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Analysis of Recombination Frequencies in Male and Female Gametes in *Cucumis melo* L.

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Summary

Sex, as a factor affecting recombination during meiosis, was investigated in *Cucumis melo* L. A pair of backcrosses, using an F_1 male parent (BCM) and an F_1 female parent (BCF) was generated. The two populations were mapped at 34 loci. Total map lengths in the respective populations were nearly indistinguishable, whereas 4 marker intervals, parts of the linkage groups in the map, were significantly different between BCM and BCF.

Key Words: *Cucumis melo* L., Linkage map, Recombination frequency, Sex.

Introduction

Sexual propagation of organisms requires a zygote derived from a female embryo sac and a sperm nucleus. Meiosis occurs in these gametes before they form a zygote. Crossing over between pairing chromosomes of each gamete occurs in the prophase of the first division in meioses; these cause gene recombination. Several factors, such as temperature (Caroline et al., 1966; Jens, 1981), chemicals (Sharma et al., 1983; Wuu and Grant, 1967), and age (Speed, 1977) affect crossing over during meiosis. Sex is an important genetic factor that affects crossing over or recombination frequency. Sex differences in recombination frequency have been well documented in animals, including human (Helen et al., 1987), mouse (Castle, 1925), and horse (Leif and Kaj, 1984). In contrast to animals, most higher plants are hermaphrodites: individuals carry both sex organs. Especially in bisexual flowers, these organs are also derived from the same meristematic tissues. In this sense, the effect of sex on the recombination in plants is not the same as in animals.

Cucumis melo L. is, in general, andromonoecious. It has both bisexual and male flowers in the same individual. Male organs of male flowers and female organs of bisexual flowers originate from the same individuals, but from different meristematic tissues. Whether or not the recombination frequency differs between female gametes of bisexual flowers and male gametes of male flowers remains unknown.

This study addresses differences of linkage map length (crossing-over value) and recombination frequency between male gamete from a male flower and female gamete of a bisexual flower by using the reciprocal backcrossed populations of *Cucumis melo* L.

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Materials and Methods

Plant material

Two melon lines were used to produce two backcross (BC) populations: the 'Andes' (Sakata Seed Co., Yokohama) double haploid line (ADH), and the 'Quincy' (The Yokohama Nursery Co. Ltd., Yokohama) double haploid line (QDH). Both were produced from a pseudo-fertilized ovule culture (Katoh et al., 1993); then they were selfed for more than three generations.

The ADH and QDH lines were fertilized on 29 September 2000 in a glasshouse kept at 30/19℃ (day/night) to produce the F_1 of QDH × ADH. Seedling of the F_1 (QDH × ADH) generation were crossed reciprocally to produce the two BC populations on 2 May 2001 in a glasshouse maintained at 30/18℃ (day/night). Recombination frequencies of male gamete and female gamete were estimated from linkage maps of ADH × F_1 (BCM) and F_1 × ADH (BCF) populations, respectively. Population sizes of BCM and BCF were 95 and 99 individuals, respectively. Genomic DNAs for analyses were isolated from young leaves, by using CTAB methods (Murray and Thompson, 1980).

DNA marker analysis

Four types of DNA markers, based on PCR method, were used for screening polymorphic markers between ADH and F_1 (QDH): 265 RAPD primer sets, 30 SRAP primer sets, 9 ISSR primer sets, and 96 AFLP primer sets. All amplifications were performed with a thermal cycler (GeneAmp PCR System 9700; Applied Biosystems, USA). Detected polymorphic markers between ADH and F_1 were used for segregation analysis of the two BC populations.

In Random Amplified Polymorphic DNA (RAPD) procedure, 265 primers were used for PCR under conditions similar to those described by Williams et al. (1990). Primers were obtained from Operon

Technologies Inc. (USA). The amplified products were separated on non-denatured 7.5% polyacrylamide gel, stained by SYBER Gold (Molecular Probes Inc., USA), and detected with FAS-III (TOYOBO Co. Ltd., Osaka). Sequence-Related Amplified Polymorphism (SRAP) procedure was performed as described by Li and Quiros (2001). SRAP mainly targets coding sequences of genes in the genome with forward and reverse primers, respectively, and containing CCGG sequence and AATT sequence. Five forward primers (me 1-5) and six reverse primers (em 1-6) were utilized. The amplified products were separated, stained, and detected according to RAPD protocol, by using the Inter Simple Sequence Repeat (ISSR) procedures according to Stepansky et al. (1999). Nine primers were used: $(TC)_8C$, $(AG)_8T$, $(GGGTG)_3$, $(AC)_8G$, $(AC)_8T$, $(ATG)_6$, $(AC)_8YC$, $(GA)_8YG$, and $(TG)_8G$. Separation and detection of amplified products were identical to those for RAPD procedure. Amplified Fragment Length Polymorphism (AFLP) procedure (Pieter et al., 1995) was performed, by using an AFLP analysis system I (Invitrogen Corp. USA), according to the manufacturer's protocol, except that the isotope-labeling step was skipped. The amplified products were separated on 7 M urea-denatured 12.5% polyacrylamide gel. After staining with SYBER Gold, the bands were detected with FLA-2000 (Fuji Photo Film Co. Ltd., Tokyo).

