Genetic Mapping of Mitochondrial Sorting of the MSC3 Mosaic Mutant of Cucumber

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Research Article

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Abstract

Passage of the highly inbred line ‘B’ of cucumber through cell cultures has produced regenerated plants with a mosaic (MSC) phenotype on cotyledons and leaves, as well as rearrangements in the mitochondrial DNA. Both of these characteristics show paternal transmission. MSC3 and MSC16 were derived from independent cell-culture experiments and have distinct mosaic phenotypes and different under-represented regions in their mitochondrial DNAs. A nuclear locus, *Psm* for paternal transmission of mitochondria, conditions a high proportion of wild type progenies when MSC16 is crossed as the male with female plants carrying the *Psm-* allele. Plants with homozygous genotypes at *Psm* were crossed with both MSC3 and MSC16, and segregation of wild-type versus mosaic progenies in these families were not consistent suggesting that sorting of wild-type progenies from crosses with MSC3 and MSC16 have different genetic bases. We identified cucumber plants that produced a high proportion of wild-type progenies in crosses with MSC3 as the male parent. Plants from a segregating F_2 family were crossed with MSC3 as the male and progenies scored for numbers of mosaic versus wild-type progenies. The same F_2 plants were genotyped-by-sequencing and single nucleotide polymorphisms identified for genetic mapping. Quantitative analysis of the proportion of wild-type testcross progenies identified a major quantitative trait locus (QTL) in the same genomic region as the *Psm* locus; however the most significant SNP associated with this QTL was located approximately 856 kilobases from *Psm*. Eventual identification of a candidate gene controlling this unique mitochondrial sorting in cucumber should reveal important aspects of mitochondrial-nuclear interactions affecting the prevalence of specific mitochondrial DNAs.

Key Message

Different nuclear loci control sorting of mitochondrial genomes from unique mosaic mutants of cucumber with the same highly inbred nuclear background.

Introduction

The cucumber mitochondrial (mt) DNA possesses several unique characteristics, including a relatively large size among plants (1.55 Mb) with approximately 13% repetitive DNA, a high degree of polymorphism even within cultivated germplasm, and paternal transmission (Havey 1997; Havey et al. 1998; Lilly and Havey 2001; Alverson et al. 2011). This last characteristic means that the three cucumber genomes have different modes of inheritance, that is maternal for chloroplast, paternal for mitochondrial, and biparental for nuclear DNAs. These different modes of inheritance allow mitochondrial traits to be experimentally distinguished from chloroplast and nuclear associated traits by crossing (Havey et al. 2002). During microspore development in cucumber, relatively few large mitochondria are present (Abreu et al. 1982) and presumably limit mitochondrial diversity delivered to progenies via the male gametophyte and may contribute to sorting of mitochondrial phenotypes (Havey et al. 2002).

There exist in cucumber multiple mitochondrial mutants conditioning paternally transmitted mosaic (MSC) phenotypes (Havey et al. 2002). These mutants were initially identified among plants regenerated from cell cultures established using the highly inbred line 'B' from Polish cultivar 'Borszczagowski', and possess malformed cotyledons and mosaic leaves (Malepszy et al. 1996; Bartoszewski et al. 2004; Havey et al. 2004). Independent MSC lines have been produced from different cell-culture experiments, and possess distinct mosaic phenotypes and different mtDNA rearrangements (Ładyżyński et al. 2002; Bartoszewski et al. 2004). Subsequent studies using independently produced MSC lines revealed that specific regions of the mtDNAs were at significantly higher or lower amounts relative to wild-type plants. In MSC3, the polycistronic region encoding exons 4 and 5 of NADH dehydrogenase subunit 5 and ATPase subunit 4 was under-represented, and in MSC16 the genomic region encoding ribosomal protein S7 (rps7) was under-represented (Del Valle-Echevarria et al. 2015) . These regions showed reduced amounts of mitochondrial transcripts, potentially useful as transcriptional knock downs of mitochondrial genes to study nuclear-mitochondrial interactions (Del Valle-Echevarria et al. 2015).

