked maternally to create four base populations (C0) that independently underwent PHE, MAS, and RAN. Each cycle of PHE or MAS included evaluation of the population (in two stages for PHE) and recombination (intermating) of selected individuals. For RAN, randomly identified individuals were intermated without evaluation



multiple lateral branching and earliness were weighted approximately 2 (multiple lateral branching) and 1.5 (earliness) times that of gynoecy and length to diameter ratio, which were weighted equally. These weights were based on their relative importance to early, uniform yield. The relative weights among the traits are illustrated by the selection differentials (difference in trait means of the selections from Stage 2 and the selections from Stage 1) for each trait in each population (Table 2). Individual plants were ranked by their values of multiple lateral branching, then earliness, and the values of gynoecy and length to diameter ratio were used to make Stage 2 selections among the highest ranked individuals. Twenty plants were selected from Stage 2 in each cycle (C1–C3) of PHE within each population, representing a standardized selection intensity (i) of 2.063, 2.219, and 2.219 for C1, C2, and C3, respectively.

The efficient recombination of Stage 2 selections required intermating in the greenhouse. The short growing season of Wisconsin did not allow enough time in the field for chemical induction of male flowers and intermating to produce mature seed from Stage 2 selections with mostly female flowers. Thus, at least two meristems of each Stage 2 selection were taken to a greenhouse in Madison, WI.

Once these cuttings were rooted, they were transplanted and were allowed to establish for at least 1 week. The apical meristems and surrounding leaves were then treated with two applications (7 days apart) of 3 mM silver thiosulfate $[\text{Ag}(\text{S}_2\text{O}_3)_2]^{3-}$ as a foliar spray to induce male flower production (Nijs and Visser 1980). Selections were then randomly mated by pollination of each female flower with five random male flowers.

To perform MAS, a total of 18 markers linked to *F* (femaleness), *de* (determinate), *ll*, and previously identified QTL (Serquen et al. 1997b; Fazio et al. 2003b) for earliness, gynoecy, multiple lateral branching, and length to diameter ratio were selected (Table 3; Fig. 2). All markers employed were drawn from Fazio et al. (2003b), except AJ6SCAR, and M8SCAR which were SCARs converted from previously mapped RAPDs (Nam et al. 2005; Robbins 2006). The strategy used to select markers for use in MAS is outlined in Robbins et al. (2002) and Robbins and Staub (2004). Briefly, many factors were taken into consideration when selecting markers such as marker type, marker inheritance (i.e., dominant or codominant), genetic distance from QTL, and number of QTL in proximity to the marker. The QTL identified for selection in this study had a relatively

Table 2 Cumulative selection differential over three cycles between Stage 1 and Stage 2 of phenotypic selection (PHE) for four traits in four populations of cucumber

Trait	Population	Selection differential ^a	<i>P</i> value ^b	Percent ^c	Result ^d
EAR ^e	1	-3.79	0.019	3.38	Gain
	2	-2.19	0.080	2.00	No difference
	3	-2.68	0.008	2.45	Gain
	4	-3.53	0.011	3.16	Gain
GYN	1	7.91	0.205	3.98	No difference
	2	38.37	0.021	18.40	Gain
	3	-20.44	0.222	10.24	No difference
	4	-25.03	0.187	13.48	No difference
MLB	1	2.94	<0.001	19.68	Gain
	2	2.40	<0.001	16.22	Gain
	3	2.51	<0.001	17.96	Gain
	4	3.20	<0.001	22.90	Gain
L:D	1	0.04	0.169	0.70	No difference
	2	0.08	0.078	0.86	No difference
	3	0.04	0.078	0.71	No difference
	4	0.08	0.027	1.00	Gain

Traits are *EAR* (number of days to anthesis of the first female flower), *GYN* gynocoey [percentage of plants classified as gynocoeious (100% female flowers in the first 10 nodes)], *MLB* multiple lateral branching (number of lateral branches at least three internodes long on the main-stem in the first 10 nodes), and *L:D* fruit length to diameter ratio (mean length to diameter ratio of 5–10 randomly selected fruit averaged over three harvests)

^a Sum over three cycles of the difference between the mean of Stage 2 selections and the mean of the selections from Stage 1 at each cycle. The harmonic mean number of individuals selected at Stage 1 over three cycles was 125.2, 128.9, 114.2, and 130.1 for Populations 1, 2, 3, and 4, respectively

^b *P* value of a *t* test for the significance of the selection differential

^c Selection differential expressed as the percent of the mean of Stage 1 selections

^d The effectiveness of two stages of selection within each cycle based on the direction of each selection differential and its *P* value. Gain = a difference between the two stages in the desired direction where $P \leq 0.05$, No difference = no significant difference ($P > 0.05$) between the means of the two stages

