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Genetic Analysis of Mitochondrial Sorting from the MSC3 Mosaic Mutant of Cucumber

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Abstract. **Cucumber (*Cucumis sativus*) plants regenerated from cell cultures occasionally possess mosaic (MSC) phenotypes on cotyledons and leaves. Lines MSC3 and MSC16 have distinct MSC phenotypes and originated from plants regenerated from different cell-culture experiments established using a highly inbred wild-type cucumber. Both the mitochondrial (mt) DNA and MSC phenotype of cucumber show paternal transmission, and MSC3 and MSC16 have different mt coding regions at significantly lower copy numbers relative to wild-type plants. A nuclear locus, *Paternal sorting of mitochondria* (*Psm*), conditions a high proportion of wild-type progenies, specifically when MSC16 is crossed as the male with wild-type female plants. During this research, we identified plants that produced a high proportion of wild-type progenies in crosses with MSC3 as the male parent. Plants from an F_2 family were crossed with MSC3 as the male, progenies were scored for numbers of MSC vs. wild-type plants, and single-nucleotide polymorphisms (SNP) were identified for genetic mapping. A major quantitative trait locus on chromosome 3 was associated with a higher frequency of wild-type progenies from MSC3 as the male parent, and the 1.5-logarithm-of-odds interval for the most significant SNP was located 627 kb from *Psm*. These results reveal that separate genetic factors control sorting to the wild-type phenotype in progenies from crosses with different MSC parents. The identification of causal genes controlling mitochondrial sorting in cucumber should provide insight regarding nuclear-mitochondrial interactions affecting the prevalence of specific mitochondrial DNA in plants.**

The mitochondrial (mt) DNA of cucumber (*Cucumis sativus*) has distinctive characteristics relative to most plants and include paternal transmission, a large size (1.685 Mb), and a high degree of structural polymorphisms within cultivated germplasm (Alverson et al., 2011; Havey, 1997; Havey et al., 1998; Lilly and Havey, 2001). Because the three genomes in cucumber show differential transmission (maternal for chloroplast, paternal for mitochondrial, and biparental for nuclear), phenotypes and polymorphisms can be assigned to genomes by reciprocal crossing (Havey et al., 1998). Passage of the highly inbred line B of cucumber through cell culture occasionally produces regenerated plants with a mosaic (MSC) phenotype on cotyledons and leaves (Malepszy et al., 1996). Genetic studies revealed that the MSC phenotype is paternally transmitted (Malepszy et al., 1996), and that MSC lines from independent cell-culture experiments have different rearrangements and under-represented regions in their mt DNA compared with their wild-type progenitor B (Bartoszewski et al., 2004, 2007; Ładyżyński et al., 2002). Mosaic cucumbers MSC3 and MSC16 were regenerated from different cell cultures and have different regions of their mt DNA at significantly lower copy numbers relative to inbred B (Del Valle-Echevarria et al., 2015). MSC3 has fewer copies of the polycistronic region encoding NADH dehydrogenase subunit 5 and ATP synthase subunit 4, whereas MSC16 has fewer copies of ribosomal protein S7 (Del Valle-Echevarria et al., 2015).

When wild-type cucumber plants are crossed as the female with MSC3 or MSC16 as the male, essentially all progenies show the MSC phenotype (Lilly et al., 2001; Malepszy et al., 1996). The reciprocal cross (MSC3 or MSC16 as the female parent with wild-type plants as the male) produces only wild-type progenies. Cucumber germplasm was screened by crossing wild-type plants with MSC16 as the male, and individual plants from the U.S. Department of Agriculture (USDA) Plant Introduction (PI) 401734 produced significantly higher numbers of wild-type progenies (Havey et al., 2004). A nuclear locus, *Paternal sorting of mitochondria* (*Psm*), controls sorting to the wild-type phenotype in progenies from crosses with MSC16 as the male parent (Havey et al., 2004; Lilly et al., 2001). When the female parent is homozygous for the *Psm*+ allele, progeny from the crossing of MSC16 as the male show the MSC phenotype. When the female parent is heterozygous at *Psm* (+/−), crossing with MSC16 as the male will produce approximately equal numbers of wild-type and MSC progenies. When female plants homozygous for the rarer *Psm*− allele are crossed with MSC16 as the male, essentially all progeny are wild-type (Havey et al., 2004). The *Psm* locus maps to chromosome 3 of cucumber (Al-Faifi et al., 2008; Calderon et al., 2012), and pentatricopeptide repeat (PPR) 336 has been proposed as a candidate gene for *Psm* (Del Valle-Echevarria et al., 2016). PPR proteins are targeted to the organelles, where they are involved with post-transcriptional modifications and translation (Barkan and Small, 2014). PPR336 has been shown to stabilize mitochondrial polyribosomes in *Arabidopsis thaliana* (Uyttewaal et al., 2008) and may help to maintain ribosomal function despite fewer copies of the ribosomal protein S7 gene and its