Rare wild-type progenies occasionally occur from crosses using MSC as the male parent, and result from sorting of the wild-type sublimon which exists at low levels in MSC plants (Lilly et al. 2001). Later it was observed that certain female cucumber plants produce significantly higher numbers of wild-type progenies in crosses with MSC16 as the male parent (Havey et al. 2004). A nuclear locus, Paternal sorting of mitochondria (*Psm*), conditioned sorting of mtDNA so that the rare wild-type molecule predominated in the progeny of crosses with MSC16 as the male parent. When a female plant with the genotype *Psm +/+* is crossed with MSC16 as the male, essentially all progenies have the MSC phenotype. When a plant that is Psm *+/-* is crossed with MSC16 as the male, approximately equal numbers of wild-type versus MSC progenies are produced. Finally when a female plant with the genotype *Psm -/-* is crossed with MSC16 as the male, essentially all progenies have the wild-type phenotype. The *Psm* locus maps to chromosome 3 of cucumber (Al-Faifi et al. 2008; Calderon et al. 2012) and *pentatricopeptide repeat 336* gene (PPR336) has been proposed as the candidate gene for *Psm* (Del Valle-Echevarria et al. 2016).

Although the *Psm* locus specifically sorts for wild-type mtDNAs in crosses with MSC16 as the male (Havey et al. 2004), mitochondrial sorting to wild-type was also observed for crosses with MSC3 as the male parent (Del Valle-Echevarria et al. 2016). MSC3 has a different genetic basis from MSC16 (Del Valle-Echevarria et al. 2015), and sorting for wild type progenies from crosses of MSC3 may provide further insights about mitochondrial-nuclear interactions affecting the predominance of specific mtDNAs transferred to progenies. The purpose of this research was to determine the genetic basis of mitochondrial sorting using MSC3 and genetically map the trait as an experimental system to study mitochondrial sorting in plants.

Materials And Methods

Cucumber plants were grown in greenhouses on the campus of the University of Wisconsin-Madison at temperatures ranging between 26° C to 31° C. Plants for flowering and crossing were initially grown in 10 cm pots until true leaves were present and then transplanted to nine-liter pots containing Pro Mix HP

general purpose soil with mycorrhizae (PRO-MIX, Quakertown, PA, USA). Plants were fertilized twice each week with Peters Professional 20-10-20 (N:P:K) containing micronutrients.

Plant Introduction (PI) 401734 from the USDA cucumber germplasm collection was previously described as producing higher frequencies of wild-type progenies in crosses with MSC16 as the male parent (Havey et al. 2004). A single plant from PI 401734 was crossed as the female with *Cucumis hardwickii* and a hybrid plant was self-pollinated to generate the F_2 population used for mapping of the *Psm* locus (Calderon et al. 2012). Plants from this same F_2 family that were homozygous for alleles at the *Psm* locus were crossed with MSC3 as the male. Seeds from these crosses were grown in sterilized vermiculite in wooden flats at 30° C and progenies scored for frequencies of wild-type versus MSC progenies. Selfed progenies from one F_2 plant (C10115HQ) with the genotype *Psm* -/- showed higher numbers of wild-type progenies in crosses with MSC3 as the male, and was crossed as the male to a doubled haploid plant from inbred 9930 (Shen et al. 2015). A single hybrid plant was self-pollinated to produce segregating F_2 progenies which were crossed with MSC3 as the male. Fifty seeds from each crossed family were grown and cotyledons scored as wild type versus mosaic. In most cases, two fruits were obtained from each F_2 plant and the numbers of wild-type and mosaic progenies were averaged. The proportions of wild-type seedlings in the testcross families were used as the phenotype for each F_2 progeny.