^e Because fewer days to anthesis is desirable for *EAR*, a negative selection differential indicates a difference in the desired direction (Stage 2 selections flower earlier). A positive selection differential is desired for all other traits

large effect (cumulative $R^2 > 37$ –85%), high LOD scores for marker linkages (>3.0 ; Table 3; Fig. 2), and were consistent over several environments (Serquen et al. 1997b; Fazio et al. 2003b). The majority of marker-QTL associations in this study were <5.0 cM (Fazio et al. 2003b). In most cases, markers flanking the QTL of interest (Edwards and Page 1994) were employed, especially in regions where marker-QTL associations were >5 cM (e.g., AK5SCAR

and M8SCAR for multiple lateral branching; Table 3; Fig. 2). Codominant markers tightly linked to QTL were given preference. Where available, SCAR, SNP, and SSR markers were chosen over RAPD and AFLP markers because of their inherent robustness, ease of use, and ability to be multiplexed (Polashock and Vorsa 2002; Tang et al. 2003; Mohring et al. 2004; Staub et al. 2004). Once the markers were chosen, the desired allele at each marker locus was identified. The desired allele was the Gy-7 allele, the H-19 allele or both Gy-7 and H-19 alleles (heterozygous) since all four parental lines carried only Gy-7 or H-19 alleles at each marker locus. The combination of desired alleles over all marker loci was identified as the ideal genotype, or ideotype (i.e., allele selected column of Table 3).

The selected markers were used to genotype individuals to make selections for each cycle of MAS. Marker genotyping, including DNA extraction, polymerase chain reaction (PCR) amplification, and agarose gel electrophoresis, was conducted according to Fazio et al. (2003b). To increase marker efficiency, the markers were multiplexed in empirically determined groups (Table 3) according to Staub et al. (2004) and Robbins (2006). All individuals within a population were genotyped at each marker locus. Those plants whose genotype matched the ideotype at the greatest number of marker loci were selected and intermated to produce the next generation. For each cycle of MAS within each of the four populations, the number of individuals tested, the selection intensity, and crossing scheme were identical to that of PHE.

Random mating was accomplished by first sowing 20 random seeds from each of the four C0 populations. Then gynocoeious plants were chemically induced to produce male flowers and all plants were intermated using the same scheme as that for MAS and PHE to create C1. The resulting seeds were equally bulked, and 20 random C1 plants were intermated in the same manner to create C2 seed and the same procedure was used to create the C3 population.

Open-field evaluation of selection

Response to selection was evaluated in an open-field trial at UWESH in the summer of 2004 with all entries replicated in two planting dates. Seeds were sown in a greenhouse in Madison, WI on June 4, 2004 and June 16, 2004, then transplanted on June 23, 2004 and July 7, 2004, respectively. Each planting date was arranged in a split-plot design with eight replications of each population (whole plot factor) in randomized complete blocks, with a combination of cycle (i.e., C0–C3) and method of selection (i.e., MAS, PHE, and RAN) completely randomized as subplots with ten plants per subplot. Plots were arranged in single rows with 18 cm between plants and 1.5 m between rows ($\sim 37,000$ plants/ha). This plant density was chosen because

Table 3 Characteristics of molecular markers defined in a genetic map of cucumber constructed by Fazio et al. (2003b) and used in marker-assisted selection for population improvement

Marker	Type	Linkage group	Map position (cM)	Parent ^a	Multiplex group ^b	Allele selected ^c	QTL (mapping parent, LOD score) and <i>gene</i> associations ^d
CSWCT28	SSR	1	5.0	G and H		G and H	EAR(G, 7.1), MLB(H, 10.4), GYN(G, 13.0), L:D(H, 5.7), <i>F</i>
L18-SNP-H19	SNP	1	7.4	H	1	H	EAR(G, 7.1), MLB(H, 10.4), GYN(G, 13.0), L:D(H, 5.7)
OP-AG1-1	RAPD	1	31.8	G		H	EAR(G, 6.4), MLB(H, 11.6), GYN(G, 7.3), <i>de</i>
AJ6SCAR	SCAR	1	61.4	G	3	H	MLB(H, 3.3)
BC523SCAR	SCAR	1	66.5	G	2	H	MLB(H, 3.3)
OP-AD12-1	RAPD	1	70.2	H		G	EAR(G, 4.1), MLB(H, 32.9), GYN(G, 3.7), L:D(G, 8.6), <i>ll</i>
AW14SCAR	SCAR	3	3.9	G and H	1	G	GYN(G, 5.1)
CSWTAAA01	SSR	4	34.1	G and H	2	H	MLB(H, 4.6)
OP-AI4	RAPD	5	101.0	G		G	GYN(G, 3.0)
OP-AO12	RAPD	5	117.3	G		G	GYN(G, 3.0)
OP-AI10	RAPD	6	22.5	H		G	L:D(G, 7.3)
AK5SCAR	SCAR	6	33.0	G	2	H	MLB(H, 3.0)
M8SCAR	SCAR	6	39.1	H		H	MLB(H, 3.0)
OP-W7-1	RAPD	6	83.4	H		G	GYN(G, 4.1)
L19-2-SCAR	SCAR	6	115.0	H	1	G	MLB(G, 4.2), GYN(G, 4.1)
NR60	SSR	6	137.4	G and H		G	MLB(G, 4.2)
BC515	RAPD	7	0.0	H		H	L:D(H, 4.2)
L19-1-SCAR	SCAR	7	9.9	H	3	H	L:D(H, 4.2)

