Single Nucleotide Polymorphisms in Linkage Disequilibrium with the Male-fertility Restoration (*Ms*) Locus in Open-pollinated and Inbred Populations of Onion

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ABSTRACT. Maintainer lines are used to seed propagate male-sterile lines for the development of hybrid onion (*Allium cepa* L.) cultivars. The identification of maintainer lines would be more efficient with molecular markers distinguishing genotypes at the nuclear male-fertility restoration (*Ms*) locus. *Ms* has been mapped to chromosome 2 of onion and linked genetic markers identified. However, linkages between these markers and *Ms* were detected using F₂ or BC₁ families at maximum linkage disequilibrium and, for many markers, their efficacy to predict genotypes at *Ms* in onion populations at or near linkage equilibrium remains unknown. In this research, near isogenic lines homozygous-dominant and -recessive at *Ms* were developed and screened for 930 single nucleotide polymorphisms (SNPs). Three SNPs tightly linked on chromosome 2 remained in linkage disequilibrium with genotypes at *Ms* among randomly selected plants from three open-pollinated populations of onion as well as among a collection of inbred lines. These SNPs should be useful for selection of the recessive *ms* allele to aid in the development of maintainer lines for hybrid onion development.

Hybrid onion seed is produced using cytoplasmic-genic male sterility (CMS) systems. The most commonly used source of CMS in onion is conditioned by male-sterile (S) cytoplasm (Havey, 1993). For plants possessing S cytoplasm, a single nuclear locus (*Ms*) controls male-fertility restoration; a dominant allele at *Ms* conditions male fertility, whereas plants homozygous-recessive at *Ms* are male-sterile (Jones and Clarke, 1943). Plants possessing normal (N) cytoplasm are always male-fertile regardless of their genotype at *Ms*. Male-sterile inbred lines are seed-propagated by crossing male-sterile plants (S *msms*) with maintainer plants that possess N cytoplasm and the homozygous-recessive genotype at *Ms* (Jones and Davis, 1944). Therefore, a main goal of hybrid onion breeding programs is to identify superior maintainer (N *msms*) lines to seed-propagate male-sterile lines for hybrid development. The identification of maintainer lines can be expensive and time-consuming because of the biennial generation time of onion and the relatively high cost of using insects for crossing (Pike, 1986). To identify maintainers, male-sterile (S *msms*) plants are crossed with male-fertile (S *Ms*– or N–) plants. The male fertility of progenies from the male-sterile parent is then established. If all testcross progenies are male-fertile, the male parent was homozygous-dominant at *Ms*. If the progenies are all male-sterile, the male parent must be N *msms*. Finally, if the testcross progenies are segregating for male fertility, the male parent must be heterozygous at *Ms*. Plants that are identified as

N *msms* or N *Msms* can be then used to develop maintainer lines for backcrossing to sources of S cytoplasm.

Numerous molecular markers distinguishing N and S cytoplasm of onion have been developed (Engelke et al., 2003; Havey, 1993, 1995; Kim et al., 2009; Sato, 1998). Identification of molecular markers tightly associated with the nuclear *Ms* locus would greatly aid the development of maintainer lines. These markers would allow breeders to identify plants carrying the recessive *ms* allele early during the first year and carry forward for testcrossing only those plants with a higher probability of possessing recessive allele(s) at *Ms*. We previously reported that the genomic region carrying a restriction fragment length polymorphism (RFLP) revealed by cDNA AOB272 is tightly linked (0.9 cM) to *Ms* and converted this marker to a polymerase chain reaction (PCR)-based polymorphism (Gökçe et al., 2002). Others have either converted our previously described markers to PCR-detectable polymorphisms (Bang et al., 2011) or developed new markers linked to *Ms* (Huo et al., 2012; Yang et al., 2012). Although these markers are useful for selection of maintainer lines after crossing (i.e., at maximum linkage disequilibrium), they may not be in linkage disequilibrium with *Ms* among plants from open-pollinated (OP) populations (Gökçe and Havey, 2002). Yang et al. (2012) identified two markers linked to the *Ms* locus that were consistent across a sample of inbreds and hybrids; however, they proposed that their markers will be useful to genotype plants from OP populations without actually testing their use. In this research, I identified SNPs that are in linkage disequilibrium with alleles at the *Ms* locus across OP and inbred populations of onion.