Young leaf tissue was harvested from each F_2 plant, immediately frozen in liquid nitrogen, and lyophilized for three days. DNA was isolated from 80 mg of tissue using a kit (NucleoSpin Plant II Midi kit, Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. DNA concentrations were quantified with a spectrophotometer (ND-1000, Nanodrop Technologies, Wilmington, DE, USA) and quality was assessed by running 100 ng of DNA on a 1% agarose gel, staining with ethidium bromide, and observing under UV light. DNAs were assessed for the presence of potential inhibitors to restriction enzymes by digesting ~300 ng with *Hin*dIII and *Eco*RI for two hours at 37° C, heat killing at 65° C for 15 minutes, and running on a 1% agarose gel to visualize the resulting fragments. A total of 122 F_2 progeny and two parental DNAs were diluted with 10 mM Tris(HCl) buffer to 50 ng per µL for genotyping-by-sequencing (GBS) at the University of Wisconsin Biotechnology center using the Illumina platform (Davey et al. 2011). DNAs were digested with *Ape*KI and GBS performed as described by Wang et al. (2018). Libraries were sequenced on a NovaSeq 6000 system (Illumina, San Diego CA, USA). The software skewer (Jiang et al. 2014) was used to trim 3' end of fragments until a Phred quality of 20 was reached. SNPs were identified using TASSEL 5.0 GBS Discovery Pipeline (Glaubitz et al. 2014) and the cucumber 9930 version 3.0 reference genome (http://cucurbitgenomics.org/organism/20) using the same approach and parameters of Wang et al. (2018). SNPs were filtered based on missing data rate ≤50% and minor allele frequencies ≥ 5%. Linkages among SNPs were detected using JoinMap software (version 5.0, Kyazma B.V., Wageningen, Netherlands) with the maximum likelihood mapping algorithm, Haldane's mapping function, and an independence LOD greater than 6.0 for linkage. QTL analysis was completed using Multiple-QTL Mapping (MQM) analysis (Ooijen 2009) of the MapQTL software (version 6.0, Kyazma B.V., Wageningen, Netherlands). A genome-wide significance threshold of LOD 4.5 was calculated using α = 0.05 and 1000 permutations.

Results

Eleven plants with the *Psm* +/+ genotype (Calderon et al. 2012) were crossed as the female with both MSC3 and MSC16 as males. For crosses with MSC16 we expected and observed very few (average proportion of 0.01) wild-type progenies (Table 1). However for families from the same plant crossed with MSC3 as the male, we observed varying proportions of wild-type and mosaic progenies with an average proportion of wild-type progenies of 0.27 ± 0.34 (Table 1). We crossed 33 plants that were Psm -/- with both MSC3 and MSC16 as males, and observed that all families from crosses with MSC16 showed high proportions of wild-type progenies with an average of 0.96 ± 0.04 (Table 1). Crosses of the same plants with MSC3 as the male produced families with variable numbers of wild-type and mosaic progenies, with an average across families of 0.58 ± 0.26 (Table 1).

All of 95 progenies from the cross of DH 9930 with MSC3 as the male showed the mosaic phenotype (Figure 1). Four progenies from self pollination of one plant (C10115HQ) that was *Psm -/-* were crossed with MSC3 as the male and produced families with an average of 0.88 ± 0.03 wild-type progenies. One of these selfed progenies from plant C10115HQ was crossed as the male to DH 9930, and one hybrid plant was self pollinated to produce a segregating F_2 family. A total of 122 F_2 progenies were crossed with MSC3 as the male, and numbers of mosaic versus wild-type phenotypes scored at the seedling stage (Figure 1). Proportions of wild-type progenies ranged from 0.00 to 0.95 with an average of 0.44 ± 0.25 for the F_2 family (Supplemental Table 1).

Using the Tassel GBS pipeline (Glaubitz et al. 2014), a total of 405,339,314 demultiplexed reads were produced across the parental and F_2 progeny DNAs with an average number of 3,166,713 demultiplexed reads per DNA sample. Results for three F_2 DNAs were discarded due to low number ($<1x10^5$) of demultiplexed reads. Reads were aligned to the 9930 version 3.0 reference (http://cucurbitgenomics.org/organism/20) and 15,497 SNPs were identified with an average of 2,214 SNPs per chromosome. Genetic mapping was completed using 4,633 SNPs after randomly selecting one SNP from groups of SNPs showing >95% identical genotypes across the F_2 progenies. Genetic mapping at LOD 6.0 produced seven linkage groups corresponding to the seven chromosomes of cucumber. Overall there was close agreement between genetic linkages among SNPs and their genomic position based on the 9930 reference sequence; however there were occasional positions at which the genetic linkages and genomic position did not agree, likely due to mis scoring of SNP genotypes resulting in double recombination events across very short genomic regions.

QTL analysis revealed one region on chromosome 3 associated with the proportion of wild-type progenies from crosses with MSC3 as the male (Figure 2, Table 3). The most highly significant genomic region encompassed 1.5 megabases, from basepair (bp) 32,761,821 to 34,254,346 on chromosome 3 based on the 9930 version 3.0 sequence. The most significant SNP associated with sorting of wild-type progenies from MSC3 was at bp 32,761,821. This SNP had a LOD score of 34.2, explained 73.4% of the phenotypic variation, and had additive and dominance effects of 0.271 and -0.188, respectively (Table 2).