SSR simple sequence repeat, SNP single nucleotide polymorphism, RAPD random amplified polymorphic DNA, and SCAR sequence characterized amplified region

^a Allelic constitution of each marker based on mapping parents H-19 and Gy-7 (synom. G421) (Fazio et al. 2003b), where G = present in Gy-7, H = present in H-19, G and H = present in Gy-7 and H-19 (codominant marker)

^b Markers used in multiplex were placed in multiplexing groups (1, 2, or 3)

^c The marker allele that was selected, which determined which QTL were under selection. G = QTL from Gy-7 were selected, H = QTL from H-19 were selected, G and H = both Gy-7 and H-19 alleles were selected

^d Markers associated with QTL for EAR = earliness, MLB = multiple lateral branching, GYN = gynoecious, and L:D = length to diameter ratio. The parentheses contain the parent contributing the QTL (G = Gy-7, H = H-19) followed by the highest LOD score for each QTL obtained from multiple field trials (Serquen et al. 1997b; Fazio et al. 2003b). Genes are *F* = femaleness, *de* = determinate, and *ll* = little leaf

it optimized potential yield in highly branched genotypes in multiple harvest operations in Wisconsin (Fredrick and Staub 1989; Staub et al. 1992). The four inbred lines that served as parents, as well as Gy-7, H-19, and the commercial cultivar ‘Vlasset’ (Semini Vegetable Seeds, Inc, Oxnard, Calif.) were included as controls for comparison.

All traits under selection were evaluated as well as yield, which was measured as the number of fruit per plant. Yield was recorded for each of four harvests at 59, 66, 76, and 96 (first planting date) and 54, 64, 75, and 91 (second planting date) days after planting to calculate four-harvest means. Each of the four harvests occurred when two to three oversized fruit (>51 mm in diameter) were observed within a plot (Wehner et al. 1989). All immature fruits >20 mm in diameter and >10 cm in length were included in total fruit number. Both multiple lateral branching and gynoecy were evaluated on each plant exactly as during PHE. Mean fruit length to diameter ratio was obtained per plot by measuring the length and diameter of 5–10 randomly selected fruits

(USDA 2B–3A grade; 2.5–3.0 cm in diameter), and then averaging over three harvests. Earliness was defined as the average number of fruits per plant in the first harvest.

Statistical analysis

All response variables were initially analyzed by analysis of variance (ANOVA) using PROC GLM of SAS (2003) to determine treatment effects. Treatments of planting date, populations, cycles, and methods were considered fixed effects, while blocks were considered random. Specific single-degree of freedom contrasts within analyses of variance were employed to determine general response to selection for biologically important comparisons (e.g., PHE and MAS). Selection responses (linear and quadratic effects) were computed by regression of trait means on selection cycles within each population for each selection method by employing single-degree of freedom contrasts within ANOVA (Steele et al. 1996). To determine the relationship

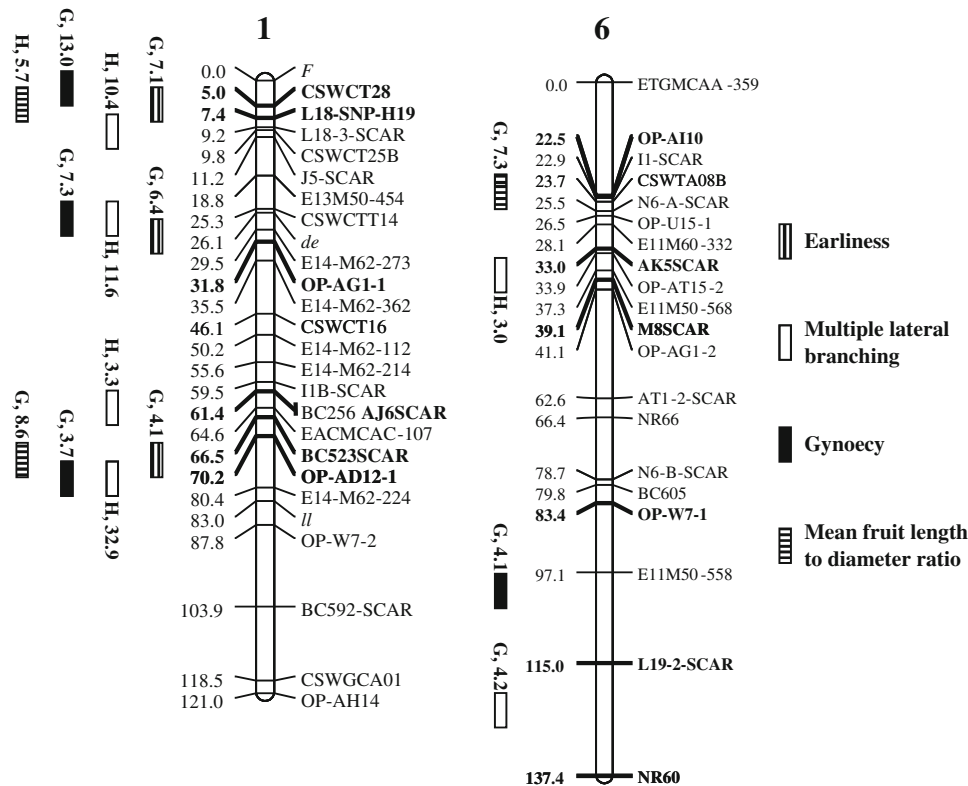


Fig. 2 Approximate map positions of QTL for yield components of cucumber (*Cucumis sativus*) on linkage groups 1 and 6, the only linkage groups that contain more than one QTL considered for selection. Linkage groups are from Fazio et al. (2003b) and consist of RAPD, SCAR, AFLP, and morphological markers (italicized). RAPDs are identified by the preceding letters OP and BC according to Serquen et al. (1997a), SSR by the preceding letters CS, CM and NR, AFLP by E_M_, and SCARs by the designation SCAR according to Fazio

