The Dominant Ms Allele in Onion Shows Reduced Penetrance

Sergio Melgar
Department of Horticulture, University of Wisconsin, Madison, WI 53706 and Escuela de Biología, Universidad de San Carlos, Guatemala City 01012, Guatemala

Michael J. Havey
U.S. Department of Agriculture, Agricultural Research Services, Department of Horticulture, University of Wisconsin, Madison, WI 53706

ABSTRACT. The most commonly used source of cytoplasmic male sterility in onion (Allium cepa) is controlled by the interaction of the cytoplasm [male-sterile (S) or normal male-fertile (N)] and one nuclear male-fertility-restoration locus (Ms). Scoring of genotypes at Ms is generally done by testcrossing male-fertile to male-sterile (S ms,ms) plants, followed by scoring of testcross progenies for male-fertility restoration. We identified two N-cytoplasmic families, one that was homozygous dominant and the other segregating at Ms. Plants from each of these two families were individually testcrossed to male-sterile onion. Nuclear restoration of male fertility in testcross progenies was evaluated in the field over 4 years. For male plants homozygous dominant at Ms, we expected testcross families to show 100% male-fertility restoration, but observed mean values between 46% and 100%. For plants segregating at Ms, we again observed lower than expected frequencies of male-fertility restoration. These results demonstrate that the dominant Ms allele shows reduced penetrance, requiring that male-fertility restoration be scored over years to more confidently assign genotypes at Ms.

Materials and Methods

CROSSES AND FIELD EVALUATIONS. Two F2—massed (M) families from a cross between ‘Brigham Yellow Globe 15–23’ and ‘Ailsa Craig (AC) 43’ were used in this study (King et al., 1998). Plants in both families possessed N cytoplasm. One family (16292) was heterozygous (Msms) and the second family (16278) was homozygous dominant (MsMs) at the Ms locus based on testcrosses to male-sterile lines and genotypes at flanking molecular markers (King et al., 1998). Individual random plants from both families were self-pollinated and used as the male parents in testcrosses to individual plants from male-sterile lines B3350A × B2352B or B1750A × B1794B (both S msms). At least 120 seeds from each testcross family were planted in commercial fields at Pymatuning, WI, under normal production conditions. Bulbs were harvested and vernalized over the winter. In May of each year, ~25 bulbs from each testcross family were planted in fields at Madison or Arlington, WI, and were allowed to flower during the years 2003, 2004, 2006, and 2007. Flowers were evaluated daily for pollen production by rubbing mature anthers with fingers or against black construction paper. Once pollen was detected, the
scape was tagged and was not evaluated further. Flowers in untagged umbels were repeatedly scored over the entire flowering period of the umbel. Multiple umbels per plant were independently scored. After flowering, plants were dug and scored as male-fertility-restored if one umbel was tagged.

**Results and Discussion**

**Field evaluations for male fertility restoration.** Family 16278 was previously scored as homozygous dominant at Ms (Gokce et al., 2002; King et al., 1998). Molecular markers surrounding Ms were all in the parental (AC43) phase, supporting the homozygous dominant genotype, and we expected all testcross progenies to be male-fertile. However, mean male fertility in testcross progenies over years varied between 46% and 100% (Fig. 1). Assuming uniform expression of the S-cytoplasmic factor, this result indicates that the dominant allele at Ms may not show complete penetrance. Jones and Clarke (1943) also reported significantly fewer male-fertile progenies for three of eight segregating families grown in greenhouses, consistent with reduced penetrance of the dominant allele at Ms.