These attributes are consistent with a locus at which codominant alleles controlling sorting to the wild-type phenotype in progenies from MSC3 as the male.

Discussion

Crossing of plants possessing the *Psm* +/+ or -/- genotype as the female with MSC16 as the male produced almost exclusively mosaic or wild-type progenies (Table 1), as expected from previous research (Calderon et al. 2012). However when these same plants were crossed with MSC3 as the male, varying proportions of wild-type and mosaic progenies were observed (Table 1). These results indicate that the *Psm* locus may not control sorting to wild-type progenies from crosses with MSC3 as the male. Genetic mapping of mitochondrial sorting from MSC3 revealed one major QTL on chromosome 3, and additive and dominance estimates for this QTL were consistent with a locus at which codominant alleles controlling sorting to the wild-type phenotype in progenies. This codominance is similar to the *Psm* locus, at which the heterozygous genotype (one *Psm-* allele) in the female parent conditions approximately one-half wild-type progenies and the homozygote for the *Psm-* alleles conditions essentially all wild-type progenies. PPR336 has been proposed as the candidate gene for *Psm* (Del Valle-Echevarria et al. 2016) and its coding region is located within this same genomic region from bp 33,618,502 to 33,619,719 of the 9930 version 3.0 sequence (Table 2). However sorting of wild-type progenies from MSC3 as the male parent is not likely conditioned by the *Psm* locus because of different segregations in progenies from MSC3 and MSC16 as male parents (Table 1) and the different under-represented coding regions in their mt DNAs (Del Valle-Echevarria et al. 2015). The most significant SNP affecting sorting from MSC3 was located 856,681 bp from the PPR336 gene.

There were 224 annotated genes in the 9930 version 3.0 sequence (http://cucurbitgenomics.org/organism/20) across the genomic region on chromosome 3 with the highest LODs (Figure 2), including five groups of PPRs (CsaV3_3G040080 through to and including CsaV3_3G040120; CsaV3_3G040390 and CsaV3_3G040400; CsaV3_3G040680; CsaV3_3G041130; and CsaV3_3G041760) and a mitochondrial outer membrane porin (CsaV3_3G041720). The PPR genes warrant further study as potential candidates for the major QTL affecting sorting of mitochondrial DNAs from MSC3, because PPR proteins are important post-transcriptional regulators in organelles (Rovira and Smith 2019). Because the family size used in this study was too small for fine mapping and candidate gene identification, further studies with larger family sizes are necessary towards elucidating the causal gene(s) for this trait. Eventual candidate gene identification should provide further insights into nuclear-mitochondrial interactions controlling the predominance of mitochondria delivered to progenies from different mitochondrial mutants.

Declarations

Authors gratefully acknowledge the financial support of USDA National Institute of Food and Agriculture grant 2016-67013-24590. The authors declare no conflicts of interest or competing interests. All data and materials are available from corresponding author. Authors' contributions: LTW produced the segregating

family, completed crosses with MSC3, scored segregations, isolated DNAs, and completed QTL analyses. MJH conceived the project, completed crosses to identify parents, produced hybrids, coordinated with UW Biotechnology Center to complete GBS and bioinformatic analyses, and produced genetic map. Both authors wrote the manuscript.

Disclaimer: Names are necessary to report factually on available data; however, the U.S. Department of Agriculture (USDA) neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Tables

Table 1. Numbers of wild-type (WT) versus mosaic progenies and proportion of WT progenies resulting from crosses of female plants with homozygous genotypes (+/+ or -/-) at the *Psm* locus with both MSC3 and MSC16 as male parents.