(2001). Vertical bars to the left of each linkage group represent the QTL regions considered for selection. Letters near the QTL regions (G = Gy-7, H = H-19) indicate the parent that provides the desired allele and numbers indicate the highest LOD score for each QTL obtained from multiple field trials (Serquen et al. 1997b; Fazio et al. 2003b). Markers utilized in marker-assisted selection are highlighted in **bold**

between the traits under selection, phenotypic correlations among traits were calculated by Pearson correlation using PROC CORR of SAS (2003).

Results

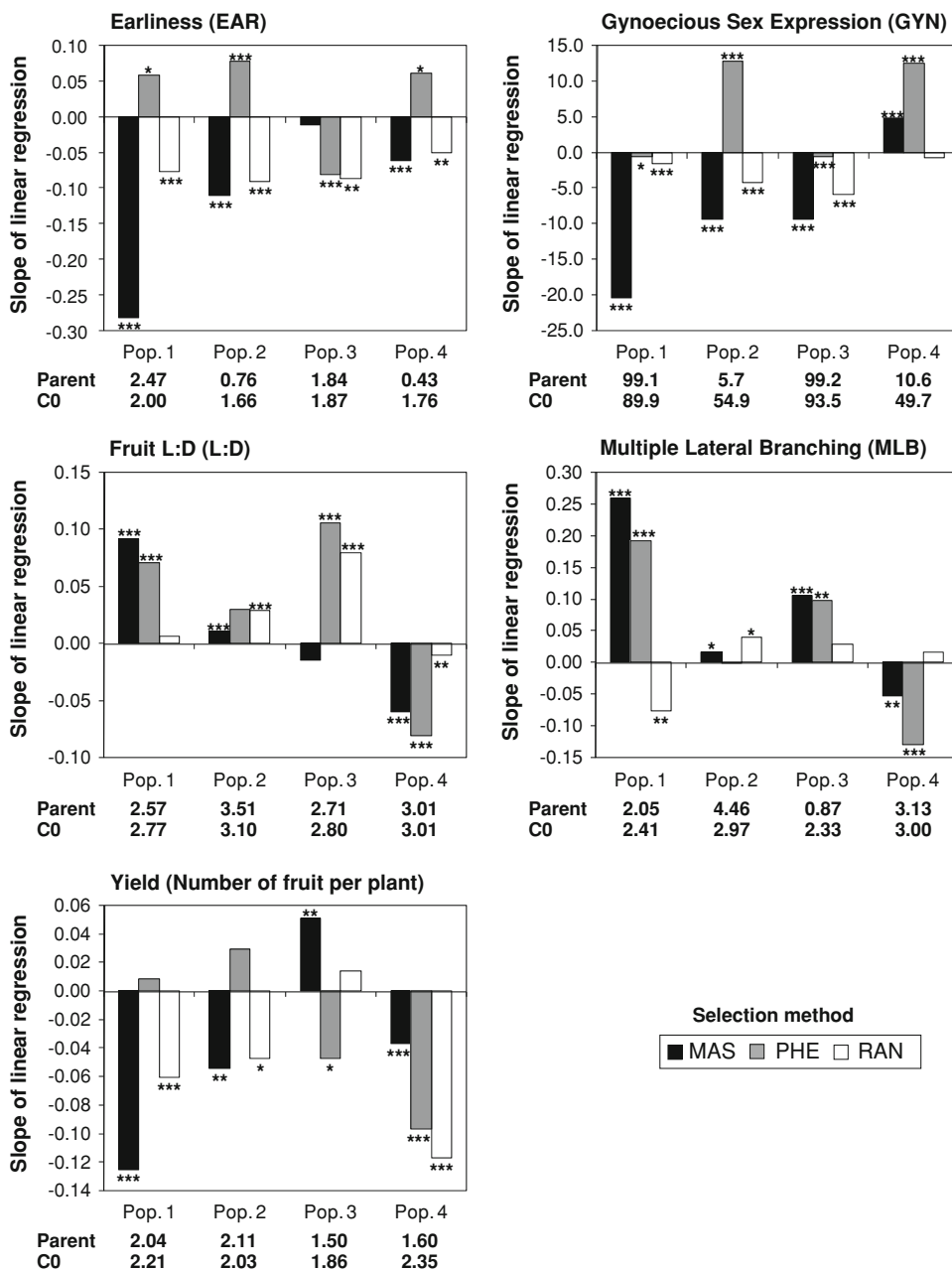
We conducted a replicated trial to determine the effect of three selection methods (MAS, PHE, and RAN) for four quantitative traits (earliness, gynoecy, fruit length to diameter ratio, and multiple lateral branching) over three selection cycles in four cucumber populations. The ANOVA of data obtained from the trial indicated that all main effects (planting date, populations, and combinations of cycles and selection methods) were highly significant ($P < 0.001$) for all traits. In general, planting date affected the magnitude of the mean value of a trait and not the entry ranking in response to selection over cycles. The means of all traits were higher for all populations in the first planting than the second, except for multiple lateral branching, which was lower. Although the planting date by population interaction