Materials and Methods

Near-isogenic lines (NILs) differing for genotypes at *Ms* were developed by self-pollinating individual plants from the

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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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OP population ‘Sapporo-Ki’ (SK) and at the same time testcrossing to the male-sterile line ‘MSU611-1AxMSU611B’. Pollinations were completed using flies (Jones and Emsweller, 1934). At least 20 progenies from each testcross family were scored in the field for male-fertility restoration over 2 to 4 years. S_1 progenies that were heterozygous at *Ms* (i.e., testcross family segregated for male-fertility restoration) were again self-pollinated and testcrossed to MSU611-1AxMSU611B. This process was repeated through the S_3 generation, selecting in each generation single heterozygous plants for self-pollination and testcrossing. Two full-sib S_3 progenies were identified as heterozygous at *Ms* and were self-pollinated to produce the S_4 generation. S_4 progenies were then identified as homozygous-dominant or -recessive at the *Ms* locus and were separately intercrossed to produce S_4 NILs that are N *MSMS* vs. N *msms*. DNA was isolated from these two NILs as described by Bark and Havey (1995). A total of 930 SNPs were scored between the two NIL populations using the KASPar assay (LGC Genomics, Beverly, MA) as previously reported by Duangjit et al. (2013).

Individual plants from OP populations ‘Brigham Yellow Globe’ (BYG), ‘Mountain Danvers’ (MD), and SK were randomly selected and paired with male-sterile lines. Pollinations were completed using flies and seed was harvested separately from the male-fertile (S_1 families) and male-sterile (testcross families) plants. At least 20 progenies from each testcross family were evaluated over at least 2 years and scored as male-fertile (male parent was *MsMs*), segregating for male fertility (male parent *Msms*) or male-sterile (male parent *msms*) as previously reported by Gökçe and Havey (2002). DNA was isolated as described by Bark and Havey (1995) from at least 25 plants from each S_1 family and a collection of inbred lines developed and released by U.S. public-sector researchers (Table 1) and genotyped for SNPs polymorphic between the two NILs using KASPar (Duangjit et al., 2013).

Results and Discussion

Testcrosses of the two NILs from SK to male-sterile MSU611-1AxMSU611B revealed 100% male-fertile and

male-sterile testcross progenies, confirming that the NILs are homozygous-dominant and -recessive at the *Ms* locus, respectively. Of 930 SNPs, 29 remained polymorphic between the NILs (Table 2) and mapped to chromosomes 1, 2, 4, 5, and 8 (Duangjit et al., 2013). SNPs on chromosomes 1, 4, and 8 were located at the ends of linkage groups (Duangjit et al., 2013) and may have remained heterozygous between the two NILs as a result of reduced crossing over. The NILs were heterozygous for numerous SNPs on chromosomes 2 and 5 (Table 2).