Data analysis

Linkage analysis was performed, by using the MAPL computer program (Ukai et al., 1995). The segregation ratio (1:1) of the genotypes at all markers was checked

Table 1. The number of polymorphic markers detected between ADH and F_1 from each type of markers.

Marker	primer	Locus	Linkage group	Number of markers detected	Number of markers mapped
flesh colour		*Flesh*	V	2	1
		E19a	III		
	E-19	E19b	VI		
	E-20	E20	III		
	G-10	G10	III		
	K-16	K16	VII		
RAPD	L-20	L20a	I	15	10
		L20b	VI		
	M-16	M16	VII		
	O-10	O10	IV		
	P-15	P15	III		
SRAP	me2/em5	me2/em5	VI	3	3
	me5/em6	me5/em6a	IX		
		me5/em6b	IX		
ISSR	$(AG)_8T$	IS2	VIII	1	1
	AAC/CAG	AAC/CAG412	II		
	AAG/CAC	AAG/CAC819	III		
	AAG/CAT	AAG/CAT265	V		
	ACA/CAT	ACA/CAT600	I		
	ACA/CTC	ACA/CTC518	IV		
	ACA/CTG	ACA/CTG1320	V		
	ACC/CTC	ACC/CTC315	III		
	ACC/CTG	ACC/CTG590	I		
	ACG/CTG	ACG/CTG504	I		
AFLP	ACT/CAC	ACT/CAC968	VI	37	19
	ACT/CTC	ACT/CTC1190	V		
		ACT/CTC459	II		
	AGA/CAG	AGA/CAG1484	VII		
	AGA/CTG	AGA/CTG251	VIII		
	AGG/CAC	AGG/CAC1026	VI		
	AGG/CTC	AGG/CTC334	VI		
	ATA/CAC	ATA/CAC710	V		
		ATA/CAC750	IV		
	AGA/CAC	AGA/CAC786	V		
Total				58	34

by using the χ^2 test; the map length of each linkage map was calculated by using the Kosambi function. Each interval recombination frequency of adjacent markers was tested with a 2 × 2 contingency table by using Fisher's exact test to compare the recombination frequencies of male and female gametes. Linkage group lengths and the total map lengths of both populations were compared, by using Wilcoxon's signed rank test and Student's t-test.

Results

Screening of polymorphic markers

The phenotypic marker, the fruit flesh color, was distinguishable as a qualitative trait for two parents. The ADH had green flesh, whereas the QDH had orange flesh. All F_1 (QDH × ADH) individuals had fruit with orange flesh. For that reason, we inferred that the QDH trait was dominant to ADH trait in fruit color. The 400 primer sets (265 for RAPD, 96 for AFLP, 30 for SRAP, 9 for ISSR), used to detect polymorphisms between ADH and F_1, resulted in mutual polymorphic markers in 53 primer sets (data not shown). Among them, 39 primer sets detected 56 polymorphic markers between ADH and F_1; 15 markers used 12 RAPD primer sets, 3 markers used 2 SRAP primer sets, 1 marker used 1 ISSR primer set, and 37 markers used 24 AFLP primer sets. In all, 57 polymorphic markers (1 phenotypic marker and 56 DNA markers) were used to construct the BCF and BCM linkage maps (Table 1). Examples of AFLP marker segregation patterns are shown in Fig. 1.