was significant for length to diameter ratio ($P = 0.01$) and earliness ($P = 0.001$), general trends over cycles were the same for both plantings for all traits. Selection was performed in each of the four populations independent of each other, and response to selection varied by population. Therefore, results are presented by population with both plantings combined (Fig. 3; Supplementary Table). Results of selection for yield components, indirect selection for yield, correlated response to selection, and temporal efficiency of selection are presented with regards to responses in each of the four populations and to evaluate the three different types of selection using population performances for comparative analyses.

Selection of yield components

Population response varied with selection method and the trait being evaluated (Fig. 3). The effectiveness of MAS was determined by comparing the regression slope of MAS to that of RAN (Fig. 3). The effectiveness of PHE was determined by a similar comparison to RAN. Comparisons

Fig. 3 Response to selection as measured by the slope of linear regression (*y-axes*) of trait values over three cycles of MAS (selection by marker), PHE (phenotypic selection) or RAN (random mating without selection) for five traits in four cucumber populations. For all traits, positive slopes indicate trait values increased, while negative slopes indicate a decrease. The symbols *, **, and *** denote slopes are significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively. To contrast the change in trait values over cycles of selection with initial trait values, the mean of the C0 population (population value before selection) and the mean of the maternal parent (6632E, 6823B, 6996A, and 6995C for Pop. 1–4, respectively) is provided under the *x-axis* label for each population. The units for each trait are fruit per plant in the first harvest (EAR), percent female flowers in the first ten nodes (GYN), mean fruit length to diameter ratio over three harvests (L:D), the number of lateral branches on the first ten nodes of the mainstem (MLB), and mean fruit per plant over four harvests (Yield)



were made separately within each Population for each trait (Fig. 3). In Population 1, PHE was more effective than MAS. The values of two traits increased after MAS (multiple lateral branching and length to diameter ratio) while PHE increased the values of three traits (multiple lateral branching, length to diameter ratio, and earliness). The most effective selection method for Population 2 was PHE. Two trait values increased after PHE (earliness and gynoecy), while none were effectively increased by MAS. The increase in length to diameter ratio and multiple lateral branching after MAS cannot be attributed to selection since a similar increase was observed after RAN. Selection from PHE was least effective in Population 3. Only one trait

value effectively increased after PHE (multiple lateral branching) compared to RAN, but trait values of earliness and gynoecy decreased. After MAS, multiple lateral branching values increased and gynoecy decreased similar to PHE, but earliness did not change. These results indicate that MAS was slightly more effective than PHE in Population 3. In Population 4, PHE increased values for earliness while MAS and PHE provided similar results for all other yield component traits. Thus, PHE was more effective than MAS in this population.

In some cases, population response to selection was dependent on the phenotypic difference between parental lines. The four inbred lines (Table 1; Fig. 1) used as parents

in this study were specifically chosen because high values for some of the traits under selection complimented low values found in other lines (e.g., 6632E is high for gynoecy and earliness, but low for multiple lateral branching and length to diameter ratio; Table 1; Fig. 3). This disparity among trait values was predictably minimized in the C0 populations (Fig. 3; Supplementary Table). In general, however, populations responded favorably to selection for traits that were inferior in maternal parents, while traits with superior values in the maternal parents either did not change or decreased after selection. For example, in Population 4 where the inbred parent (6995C) was inferior for earliness and gynoecy but superior for length to diameter ratio and multiple lateral branching, both earliness and gynoecy increased after PHE while length to diameter ratio and multiple lateral branching decreased (Fig. 3; Supplementary Table). Populations responded better overall to PHE than MAS at increasing inferior trait values. In contrast, trait values generally decreased or remained unchanged after RAN, regardless of maternal parent values. The three exceptions were an increase in trait values after RAN for length to diameter ratio in Population 2, multiple lateral branching in Population 2, and length to diameter ratio in Population 3.

Indirect selection for yield

Selection for yield components by either MAS or PHE did not increase yield (number of fruit per plant) in the majority of the populations (Fig. 3; Supplementary Table). Indirect selection by PHE was most effective at maintaining yield (Populations 1 and 2), while the only increase in yield came from MAS (Population 3).