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transcript in MSC16 mitochondria (Del Valle-Echevarria et al., 2016).

When plants with different genotypes at *Psm* were crossed with both MSC3 and MSC16 as males, segregation of MSC vs. wild-type progenies differed between families from these two MSC parents (Del Valle-Echevarria et al., 2016). Because MSC3 and MSC16 have different under-represented regions of their mt DNA, we were interested in determining whether the *Psm* locus has a role in sorting to wild type from different MSC mitochondrial mutants. The goals of this research were to study the genetic basis of mitochondrial sorting in progenies from crosses with MSC3 and to determine if independent genetic control of mitochondrial sorting from different MSC parents exists.

Materials and Methods

Plants were grown in greenhouses at the University of Wisconsin in 9-L pots containing a soilless mix (Pro-Mix HP Mycorrhizae; Premier Tech Horticulture, Quakertown, PA) at temperatures between 26 and 31 °C and fertilized twice per week with 20N–8.7P–16.6K (Peters Professional 20–10–20; Everris, Dublin, OH). PI 401734 from the USDA germplasm collection was previously identified as producing higher frequencies of wild-type progenies in crosses with MSC16 as the male parent (Havey et al., 2004). *Cucumis sativus* var. *hardwickii* is a feral relative of cucumber and produces only mosaic progenies from crosses with MSC16 as the male (Calderon et al., 2012). A single plant from PI 401734 was crossed as the female with *C. sativus* var. *hardwickii*, one hybrid was self-pollinated to generate a segregating family, and F_2 plants were crossed with MSC16 as the male. Frequencies of wild-type vs. mosaic progenies were used to map the *Psm* locus (Calderon et al., 2012). Plants from this same family that were homozygous at *Psm* (+/+ or −/−) were crossed with both MSC3 and MSC16 as the males. Seeds from these crosses were grown in sterilized vermiculite at 30 °C and progenies were scored as wild-type vs. mosaic. One plant (C10630) with the genotype *Psm*−/− showed higher numbers of wild-type progenies in crosses with MSC3 as the male, and it was crossed as the male to a plant from the doubled haploid (DH) 9930 (Shen et al., 2015). One hybrid was self-pollinated to produce F_2 progenies that were crossed as the female with MSC3 as the male. Fifty seeds from each family were grown and cotyledons were scored as wild type vs. mosaic. Two fruits were produced from many of the F_2 plants, and the numbers of wild-type and mosaic progenies from each fruit were averaged. The proportion of wild-type progenies in each family from crossing with MSC3 was calculated after requiring at least 50% germination of seed and used for genetic analyses.