Family 16292 segregated at Ms. Random plants from 16292 were crossed with male-sterile plants and these testcross families were expected to segregate 1:2:1 for all male-sterile to equal numbers of male-fertile and male sterile to all male-fertile progenies. We observed 10 testcross families with no or very few male-fertile progenies and were scored as S msms (Fig. 2). The differences were less clear for testcross families presumably originating from crosses with males heterozygous or homozygous dominant at Ms. To objectively determine the point where a change in slope occurred, the average coefficient of determination of the two slopes was calculated from the best fit curve for testcross families from heterozygous and the homozygous dominant male parents. The best fit was obtained after scoring testcross family 20187 as segregating at Ms (Fig. 2). Based on this criterion, segregations fit the expected 1:2:1 ratio ($\chi^2 = 1.04, P = 0.59$). For 28 testcross families from male parents scored as heterozygous at Ms (families 20249 to 20187 in Fig. 2), 11 fit the expected 1:1 ratio of male-fertile (S Msms) to male-sterile (S msms) testcross progenies. For the remaining 17 families that showed poor ($P < 0.05$) fits to the expected 1:1 ratio, 14 had too many male-sterile and only three families had too many male-fertile testcross progenies, consistent with reduced penetrance of Ms.

Environmental factors (such as nutritional and water deficiencies or high temperatures), pests (insects or diseases), and/or other genetic factors could affect pollen production (Barham
Barham and Munger (1950) studied temperature effects on pollen production in S-cytoplasmic male-sterile lines and found that high temperatures after emergence of scapes increased the amount of viable-appearing pollen; however, no selfed seeds were produced on these S-cytoplasmic plants. In chive (Allium schoenoprasum), a restorer locus has been reported that produces viable pollen at high temperatures (Engelke et al., 2004). In spite of these complicating factors, we observed three testcross families (20187, 20235, and 20275) from male parents heterozygous at Ms that showed average proportions of male-fertile plants close to expected 0.5 value with lower variation across years for male-fertility restoration (indicated with arrows in Fig. 2). This consistent expression of male-fertility restoration could be due to fewer environmental effects on expression of the dominant Ms allele in these specific genetic backgrounds.

Some pollen production was detected in testcross families scored as male-sterile (families 20253 to 20197 in Fig. 2), indicating that production of pollen may occur even if the plant is putatively homozygous recessive at Ms. A similar observation was reported by Clarke and Pollard (1949), who observed an average of 4% self-pollination in male-sterile lines. However, the occasional production of fertile pollen in the male-sterile lines of Clarke and Pollard (1949) could be due to low-frequency contamination of the maintainer line by the dominant Ms allele. In rapeseed, a similar phenomenon was observed and partially maintaining genotypes were identified (Pahwa et al., 2004).

**Microscopy.** Microscopic analyses of pollen from male-fertility-restored plants showed normal pollen grains with elliptical shape and two obvious nuclei (Fig. 3A). Male-sterile anthers possessed misshapen microspores often clumped together (Fig. 3B) that did not mature to viable pollen. Microscopic analysis also revealed differences for production of viable pollen in male-fertility-restored plants (Fig. 4), with viable and empty pollen grains being produced. This variation among male-fertility-restored plants may be due to genetic background or environmental factors such as high temperatures at critical times of flower development (Barham and Munger, 1950; Meer and Bennekom, 1969). Ockendon and Gates (1976) reported variation for pollen viability among flowers from the same plant. In Beta vulgaris ssp. maritima, a similar phenomenon was observed and male-fertility-restored plants showed variability for pollen production and viability (Dufoy et al., 2008). Similar variation was found in the Tournefortii male sterility of rapeseed (Brassica napus), in which some genotypes showed different degrees of male-fertility-restoration and were explained as interactions with genetic background (Pahwa et al., 2004).

**Conclusions**

Reduced penetrance of the dominant Ms allele and occasional occurrence of viable pollen in S msms plants makes it difficult to confidently evaluate testcross families. To correctly score genotypes at Ms, evaluations must be done over environments, the entire flowering period, and across numerous testcross progenies. An interesting observation was that some testcross families (20187, 20235, and 20275 in Fig. 2) showed more consistent male-fertility restoration over years. These families should be studied further to determine if this...
phenotypic stability is heritable, which would be useful for identifying genetic markers in linkage disequilibrium with the Ms locus.

**Literature Cited**