Correlated response to selection

Phenotypic trait correlations are important since they can have a dramatic effect on cucumber fruit yield (i.e., source/sink relationships) depending on plant architecture, and the type and intensity of selection. Strong positive and negative phenotypic correlations (r) between yield components were identified after both MAS and PHE and are presented by population in Table 4. Consistent, positive correlations were detected for earliness with gynoecy and yield ($r = 0.25–0.70$), but earliness was always negatively correlated with multiple lateral branching ($r = -0.26$ to -0.54). Earliness and length to diameter ratio were usually not correlated. Negative correlations were generally detected for gynoecy with length to diameter ratio and multiple lateral branching ($r = -0.07$ to -0.64), but gynoecy was typically not correlated with yield. Generally, length to diameter ratio was positively correlated with multiple lateral branching ($r = 0.06–0.38$). Consistent, positive correlations were identified between length to diameter ratio and yield only in

Populations 2 and 4 ($r = 0.28–0.34$). Yield and multiple lateral branching were normally not correlated.

Temporal efficiency of selection

There can be dramatic differences in the cost of selection (i.e., labor and time) given the life cycle time of cucumber. For instance, selection by MAS required less time to complete than PHE (Fig. 1). Evaluation by PHE under Wisconsin conditions required open-field evaluations of mature plants during the growing season. All four populations were evaluated by PHE simultaneously for each cycle and required 3 months (June–August) from seeding until all data were collected. Recombination required 1 month to establish roots and transplant cuttings and 3 months to induce male flowers and intermate selections to obtain mature seed. Since a field season was necessary for each cycle of PHE, 31 months (June 2001–December 2003) were required to complete three selection cycles. Evaluation by MAS generally required 1 month from seeding to collect all genotypic information. Populations were too large to genotype simultaneously, so they were offset such that genotyping usually occurred in one population while other populations were intermated. Selections from MAS required 3 months for transplanting, induction of male flowers, and intermating to obtain mature seed. In contrast to PHE, all three cycles of MAS were performed for all four populations in a total of 19 months (September 2002–March 2003 and June 2003–May 2004).

Discussion

Empirical studies comparing MAS and PHE for increasing gain from selection in various plant species have provided mixed results. In some cases, MAS was more effective and/or efficient than PHE (e.g., Yousef and Juvik 2001; Yu et al. 2000; Fazio et al. 2003a; Zhang et al. 2006). In other studies, the two methods were considered equal (e.g., Stromberg et al. 1994; Romagosa et al. 1999; Van Berloo and Stam 1999; Willcox et al. 2002; Moreau et al. 2004). In additional studies, MAS was not as effective and/or efficient as PHE (e.g., Hoeck et al. 2003; Davies et al. 2006). In other comparisons, the effectiveness of MAS and PHE varied within the same study (e.g., Eathington et al. 1997; Schneider et al. 1997; Flint-Garcia et al. 2003). Most of these studies, however, did not evaluate selection methods for their efficacy in the improvement of multiple, quantitatively inherited traits over multiple cycles of recurrent selection. We present data herein that provide a comprehensive, comparative evaluation of MAS and PHE for quantitative traits in a vegetable crop species using a selection scheme that is representative of a breeding program.

Table 4 Phenotypic correlations (r) among traits in cucumber over three cycles of selection by markers (MAS) and phenotype (PHE)

Trait	EAR	GYN	L:D	MLB
Selection by markers (MAS)				
Population 1 ^a				
GYN	0.70***			
L:D	-0.26*	-0.57***		
MLB	-0.53***	-0.53***	0.13	
Yield	0.56***	0.47***	-0.18	-0.04
Population 2				
GYN	0.37**			
L:D	0.21	-0.44***		
MLB	-0.54***	-0.43***	0.14	
Yield	0.54***	0.17	0.34**	-0.07
Population 3				
GYN	0.30*			
L:D	0.30*	-0.26*		
MLB	-0.38**	-0.37**	0.06	
Yield	0.54***	0.12	-0.01	-0.17
Population 4				
GYN	0.37**			
L:D	0.03	-0.53***		
MLB	-0.46***	-0.41***	0.32**	
Yield	0.39**	0.08	0.32**	0.27*
Selection by phenotype (PHE)				
Population 1				
GYN	0.35**			
L:D	0.19	-0.33**		
MLB	-0.29*	-0.31*	0.36**	
Yield	0.25*	0.04	0.17	-0.02
Population 2				
GYN	0.30*			
L:D	0.01	-0.20		
MLB	-0.35**	-0.32**	0.38**	
Yield	0.56***	-0.01	0.28*	0.12
Population 3				
GYN	0.45***			
L:D	-0.11	-0.07		
MLB	-0.26*	-0.14	0.31*	
Yield	0.55***	0.16	-0.03	-0.08
Population 4				
GYN	0.42***			
L:D	0.07	-0.64***		
MLB	-0.43***	-0.42***	0.23	
Yield	0.49***	-0.12	0.32**	0.13

EAR earliness (fruits per plant in first harvest), *GYN* gynoecy (percent female flowers in the first ten nodes), *L:D* fruit length to diameter ratio (mean length to diameter ratio of 5–10 randomly selected fruit averaged over three harvests), *MLB* multiple lateral branching (number of lateral branches at least three internodes long on the mainstem in the first 10 nodes), and *Yield* (mean number of fruits per plant over four harvests)

* $P \leq 0.05$, ** $P \leq 0.01$,

*** $P \leq 0.001$

^a Populations were created by intermating four inbred lines, and then bulking by the maternal parent (Fig. 1)

