Efficacy of Molecular Markers Jnurf13 and AcPms1 for Prediction of Genotypes at the Nuclear Ms Locus in Three Open-pollinated Populations of Onion from North America

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Abstract. Seed of hybrid onion (Allium cepa L.) is produced using cytoplasmic male sterility (CMS). For the most widely used source of onion CMS, male sterility is conditioned by the interaction of male sterile (S) cytoplasm and the homozygous recessive genotype at the nuclear male fertility locus Ms. Because of the biennial generation time of onion, classical crossing and segregation analyses take years to establish cytoplasmic and genotypes at Ms. Numerous molecular markers have been developed to distinguish onion cytoplasms and estimate genotypes at Ms. Two nuclear markers (jnurf13 and AcPms1) have been reported to cosegregate with Ms and correctly predict genotypes in commercial breeding lines and diverse onion germplasm; however, these markers were less predictive for open-pollinated (OP) populations from India. We evaluated the efficacy of jnurf13 and AcPms1 to correctly classify genotypes at Ms using 144 random plants from three OP populations of long-day onion from North America. No recombination events were detected between AcPms1 and the Ms locus and three events occurred between jnurf13 and Ms. Our results support either marker as a useful tool to predict genotypes at Ms in North American populations of onion, with AcPms1 being the better of the two.

Hybrid onion (Allium cepa L.) cultivars are widely grown around the world due in part to hybrid vigor and greater uniformity. Because onion has perfect flowers, production of hybrid seed is based on systems of CMS. For the most widely used CMS in onion, male sterility is conditioned by the interaction of male sterile (S) cytoplasm and the homozygous recessive genotype (msms) at the nuclear male fertility locus Ms (Jones and Clarke, 1943). Plants with dominant allele(s) at Ms and S cytoplasm are male fertile. All plants possessing normal (N) male fertile cytoplasm are male fertile regardless of genotypes at Ms. Because of the biennial generation time of onion, the use of classical crossing to determine cytoplasms and genotypes at Ms is a slow process requiring years. Significant effort has been made to identify molecular markers that distinguish onion cytoplasms and predict genotypes at Ms. S cytoplasm is an alien cytoplasm that was introgressed into onion populations (Havey, 1993), and therefore, many molecular markers in the chloroplast and mitochondrial DNAs confidently distinguish N and S cytoplasms (Havey, 1993, 1995; Kim et al., 2009; Sato, 1998; von Kohn et al., 2013). Many molecular markers have also been described that show linkage to the nuclear Ms locus (Bang et al., 2013; Gökçe et al., 2002; Havey, 2013; Hoo et al., 2012; Kim, 2014; Kim et al., 2015; Park et al., 2013; Yang et al., 2012), even though in some cases few polymorphisms were screened on relatively small numbers of segregating progenies. The plethora of nuclear markers showing linkage to Ms can be explained by the location of this locus close to the centromere of chromosome 2 in a region of relatively low recombination (Khrustaleva et al., 2016). Nevertheless these nuclear markers may be in linkage disequilibrium with Ms and useful to predict genotypes at Ms, allowing breeders to advance for crossing only plants likely to possess the desired genotype. Kim (2014) and Kim et al. (2015) identified two indel markers (jnurf13 and AcPms1, respectively) that cosegregated with Ms in a large F2 family and consistently predicted genotypes at Ms in commercial breeding lines and diverse onion germplasm. The marker AcPms1 resides in the onion homolog of mismatch repair (Msh1), which was proposed as a candidate gene for Ms (Kim et al., 2015). However Khar and Saini (2016) used markers jnurf13 and AcPms1 to genotype random plants from Indian populations of onion and reported that both markers did not always predict correct genotypes at Ms. In this study, we establish the efficacy of markers jnurf13 and AcPms1 to classify genotypes at Ms using random plants from three OP populations of North American onion.

Materials and Methods

Genotypes at Ms were determined for 144 random plants from three OP populations of long-day storage onions (‘Brigham Yellow Globe’, ‘Mountain Danvers’, and ‘Sapporo-Ki’) and DNAs were isolated as previously described (Gökçe et al., 2002). Primers and conditions for the polymerase chain reaction (PCR) for marker jnurf13 were as described by Kim (2014). For marker AcPms1, we obtained inconsistent results using conditions for PCR reported by Kim et al. (2015) and therefore developed a touchdown PCR which more consistently produced amplicons of expected sizes. Reactions consisted of 1x PCR Buffer of the manufacturer (Promega, Fitchburg WI), 2.5 mM MgCl2, 0.25 mM NTP, 1.25 unit Taq polymerase, and 1 μM of primers with 50 ng of onion DNA. Cycling conditions were 95 °C for 5 min; six cycles of 95 °C for 30 s, 69 °C for 1 min with a decrease of 2 °C per cycle, and 72 °C for 45 s; seven cycles of 95 °C for 30 s, 59 °C for 1 min with a decrease of 1 °C per cycle, and 72 °C for 45 s; and then 27 cycles of 95 °C for 30 s, 52 °C for 1 min, and 72 °C for 45 s. Amplicons for both markers were resolved through 8% acrylamide gels and visualized by silver staining.

Results and Discussion

The jnurf13 and AcPms1 markers were reported to cosegregate with alleles at Ms for a large F2 family and to predict genotypes at Ms across diverse onion germplasm (Kim, 2014; Kim et al., 2015). We genotyped these two markers using 144 random plants from three OP populations of long-day onions from North America, which had been previously scored for genotypes at Ms (Gökçe et al., 2002) and showed evidence of linkage equilibrium near Ms (Gökçe and Havey, 2002). No recombination events were detected between AcPms1 and the Ms locus for all plants from the three OP populations. We identified three recombination events between jnurf13 and Ms (Fig. 1). Kim et al. (2015) reported recombination between jnurf13 and AcPms1 in USDA PI 233186.
These results clearly support either marker as a useful tool to predict genotypes at Ms, with AcPms1 being the better of the two.

Kim et al. (2015) proposed AcPms1 as a candidate gene for Ms; however, Khar and Saini (2016) reported that this marker did not accurately predict male fertility restoration for random S-cytoplasmic plants from Indian populations. This inconsistency could be due to rare recombination event(s) between AcPms1 and Ms, or possibly different male fertility restoration locus (loci) for S-cytoplasmic onion in Indian populations. The genetics of male fertility restoration for S cytoplasm were studied by Jones and Clarke (1943) using crosses of the original source of S cytoplasm (Italian Red 13-53) with a few North American populations. Onion was likely domesticated in Central Asia and spread in a western direction toward the Mediterranean region, through Europe and North Africa, to North and South America, and ultimately from North America to Japan and East Asia (Goldman et al., 2000). Onion also spread in a south-eastern direction from Central Asia into the Indian subcontinent. As a result, onion germplasm commonly grown in East Asia may be of European origin and share the same male fertility restoration locus for S cytoplasm as European and American (Northern and Southern) germplasm, as evidenced by the effectiveness of jnurf13 and AcPms1 to predict genotypes at Ms for North American (this research) and East Asian (Kim et al., 2015) onions. Robust cytoplasmic [such as the accD polymorphism (Khar and Saini, 2016; von Kohn et al., 2013)] and nuclear (AcPms1) markers should help to resolve inconsistencies regarding the genetics of male fertility restoration across different onion populations.

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