DNA was isolated from 80 mg of lyophilized leaves from parental plants and F_2 progenies using a kit (NucleoSpin Plant II Midi kit; Macherey-Nagel, Düren, Germany). Concentrations were determined spectrophotometrically (NanoDrop 1000; Thermo Fisher Scientific, Waltham, MA). DNA of the two parents and 122 F_2 progenies were digested with *Ape*KI (Wang et al., 2018) and genotyped by sequencing (Davey et al., 2011) at the University of Wisconsin Biotechnology Center (NovaSEq. 6000; Illumina, San Diego, CA). Skewer software (Jiang et al., 2014) was used to trim the 3′ end of fragments until a Phred quality of 20 was reached. The SNPs were identified using TASSEL 5.0 GBS Discovery Pipeline (Glaubitz et al., 2014) and the cucumber 9930 version 3.0 reference genome (Li et al., 2019) as described by Wang et al. (2018). SNPs were filtered based on ≤50% missing data and minor allele frequencies ≥5%. Linkages among SNPs were detected using JoinMap version 5.0 (Van Ooijen, 2018) with the maximum likelihood mapping algorithm and an independence logarithm of odds (LOD) greater than 6.0 for linkage. Quantitative analysis of mitochondrial sorting was completed using the R/qtl package (Broman and Sen, 2009; Broman et al., 2003) in R Studio (R Foundation for Statistical Computing, Vienna, Austria). Quantitative trait loci (QTL) were detected using the scanone, scantwo, and stepwiseqtl functions, and a LOD threshold at $P = 0.05$ was computed after 1000 permutations. The fitqtl and refineqtl functions were used to estimate effects of the candidate QTL, percentage of the phenotypic variation explained by the QTL, and the LOD-1.5 interval surrounding the most significant SNP.

Results and Discussion

Different frequencies of wild-type vs. MSC progenies were observed from crossing the same female plants with MSC3 and MSC16 as males. For 11 plants with the *Psm*+/+ genotype, crosses with MSC16 as the male produced very few (average proportion, 0.01) wild-type progenies (Table 1). When the same plant was crossed with MSC3, we observed varying numbers of wild-type and mosaic progenies (Fig. 1), with an average proportion of 0.27 ± 0.34 wild-type progenies (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 (average, 0.96 ± 0.04) (Table 1). Crosses of the same plants with MSC3 produced families with variable numbers of wild-type and mosaic progenies, with an average proportion of wild-type of 0.58 ± 0.26 (Table 1). Different segregations for wild-type vs. MSC progenies from crosses with MSC3 and MSC16 indicate that the *Psm* locus may not control sorting to the wild-type phenotype in progenies from crosses with different MSC parents.

All of the 95 progenies from the cross of DH9930 with MSC3 as the male had the mosaic phenotype (Fig. 1). Progenies from crossing plant C10630 with MSC3 as the male produced an average of 0.88 ± 0.03 wild-type progenies. DH9930 was crossed with C10630, and a single F_1 plant was self-pollinated to produce an F_2 family. A total of 122 F_2 progenies were crossed with MSC3 as the male, and the proportions of wild-type progenies (Fig. 1) ranged from 0.00 to 0.95 (Supplemental Table 1), with an average of 0.44 ± 0.25.

A total of 405,339,314 demultiplexed sequencing reads were produced across all DNAs, with an average number of 3,166,713 reads per DNAs sample. Results of three F_2 DNA were discarded because of low numbers ($<1 \times 10^5$) of demultiplexed reads. Reads were aligned to the 9930 version 3.0 reference sequence and 15,497 SNPs were identified, with an average number of 2214 SNPs per chromosome. Genetic mapping was completed using 4633 SNPs that fit the expected 1:2:1 ratio ($P > 0.05$) and after randomly selecting one SNP from groups of SNPs showing >95% identical genotypes across the progeny DNAs. 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 positions in the reference sequence; however, there were

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

	Male parent					
	MSC3			MSC16		
Genotype of female parent	WT	MSC	Prop WT	WT	MSC	Prop WT
	Progenies (no.)			Progenies (no.)		
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

Psm = Paternal sorting of mitochondria.

occasional positions at which the genetic linkages and genomic position did not agree, possibly because of mis-scoring of SNP genotypes across short genomic regions.

Fig. 1. Mosaic (MSC) progenies (left) from the crossing of cucumber doubled haploid 9930 as the female with MSC3 as the male. Wild-type progenies (right) were from the cross of an F_2 plant with MSC3 as the male.

Quantitative analysis revealed one region on chromosome 3 associated with the proportion of wild-type progenies from crosses with MSC3 as the male (Fig. 2). The most significant SNP was at basepair (bp) 34,246,720 on chromosome 3, with a LOD score of 40.0 (LOD threshold = 4.87); this explained 70.6% of the phenotypic variation. Additive and dominance effects were both significant ($P < 0.05$) and increased the numbers of wild-type progenies by 32.2% and 14.5%, respectively. These attributes are consistent with a locus at which partially dominant alleles control sorting to the wild-type phenotype in progenies from MSC3 as the male parent and differ from the co-dominance of alleles at the *Psm* locus (Calderon et al., 2012; Havey et al., 2004).