Considerations for MAS

When selecting for multiple, quantitative traits, the determination of which marker-QTL associations to use in selection may affect the outcome of MAS. In several instances, QTL were so tightly clustered that multiple QTL for differ-

ent traits were located between adjacent marker loci (e.g., QTL for all traits were linked to CSWCT28 and L18-SNP-H19 as well as OP-AD12-1; Table 3; Fig. 2). As the desired QTL allele came from different parental lines for separate traits (e.g., earliness and gynoecy from Gy-7; multiple lateral branching and length to diameter ratio from H-19 at

CSWCT28), strategic decisions were made based on QTL effects and neighboring genes to determine the most appropriate parental type for each marker locus. For CSWCT28, a codominant marker, heterozygotes were selected in an effort to carry QTL alleles from both parents in this region. In another example, the Gy-7 allele was selected at OP-AD12-1, the marker linked to the little leaf gene (*ll*) from H-19, in order to avoid the deleterious effects of the little leaf type on gynoecy and earliness (Fazio et al. 2003b). Little leaf types, however, typically have more branches than standard leaf types, and the QTL (from H-19) with the greatest effect on multiple lateral branching (LOD = 32.9, $R^2 = 32\%$) is tightly linked (0.7 cM) to *ll* (Fazio et al. 2003b). Selection of the H-19 allele at OP-AD12-1, therefore, may have resulted in greater gains in multiple lateral branching from MAS, but may, in turn, have negatively affected earliness, gynoecy, and length to diameter ratio, which are associated with the Gy-7 allele.

Another consideration for MAS is marker type. The majority of the RAPD markers used in this study were repeated to provide certainty during genotyping. In contrast, all but one (M8SCAR) of the SNP and SCAR markers could be multiplexed (Table 3), allowing for increased genotyping efficiency. The low repeatability of RAPDs and the advantage of multiplexing for high-throughput genotyping demonstrate the need for SNP, SCAR, and SSR markers for more efficient MAS in cucumber.

Effectiveness of selection for yield components

Each of the four base populations underwent RAN to provide four estimates of genetic drift. Since crosses from RAN followed the same mating scheme as MAS and PHE, RAN serves as a reference to determine the effectiveness of selection by MAS or PHE. When considering all five traits in each of the four populations, 15 of the 20 slopes were significant after RAN, but in only 3 instances did trait values increase (Fig. 3; Supplementary Table). These changes in trait values are most likely due to genetic drift or physiological factors such as source–sink relationships. In the three instances where trait values increased, the similar increase from MAS or PHE cannot be attributed to selection. Although trait values were generally not static in the absence of selection, their general reduction indicates that increases after MAS or PHE can be attributed to a response from selection.

Both MAS and PHE provided improvements in all traits under selection in at least one population, except earliness by MAS (Fig. 3; Supplementary Table). Generally, PHE was most effective for gynoecy, earliness, and length to diameter ratio, while MAS was slightly more effective for multiple lateral branching. Both PHE and MAS were generally effective at improving populations with inferior traits,

but not as effective at maintaining traits with high values. Based on trait value changes in response to selection, PHE was more effective than MAS in Populations 1, 2, and 4, but MAS was slightly more effective than PHE in Population 3. Thus, the choice of selection methods for cucumber improvement through plant architectural manipulation (i.e., yield components) will depend upon the populations and traits under selection.

Our results are complementary to those of Fazio et al. (2003b) and Fan et al. (2006), which explore different aspects of incorporating MAS into cucumber breeding programs. Fazio et al. (2003a) compared MAS and PHE for a single trait during backcrossing typically utilized in developing superior cucumber breeding lines. Both selection methods equally improved multiple lateral branching, but MAS was more efficient. We observed a similar response in multiple lateral branching from MAS and PHE in four populations even though we were selecting for multiple traits in a recurrent selection scheme focused on population development. Fan et al. (2006) tested whether MAS could improve multiple yield component traits during backcrossing after two cycles of PHE. The base population and the three cycles of PHE by recurrent selection reported by Fan et al. (2006) are the same as Population 1 described herein. Specific selections from PHE cycle 2 of Population 1 were utilized by Fan et al. (2006) to test the effectiveness of MAS for two backcrossing cycles after PHE. The structure and focus of the Fan et al. (2006) study (tandem selection of PHE in one population by recurrent selection then MAS during backcrossing for breeding line development) is distinct from that presented herein (MAS and PHE in parallel for direct comparison in four populations to mitigate trait correlations using recurrent selection for population improvement). Both studies, nevertheless, indicate the potential of MAS. After two cycles of PHE improved multiple lateral branching and length to diameter ratio in Population 1, Fan et al. (2006) demonstrated that subsequent use of MAS continued to improve these two traits during backcrossing. Although gynoecy was not improved by PHE in Population 1, Fan et al. (2006) showed that MAS can increase femaleness during backcrossing. In this study, MAS increased gynoecy, length to diameter ratio, and multiple lateral branching in at least one population. These combined results confirm the potential value of the marker-QTL associations for selection of these three traits using several breeding strategies. However, the effectiveness of MAS is population-dependent, especially during recurrent selection.