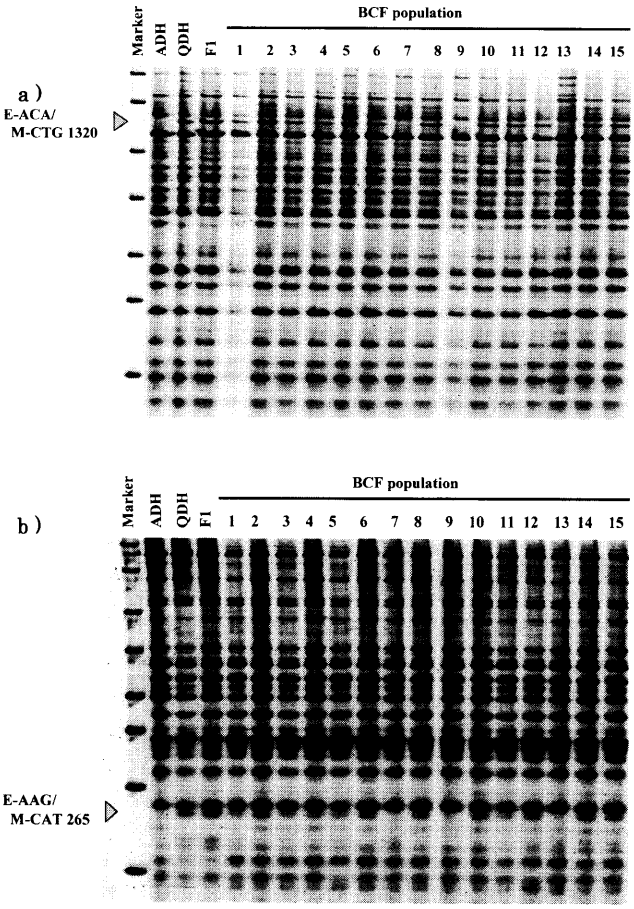

Fig. 1. Examples of the segregation of two AFLP markers on the map. The DNAs were isolated from BCF population and amplified by selective-primers E-ACA/M-CTG (a) and E-AAG / M-CAT (b). First 4 lanes from the left are molecular marker (100-2000 bp ladder marker), parents ADH, QDH and F_1, respectively; the remaining lanes are the individuals of BCF population. Arrows indicated E-ACA / M-CTG1320 (a) and E-AAG / M-CAT265 (b).

Map construction

The two melon linkage maps for BCF and BCM (Fig. 2) consists of nine linkage groups. From two BC populations, 33 DNA markers were mapped; the other 23 DNA markers were solitary markers. The morphological marker of flesh (*Flesh*) was mapped in linkage group V. These maps represented 279.1 cM for BCM and 295.7 cM for BCF. Segregation distortion was determined based on χ^2 analysis (Fig. 2). Of the 34 markers on the map, 3 markers showed segregation distortion of expected 1:1 segregation at $P < 0.05$. Those three skewed markers were distributed randomly throughout linkage groups and one marker was solitary.

Comparison of linkage map between BCF and BCM

Comparison of the linkage maps constructed in the BCM and BCF population indicates the recombination frequencies in the male gamete, whereas the marker segregations of BCF represent the female gamete. Four marker intervals showed significantly different recombination frequencies for BCM and BCF (Table 2). Recombination frequencies of linkage groups II and V of BCM were higher than those of BCF, but fewer in groups VI and VIII. The tendency in height of recombination frequencies was not biased toward either male or female. Furthermore, these significantly different regions between male and female gametes were scattered around the linkage map and not located at a certain region.

Map length difference of each linkage group between that of BCM and BCF ranged from 0.3 to 24.6 cM; their average was 1.8 cM (Table 3). The total map length in BCF (295.7 cM) is slightly longer than that in BCM (279.1 cM). However, the total map length between two maps did not differ significantly.

Discussion

Correspondence of this map with other maps already published

Perin et al. (2002) compared their map with maps produced in different laboratories by using anchor loci such as RFLP/SSR/phenotype loci. On comparing our map with other published maps, the locus of flesh color (*Flesh*) was assigned to linkage group V (Fig. 2) and