The *Psm* locus controls sorting to wild type in progenies from crosses with MSC16 as the male (Calderon et al., 2012; Havey et al., 2004), and PPR336 has been proposed as the candidate gene for *Psm* (Del Valle-Echevarria et al., 2015). This gene is located from bp 33,618,502 to 33,619,719 on chromosome 3 in the 9930 reference sequence (Li et al., 2019). The LOD-1.5 interval surrounding the most significant SNP associated with sorting to wild-type progenies from crosses with MSC3 encompassed 134 kilobases (kb) from bp 34,246,720 to 34,380,732 on chromosome 3; this region is located 627 kb from PPR336. There

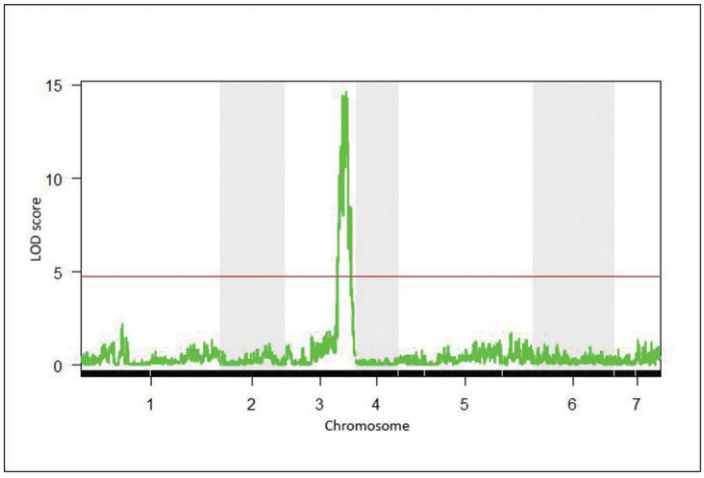

Fig. 2. Plot of logarithm of odds (LOD) scores (y-axis) for the sorting of wild-type progenies vs. marker positions across the seven chromosomes (x-axis) of cucumber. The genome-wide threshold as determined by permutation analysis at $P = 0.05$ is shown by the red line at LOD 4.87.

Table 2. Locations and annotations of cucumber genes from version 3 of the Chinese Long 9930 genomic sequence (Li et al., 2019) across the logarithm-of-odds (LOD)-1.5 interval on chromosome 3 associated with proportions of wild-type and mosaic (MSC) progenies from crosses of F_2 progenies as the female with MSC3 as the male and the corresponding most similar annotated genes in *Arabidopsis thaliana*.