The *Ms* locus has been mapped to chromosome 2 and showed tight linkage to an RFLP revealed by cDNA clone AOB272 (Gökçe et al., 2002). SNPs isotig34671_610, isotig30856_1351, isotig29186_1830, isotig17237_4883, isotig28524_1455, isotig39878_664, and isotig29609_387 mapped to the same region on chromosome 2 as AOB272 in either or both the BYG15-23 × AC43 or OH1 × 5225 families (Duangjit et al., 2013). To estimate the degree of linkage disequilibrium with *Ms*, DNAs from 148 plants from OP populations BYG (31), MD (54), and SK (63) were genotyped for the 29 SNPs. These plants had been previously genotyped at *Ms* (Gökçe and Havey, 2002) and the estimated allelic frequencies combined across all three populations for *Ms* and *ms* were 0.59 and 0.41, respectively. Because these three onion populations have been maintained by open pollination, only markers very near *Ms* should remain in linkage disequilibrium. As expected, all SNPs on chromosomes 1, 4, 5, and 8 and many of those on chromosome 2 were over 30% recombinant between genotypes at *Ms* (Table 2). Importantly three SNPs on chromosome 2 [isotig34671_610, isotig30856_1351, and isotig29186_1830 (Table 3)] remained in disequilibrium with *Ms* across the three OP onion populations, with 11.0%, 13.0%, and 15.7% recombinant gametes from this random sample of plants (Table 2). No recombination among these SNPs was observed in segregating families from BYG15-23 × AC43 and OH1 × 5225 (Duangjit et al., 2013). These three SNPs were also consistent for genotypes at *Ms* across a collection of public-sector inbred lines of onion, although there was recombination detected in B1828B, MSU8155B, and W52 for SNP isotig34671_610 (Table 1). Putative annotations of and

Table 1. Genotypes at the nuclear male-fertility restoration locus (*Ms*) and single nucleotide polymorphisms (SNPs) among public-sector onion inbreds.^z

Inbred	Source ^y	Genotype at <i>Ms</i>	SNP genotype ^x		
			isotig34671_610	isotig30856_1351	isotig29186_1830
YB986A	USDA	<i>msms</i>	T:T	A:A	G:G
B1750A	USDA	<i>msms</i>	T:T	A:A	G:G
B1750B	USDA	<i>msms</i>	T:T	A:A	G:G
B1789B	USDA	<i>msms</i>	T:T	A:A	G:G
B1794B	USDA	<i>msms</i>	T:C	A:A	G:G
B1828B	USDA	<i>msms</i>	T:C	A:A	G:G
B2117B	USDA	<i>msms</i>	T:T	A:A	G:G
B2215C	USDA	<i>MSms</i>	T:C	G:A	G:A
MSU2399B	MSU	<i>msms</i>	T:T	A:A	G:G
MSU611-1B	MSU	<i>msms</i>	T:T	A:A	G:G
MSU8155B	MSU	<i>msms</i>	T:C	A:A	G:G
W101B	UW	<i>msms</i>	T:T	A:A	G:G
W205B	UW	<i>msms</i>	T:T	A:A	G:G
W404B	UW	<i>msms</i>	T:T	A:A	G:G
W52B	UW	<i>msms</i>	T:C	A:A	G:G

^zSNP genotypes shown in bold indicate that recombination was detected between the SNP and the recessive *ms* allele.

^yUSDA = U.S. Department of Agriculture, Beltsville, MD; MSU = Michigan State University, East Lansing; UW = University of Wisconsin, Madison.

^xSequences flanking SNPs and primers used for genotyping were reported by Duangjit et al. (2013).

Table 2. Percent recombination detected between the recessive *ms* allele and single nucleotide polymorphisms (SNPs) among random plants from three open-pollinated populations of onion.

SNP ^z	Recombination (%)	Frequency of nucleotide in coupling phase with recessive <i>ms</i> allele	Chromosome no.
isotig34671_610	11.0	T (0.37)	2
isotig30856_1351	13.0	A (0.46)	2
isotig29186_1830	15.7	G (0.45)	2
isotig35505_474	30.0	G (0.43)	Not mapped
isotig33680_1027	32.5	T (0.45)	5
isotig17237_4883	34.6	T (0.44)	2
isotig30879_537	35.5	G (0.55)	2
isotig32346_843	37.5	A (0.47)	Not mapped
isotig30110_847	37.8	A (0.70)	Not mapped
isotig28524_1455	38.0	G (0.64)	2
isotig25881_1343	38.1	C (0.40)	5
contig00676_1004	38.8	A (0.48)	1
isotig36793_769	39.0	A (0.59)	4
isotig27259_617	40.5	C (0.70)	1
isotig32358_978	40.6	C (0.58)	Not mapped
isotig34668_1112	41.0	G (0.63)	Not mapped
isotig42686_290	41.3	T (0.66)	8
isotig39878_664	41.8	C (0.66)	2
isotig35125_241	42.0	C (0.57)	5
isotig32712_566	43.1	A (0.62)	3
isotig15820_1734	43.2	A (0.66)	Not mapped
isotig29167_1843	44.8	T (0.61)	5
isotig26178_547	45.5	G (0.67)	5
isotig29102_2197	45.5	A (0.81)	1
isotig34751_952	46.9	A (0.68)	5
isotig31622_435	49.0	A (0.78)	Not mapped
isotig29609_387	50.3	T (0.74)	2
isotig37821_673	52.4	G (0.82)	4
isotig12617_836	56.5	G (0.79)	2