Genotype of	Crossed with MSC3			Crossed with MSC16		
female parent	WT	Mosaic	Prop WT	WT	Mosaic	Prop WT
Psm +/+	0	48	0.00	0	48	0.00
Psm +/+	0	52	0.00	2	48	0.04
Psm +/+	1	49	0.02	0	54	0.00
Psm +/+	1	51	0.02	2	46	0.04
Psm +/+	2	48	0.04	1	49	0.02
Psm +/+	3	46	0.06	0	54	0.00
Psm +/+	5	48	0.09	0	48	0.00
Psm +/+	29	21	0.58	0	51	0.00
Psm +/+	33	17	0.66	0	46	0.00
Psm +/+	35	13	0.73	0	51	0.00
Psm +/+	45	10	0.82	0	46	0.00
Total	**154**	**403**	**0.28**	**5**	**541**	**0.01**
Psm -/-	0	50	0.00	45	4	0.92
Psm -/-	0	46	0.00	47	3	0.94
Psm -/-	8	41	0.16	48	2	0.96
Psm -/-	10	39	0.20	50	0	1.00
Psm -/-	13	36	0.27	46	2	0.96
Psm -/-	22	26	0.46	46	3	0.94
Psm -/-	23	27	0.46	39	9	0.81
Psm -/-	23	27	0.46	39	3	0.93
Psm -/-	24	26	0.48	53	2	0.96
Psm -/-	25	26	0.49	49	1	0.98
Psm -/-	25	25	0.50	25	3	0.89
Psm -/-	26	27	0.49	41	5	0.89
Psm -/-	26	24	0.52	47	4	0.92
Psm -/-	26	23	0.53	47	2	0.96

Psm -/-	26	22	0.54	46	2	0.96
Psm -/-	27	23	0.54	47	0	1.00
Psm -/-	28	20	0.58	48	2	0.96
Psm -/-	29	21	0.58	47	3	0.94
Psm -/-	31	18	0.63	47	2	0.96
Psm -/-	31	14	0.69	46	1	0.98
Psm -/-	34	16	0.68	50	0	1.00
Psm -/-	35	14	0.71	49	1	0.98
Psm -/-	35	15	0.70	47	2	0.96
Psm -/-	36	9	0.80	47	3	0.94
Psm -/-	37	13	0.74	45	5	0.90
Psm -/-	39	11	0.78	49	0	1.00
Psm -/-	39	10	0.80	50	0	1.00
Psm -/-	40	10	0.80	50	0	1.00
Psm -/-	43	7	0.86	50	0	1.00
Psm -/-	45	2	0.96	49	1	0.98
Psm -/-	46	4	0.92	50	0	1.00
Psm -/-	46	0	1.00	27	0	1.00
Psm -/-	47	3	0.94	50	0	1.00
Total	**945**	**675**	**0.58**	**1516**	**65**	**0.96**

Table 2. Nucleotide positions of single nucleotide polymorphisms (SNP) on chromosome 3 of cucumber across the region with the highest logarithm of odds (LOD) for sorting to the wild-type phenotype in a segregating family crossed with MSC3 as the male, percent variation (% Var) explained and additive (Add) and dominance (Dom) effects of SNP. The permutation threshold at α = 0.05 was LOD 4.5. PPR336 is the proposed candidate gene for the *Psm* locus (Del Valle-Echevarria et al., 2016) and its position on chromosome 3 of the 9930 version 3.0 sequence is shown.

Nucleotide position of SNP	LOD	% Var Explained	Add Effect	Dom Effect
32057082	23.4	59.6	0.239	-0.177
32220379	28.0	66.1	0.266	-0.181
32232276	19.3	52.5	0.228	-0.164
32761821	34.2	73.4	0.271	-0.188
33312662	25.3	62.4	0.235	-0.172
33364232	28.7	67.1	0.255	-0.143
33368892	31.4	70.3	0.260	-0.151
33593655	23.0	58.9	0.232	-0.144
33618502 (PPR336)				
33720971	33.4	72.5	0.274	-0.165
33833447	24.3	60.9	0.232	-0.139
33917999	25.2	62.2	0.242	-0.149
33918155	25.5	62.7	0.248	-0.155
33923790	30.6	69.4	0.262	-0.175
33929005	24.1	60.6	0.239	-0.134
34246720	30.6	69.4	0.264	-0.172
34254346	26.2	63.8	0.241	-0.168
34380732	22.9	58.7	0.244	-0.154

Figures

Figure 1

Mosaic progenies (left image) from crossing of doubled haploid 9930 with MSC3 as the male. Wild-type progenies (right image) were from the cross of an F2 plant with MSC3 as the male.