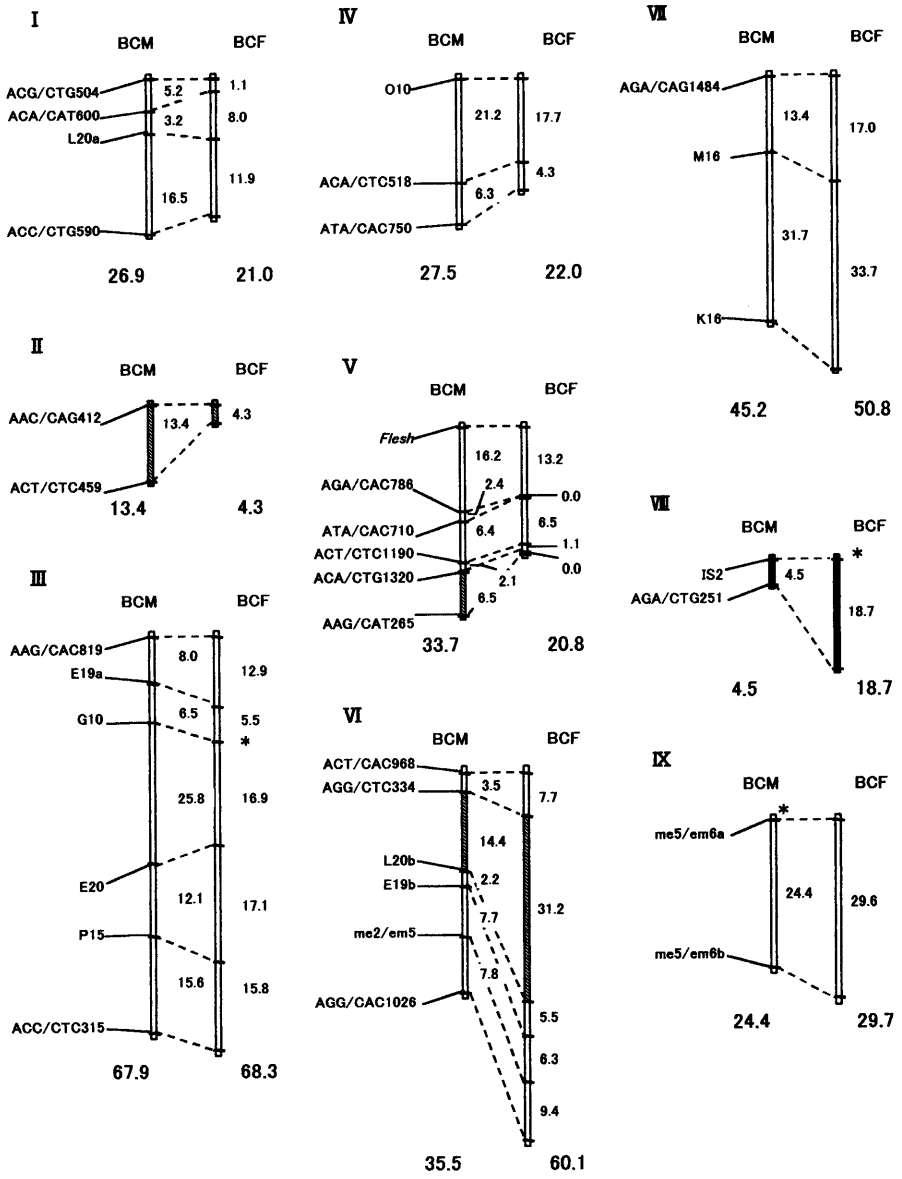

Fig. 2. Comparison of melon linkage maps from two BC populations. Map distances between markers and respective linkage groups (cM; right side and bottom of the maps). Hatched and black regions within the bars, respectively, indicate significantly different intervals in recombination at the $P<0.05$ and $P<0.01$. Asterisks indicate distorted markers at the $P<0.05$.

also to linkage group IX in the map constructed by Perin et al. (2002). This correspondence assigns our group to linkage group 5 by Baudracco-Arnas and Pitrat (1996), linkage group II by Danin-Poleg et al. (2000) and linkage group 11 by Oliver et al. (2001). Other genes, *Fom-1* (Fusarium race 0 and 2 resistance), *gf* (green flesh color), *Prv* (Papaya ringspot virus resistance) and *Al-4* (Abscission layer-4) are clustered on this linkage group. Therefore, this linkage group is very important for agronomical trait.

Comparison of recombination frequency in male and female gametes

We generated each pair of BCM and BCF populations using F_1 (ADH × QDH) as male or female parents. Therefore, the recombination detected in these backcross populations reflects the crossing over that occurred in male or female gametes during meiosis. Several factors, including temperature, age, and genotype, are known to influence recombination frequency that this report was designed to eliminate. F_1 and recurrent parents were seeded on the same day, grown in identical conditions and backcrossed on only one day. Furthermore, pure lines were used as parents; a single F_1 plant was used as the male and the female in backcrossing. Therefore, the main cause of difference in male and female maps is considered to be sex.

The differences in recombination frequencies at whole-genome level between the male and female gametes have been reported for some plant species. Re-

Table 2. The significant difference of recombination frequency between BCM and BCF.