^zNames and sequences flanking SNPs and primers used for amplifications were reported by Duangjit et al. (2013).

sequences flanking the three cDNAs in linkage disequilibrium with *Ms* are listed in Table 3. Although isotig30856_1351 shows significant similarity to pentatricopeptide repeat-containing proteins, a class of nuclear genes often conditioning male-fertility restoration (Schmitz-Linneweber and Small, 2008), recombination between the SNP in this cDNA and *Ms* was detected (Table 2).

The SNPs listed in Table 3 must be tightly associated with the *Ms* locus to remain in linkage disequilibrium in OP populations

and should be useful for selecting plants carrying the recessive *ms* allele for development of maintainer lines. However, because recombination between these SNPs and the *Ms* locus was detected among the OP and inbred populations, the success of these SNPs to predict genotypes at *Ms* will vary among onion populations. I recommend that an onion population be crossed with S-cytoplasmic, male-sterile line(s) and testcross seed evaluated for male-fertility restoration. Once it is established that the recessive *ms* allele is present in the population, individual

Table 3. Annotations and complementary (c) DNA sequences flanking single nucleotide polymorphisms (SNPs) tightly associated with the male-fertility restoration (*Ms*) locus of onion.^z

Onion cDNA	Annotation	Sequence ^y
isotig34671_610	Auxin-induced protein	TGCAGCAACTGTCAAANATTGGATCAATCCGAAAAAGCAAGTGAAGGC TGTTACAGAAAG[C/T]CTGGCTGGATACTGCTTTAGCACTGGAACCTG AAGAACCATCCATCCAGACCAGGCGAGA
isotig30856_1351	Pentatricopeptide repeat-containing protein	GTTGAAGCACTATGGATGCATGGCTGATCTGTATGGACGGTCTGGATTG GTTGATGAGGC[G/A]aTGGAGATGATAGAAGGGATGCCGATGAAGGC GGATGAGTATGTGTGGGGAGCGGTGCTT
isotig29186_1830	RNA polymerase II C-terminal domain phosphatase-like 2	GAATGATCCGAGTAAGAACAATGTTACGATCTGACAATCGAATAACCGG ACGAGAAGCAC[A/G]GgTTTGTGCACCAGGTAATTGCAAGACTTGTTCG TTTTGTGCAGATACTGGCATCCCCATT

^zPolymorphic nucleotides are shown in brackets. These sequences were previously reported by Duangjit et al. (2013) and are included here for convenience only.

^yLower case letters indicate nucleotides with lower quality (<Q40) calls as determined by Newbler software (Duangjit et al., 2013).

plants should be genotyped for isotig34671_610, isotig30856_1351, and isotig29186_1830 to determine if these SNPs are polymorphic, and to select individual plants carrying the SNP allele(s) putatively in coupling phase with the recessive *ms* allele (Table 2). If these markers remained in linkage disequilibrium with the *Ms* locus in the population, discarding plants with SNP alleles in coupling phase with the dominant *Ms* allele should allow for efficient development of maintainer lines from selected plants or increase the frequency of the recessive *ms* allele in progenies from intercrossing among the selected plants.

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