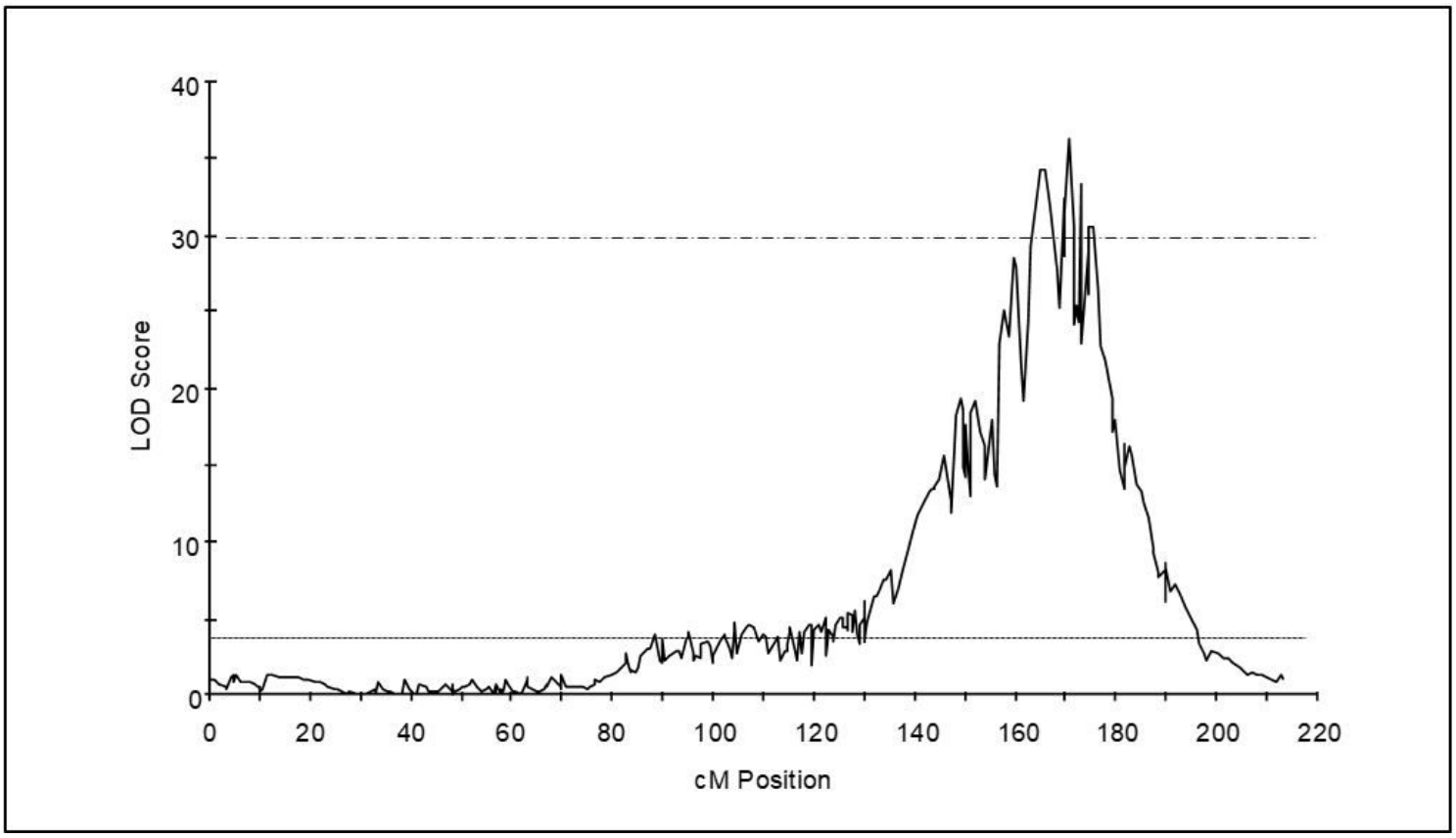

Figure 2

Plot of logarithm of odds (LOD) scores (y-axis) for sorting of wild-type progenies versus marker positions in cM on chromosome 3 (x-axis) of cucumber. Genome-wide logarithm of odds (LOD) threshold as determined by permutation analysis is shown by dotted line at LOD 4.5. The peak region with LOD scores greater than 30 is shown by the dashed line.

Supplementary Files

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- SupplementalTable1.docx