Effectiveness of indirect selection for yield

Yield was not under direct selection in this study, but was evaluated to test the efficacy of indirect improvement by

selection for yield components. Indirect selection by MAS or PHE was generally not effective at increasing yield. Nevertheless, the hypothesis that yield increases with the improvement of all four yield components cannot be rejected, since in no instance did improvement of all four traits occur. The challenge to improve yield in cucumber will likely be the simultaneous improvement of yield components using both MAS and PHE.

The simultaneous increase in all four traits under selection in this study is predictably difficult given the negative correlations among some yield component traits. The strength and direction of these correlations have been documented in a wide range of genetic backgrounds (Kupper and Staub 1988; Serquen et al. 1997a; Cramer and Wehner 1998; Cramer and Wehner 1999; Cramer and Wehner 2000b; Fazio et al. 2003b). To mitigate negative correlations among yield components, we intermated four parental inbred lines and employed recurrent selection in four different populations. This strategy was generally ineffective, however, because gynoecy and earliness were positively correlated as were multiple lateral branching and length to diameter ratio in all four populations (Table 4). The correlations among these yield components are most likely due to a combination of pleiotropy with the *F*, *de*, and *ll* genes (Fazio et al. 2003b), and linkage among individual QTL (Robbins and Staub 2004). Fine mapping in regions with clustered QTL would assist in determining the extent of linkage between QTL and identifying molecular markers that could be useful for selecting recombinants between tightly linked QTL (Nam et al. 2005).

We observed a heterotic yield effect in the cucumber populations examined. Yield was higher in every C0 population than the maternal parent that produced it, except in Population 2, which was derived from the highest yielding parent, 6823B (Table 1; Fig. 3). Cucumber is considered a cross-pollinated crop, and although it exhibits little inbreeding depression, heterosis for yield has been observed in a number of cases (Wehner 1989). Using the mean of the four parents (1.81) as the mid-parent value, the mid-parent percent heterosis for yield is 22, 12, 2.6, and 27% for Populations 1–4, respectively. These values are similar to those reported for fruit number in previous studies (Wehner 1989). Given this heterotic yield effect, and the difficulty of simultaneously increasing several yield components, inbred lines with high values for specific yield component combinations could be developed in parallel, and then crossed to create high yielding hybrids. Our results indicate that, while it is difficult to improve all four yield component traits simultaneously, both MAS and PHE can be utilized to improve specific trait combinations such as length to diameter ratio with multiple lateral branching or gynoecy with earliness. This approach would involve extensive combining ability or test cross evaluation of

inbred lines in multiple environments, and would likely be population specific.

Efficiency of selection methods in breeding programs

For MAS to be employed in plant improvement programs, it must provide resource (cost/benefit), technical (improved effectiveness), or temporal (efficiency) advantages over PHE. In this study, the cumulative time required to complete three cycles of MAS in all four populations was 19 months as compared to 31 months for PHE (Fig. 1). The increased efficiency of MAS may, in some cases, be an advantage over PHE. For example, the improvement of gynoecy per year in Population 4 was similar between MAS (4.9% per cycle \times 3 cycles per year = 14.7% per year) and PHE (12.6% per cycle \times 1 cycle per year = 12.6% per year). The efficiency of MAS could be further improved by the use of codominant, single-copy markers that can be multiplexed, such as SCARs, SNPs, and SSRs in combination with high-throughput technologies such as robotics, gel-less assays, microarrays, and pyrosequencing (Gupta et al. 2001; Collard et al. 2005). The resources and methods available at the inception of this project have changed dramatically. A large amount of genomic resources will soon be available for cucumber (Huang et al. 2008) that could greatly increase the efficiency of MAS in marker-assisted recurrent selection (MARS) or genome-wide selection approaches (Bernardo and Charcosset 2006; Bernardo and Yu 2007). However, substantial investments required for high-throughput technologies are currently cost limiting for minor crops such as cucumber. The efficiency of MAS will most likely increase as these genomic tools become more available and affordable.

Recurrent selection is the method of choice for traits with low heritability and has been used extensively for yield improvement in cucumber (Lower and Edwards 1986; Wehner 1989; Cramer and Wehner 1998). Two important considerations for recurrent selection are selection intensity and genetic drift. Selection intensity must be stringent enough to increase desired allele frequencies (make gain from selection), but modest enough to allow diversity to continue improvement in subsequent cycles of selection (Casler 1999; Bernardo 2002). Our results from RAN indicated that selecting 20 out of 600 individuals to obtain high selection intensities resulted in genetic drift for some traits (Fig. 3; Supplementary Table). The evaluation of 600 individuals in each population was the maximum allowable for each method with the resources available in this study. However, evaluating 600 individuals by MAS and 600 by PHE in the same cycle and intermating 40 selections is possible. Using this approach, high selection intensities are maintained while intermating more individuals may overcome

genetic drift. In addition, evaluating a greater number of individuals may allow for recombination among tightly linked QTL to overcome negative correlations among traits due to linkage. Thus, selection for improved yield in cucumber may be most effective by combining both MAS and PHE, a conclusion that is supported by previous studies comparing MAS and PHE (Eathington et al. 1997; Bohn et al. 2001; Davies et al. 2006).

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