Cucumber			Arabidopsis	
Gene	Location (bp)	Annotation	Gene	Annotation
CsaV3_3G042090	34238755.34249961	BAH domain-containing protein	AT3G48050.1	BAH domain; TFIIS helical bundle-like domain
CsaV3_3G042110	34250818.34254596	Trichome birefringence-like family	AT3G28150.1	Trichome birefringence-like 22
CsaV3_3G042120	34255050.34259140	Alpha dioxygenase 1	AT3G01420.1; AT1G73680.2	Alpha dioxygenase
CsaV3_3G042130	34261926.34265783	Alpha dioxygenase 1	AT3G01420.1; AT1G73680.2	Alpha dioxygenase
CsaV3_3G042140	34267524.34273157	Alpha dioxygenase 1	AT3G01420.1; AT1G73680.2	Alpha dioxygenase
CsaV3_3G042150	34278738.34282283	WAT1-related protein transmembrane	AT5G40240.2	Nodulin MtN21/EamA-like transporter family protein
CsaV3_3G042160	34287103.34290898	WAT1-related protein transmembrane	AT5G40230.1	Nodulin MtN21/EamA-like transporter family protein
CsaV3_3G042170	34294356.34301095	WAT1-related protein transmembrane	AT5G40230.1	Nodulin MtN21/EamA-like transporter family protein
CsaV3_3G042180	34301396.34310955	DNA repair protein uvh3	AT3G28030.1	5′-3′ exonuclease family protein
CsaV3_3G042190	34311732.34316260	NHL domain-containing protein	AT1G70280.2	NHL domain-containing protein
CsaV3_3G042200	34316948.34322584	Potassium transporter	AT5G14880.1	Potassium transporter family protein
CsaV3_3G042210	34340523.34341587	Transmembrane protein	AT1G23840.1	Unknown protein
CsaV3_3G042220	34341881.34342374	Unknown protein	No hit	
CsaV3_3G042230	34342794.34343783	Transmembrane protein	AT1G23850.1	Unknown protein
CsaV3_3G042240	34344343.34347962	Cyclic nucleotide-gated channel	AT5G14870.1	Cyclic nucleotide-gated channel 18
CsaV3_3G042250	34349241.34352808	ARM repeat superfamily protein isoform 1	AT5G14790.1	ARM repeat superfamily protein
CsaV3_3G042260	34351451.34351606	Unknown protein	No hit	
CsaV3_3G042280	34360054.34360270	Unknown protein	No hit	
CsaV3_3G042270	34360013.34378906	Methyl-CpG-binding domain-containing protein 9	AT3G01460.1	Methyl-CPG-binding domain 9
CsaV3_3G042290	34379311.34383476	Homeobox-leucine zipper protein HAT5	AT3G01470.1	Homeobox 1

are 20 annotated genes (Table 2) in the 9930 reference sequence (Li et al., 2019) across the LOD-1.5 interval, of which two were identified as possible candidate genes. The first candidate is alpha-dioxygenase, which has three copies (CsaV3_3G042120, CsaV3_3G042130, and CsaV3_3G042140) in the genomic region of the QTL. Alpha-dioxygenase genes are important in lipid biochemical pathways in organelles because they add oxygen to lipids to produce oxylipins, which have roles in defense responses, senescence, and stress tolerance (Machado et al., 2015). The second candidate gene (CsaV3_3G042250) encodes for an isomer of the ARM repeat superfamily proteins, which have repetitive motifs similar to PPRs and are involved with plant cell physiology, stress, and development (Manisha and Pandey, 2016). Candidate genes involved with stress response may help to mitigate the deleterious effects of the MSC phenotype, thereby enhancing the survival of MSC progenies. Genetic variants that are less effective at mitigating the stress caused by MSC may allow relatively rare wild-type mitochondrial DNA to become more predominant, resulting in wild-type progenies.

This research demonstrates that different nuclear genomic regions affect sorting to the wild-type phenotype in progenies from different MSC male parents. Larger family sizes will be necessary to confidently identify the causal genes for mitochondrial sorting from MSC3. The eventual identification of genes controlling mitochondrial sorting in cucumber should provide insight regarding specific nuclear–mitochondrial interactions affecting the predominance of mitochondria delivered to progenies and subsequent effects on the growth and vigor of plants.

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Supplemental Table 1. Total numbers of cucumber progenies and proportions of wild-type (WT) vs. mosaic (MSC) progenies from crosses of F_2 plants as the female with MSC3 as the male.