Linkage group	Interval	Locus[z]		BCM population		BCF population		Level[y]
		L	R	Number of individuals	Recombination frequency	Number of individuals	Recombination frequency	
II	ACC/CAG412 - ACT/CTC459	+	+	43		40		
		-	-	37		49		
		+	-	7	0.130	3	0.043	*
		-	+	5		1		
V	ACA/CTG1320 - AAG/CAT265	+	+	44		52		
		-	-	45		47		
		+	-	3	0.063	0	0.000	*
		-	+	3		0		
VI	AGG/CTG334 - L20b	+	+	38		28		
		-	-	42		33		
		+	-	6	0.140	16	0.291	*
		-	+	7		9		
VIII	IS2 - AGA/CTG251	+	+	39		29		
		-	-	47		35		
		+	-	3	0.044	4	0.190	**
		-	+	1		11		

[z] L, R; Flanking markers of each interval. +, -; presence and absence of marker band.
[y] * and ** indicate the significant differences at $P<0.05$ and $P<0.01$ by Fisher's exact test, respectively.

Table 3. Comparison of the map length (cM) of each linkage group.

Linkage group	BCM	BCF	Difference[z]
LG I	26.9	21.0	5.9
LG II	13.4	4.3	9.1
LG III	67.9	68.3	-0.3
LG IV	27.5	22.0	5.5
LG V	33.7	20.8	12.9
LG VI	35.5	60.1	-24.6
LG VII	45.2	50.8	-5.6
LG VIII	4.5	18.7	-14.2
LG IX	24.4	29.7	-5.3
Total	279.1	295.7	-16.6
Average	31.0	32.9	-1.8

[z] Difference indicates the numeric taking BCF value away from BCM value.

combination frequencies were higher in the female gamete than in the male gamete in tomato (de Vicente and Tanksley, 1991), *Tulbaghia* (Vosa, 1972) and *Brassica oleracea* (Kearsey et al., 1996). In contrast, in rye (Benito et al., 1996), *Allium* (Gohil and Kaul, 1980) and *Arabidopsis* (Vizir and Korol, 1990), they are higher in the male gamete than in the female gamete. In *Coffea canephora* (Lashermes et al., 2001) and pearl millet (Busso et al., 1995), recombination frequencies at the whole-genome level were found to be nearly indistinguishable in either sex, although some of those within individual marker intervals are significantly different. Our results with the melon are similar to those obtained for *Coffea canephora* (Lashermes et al., 2001), pearl millet (Busso et al., 1995), *Brassica napus* (Kelly et al., 1997), and barley (Miyazaki et al., 2000; Pierre et al., 1995). In our report, four marker intervals showed significantly different recombination frequencies for BCF and BCM (Table 2). Recombination frequencies of two intervals of BCM were more numerous than those of BCF, but few recombinations occurred at two other intervals in BCM. The map from male meiosis gave a total length of 279.1 cM versus 295.7 cM for female meiosis. No significant difference was found between them at $P=0.05$. No difference in recombination frequency was observed at the whole-genome level.

Difference in male-female recombination can potentially be exploited for practical purposes (de Vicente and Tanksley, 1991). Backcross breeding is the normal method used to introduce genes for desirable traits from one variety or species to another. One drawback of this technique is "linkage drag", which is the simultaneous introgression of undesirable genes that are linked to the genes being introduced (Zeven et al., 1983). For example, recombination frequencies are higher in females; consequently, exercising back cross breeding by using the recurrent parent as male should minimize linkage drag. From our research on the melon, the equivalent recombination frequencies between male and female gametes at the whole-genome level suggest that a backcrossing program can be carried out in the custom-

ary manner, rather than by using F_1 as female, as would be suggested from tomato (de Vicente and Tanksley, 1991). However, some intervals showed different recombination frequencies for each of the two gametes. The melon breeder should use recurrent parents with a less recombinant gamete to minimize linkage drag, if desirable traits to be introduced by backcrossing are closely linked to these markers. Unfortunately, we have not determined the desirable traits to which these markers are closely linked. Further research on higher-resolution genetic mapping and sex difference in the recombination process, by utilizing a more elegant backcrossing program, seems necessary.

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メロンの雄性および雌性配偶子における組換え頻度について

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摘　要

植物の性が減数分裂時の組換えに与える影響について，メロンを供試して調査した．F_1 を花粉親および種子親に用いることで，雄性配偶子および雌性配偶子の組換えを検出する2組の戻し交配集団を育成し，34のDNAマーカーから成る連鎖地図をそれぞれ作成した．両地図の全体の地図距離に有意な差は認められなかった．4つのマーカー区間については地図距離に有意な差が認められ，雄性配偶子と雌性配偶子の間における組換え頻度に差があることが示された．