F_2 plant	Total progenies (no.)	Proportion of progenies	
		WT	MSC
C10627A	99	0.44	0.56
C10627B	93	0.37	0.63
C10627C	50	0.00	1.00
C10627D	98	0.39	0.61
C10627E	45	0.42	0.58
C10627F	61	0.12	0.89
C10627G	99	0.01	0.99
C10627H	49	0.51	0.49
C10627I	73	0.53	0.47
C10627J	50	0.18	0.82
C10627K	99	0.54	0.47
C10627L	62	0.00	1.00
C10627M	92	0.49	0.51
C10627N	31	0.71	0.29
C10627O	96	0.02	0.98
C10627P	100	0.44	0.56
C10627Q	98	0.56	0.44
C10627R	70	0.81	0.19
C10627S	49	0.45	0.55
C10627T	18	0.28	0.72
C10627U	99	0.55	0.46
C10627V	99	0.52	0.49
C10627W	51	0.00	1.00
C10627X	77	0.49	0.51
C10627Y	80	0.50	0.50
C10627Z	80	0.49	0.51
C10627AA	74	0.60	0.41
C10627AB	75	0.57	0.43
C10627AC	84	0.67	0.33
C10627AD	62	0.47	0.53
C10627AE	54	0.48	0.52
C10627AF	82	0.01	0.99
C10627AG	81	0.49	0.51
C10627AH	28	0.57	0.43
C10627AI	85	0.44	0.57
C10627AJ	56	0.32	0.68
C10627AK	50	0.68	0.32
C10627AL	62	0.26	0.74
C10627 AM	69	0.51	0.49
C10627AN	50	0.70	0.30
C10627AO	35	0.00	1.00
C10627AP	68	0.62	0.38
C10627AQ	67	0.52	0.48
C10627AR	39	0.67	0.33
C10627AS	65	0.69	0.31
C10627AT	83	0.34	0.66
C10627AU	38	0.68	0.32
C10627AV	73	0.36	0.64
C10627AW	54	0.56	0.44
C10627AX	71	0.54	0.47
C10627AY	27	0.44	0.56
C10627AZ	51	0.94	0.06
C10627BA	74	0.53	0.47
C10627BB	66	0.56	0.44

Supplemental Table 1. Continued.

F_2 plant	Total progenies (no.)	Proportion of progenies	
		WT	MSC
C10627BC	47	0.70	0.30
C10627BD	51	0.41	0.59
C10627BE	28	0.64	0.36
C10627BF	70	0.00	1.00
C10627BG	94	0.53	0.47
C10627BH	38	0.61	0.40
C10627BI	32	0.84	0.16
C10627BJ	81	0.52	0.48
C10627BK	54	0.02	0.98
C10627BL	55	0.95	0.06
C10627BM	27	0.67	0.33
C10627BN	48	0.46	0.54
C10627BO	66	0.55	0.46
C10627BP	73	0.52	0.48
C10627BQ	72	0.07	0.93
C10627BR	66	0.49	0.52
C10627BS	54	0.04	0.96
C10627BT	68	0.78	0.22
C10627BU	27	0.56	0.44
C10627BV	72	0.53	0.47
C10627BW	59	0.56	0.44
C10627BX	59	0.51	0.49
C10627BY	62	0.47	0.53
C10627BZ	78	0.64	0.36
C10627CA	68	0.41	0.59
C10627CB	74	0.01	0.99
C10627CC	89	0.52	0.48
C10627CD	72	0.49	0.51
C10627CE	89	0.01	0.99
C10627CF	36	0.06	0.94
C10627CG	76	0.55	0.45
C10627CH	78	0.00	1.00
C10627CI	80	0.50	0.50
C10627CJ	70	0.53	0.47
C10627CK	38	0.53	0.47
C10627CL	8	0.00	1.00
C10627CM	82	0.04	0.96
C10627CN	46	0.76	0.24
C10627CO	16	0.06	0.94
C10627CP	87	0.39	0.61
C10627CQ	81	0.56	0.44
C10627CR	17	0.00	1.00
C10627CS	49	0.45	0.55
C10627CT	90	0.01	0.99
C10627CU	45	0.58	0.42
C10627CV	60	0.08	0.92
C10627CW	51	0.51	0.49
C10627CX	71	0.38	0.62
C10627CY	23	0.74	0.26
C10627CZ	41	0.63	0.37
C10627DA	80	0.40	0.60
C10627DB	45	0.49	0.51
C10627DC	77	0.01	0.99
C10627DD	63	0.43	0.57
C10627DE	90	0.61	0.39

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Supplemental Table 1. Continued.

		Proportion of progenies	
F_2 plant	Total progenies (no.)	WT	MSC
C10627DF	96	0.48	0.52
C10627DG	81	0.57	0.43
C10627DH	58	0.53	0.47
C10627DI	86	0.65	0.35
C10627DJ	60	0.40	0.60
C10627DK	76	0.75	0.25
C10627DL	34	0.00	1.00
C10627DM	44	0.30	0.71
C10627DN	18	0.67	0.33
C10627DO	51	0.55	0.45
C10627DP	75	0.53	0.47
C10627DQ	42	0.60	0.41
C10627DR	69	0.58	0.42