Seed Yield, Floral Morphology, and Lack of Male-fertility Restoration of Male-sterile Onion (*Allium cepa*) Populations Possessing the Cytoplasm of *Allium galanthum*

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ABSTRACT. The primary source (S cytoplasm) of cytoplasmic-genic male sterility (CMS) used to produce hybrid-onion (*Allium cepa* L.) seed traces back to a single plant identified in 1925 in Davis, California. Many open-pollinated populations also possess this cytoplasm, creating an undesirable state of cytoplasmic uniformity. Transfer of cytoplasm from related species into cultivated populations may produce new sources of CMS. In an attempt to diversify the cytoplasm conditioning male sterility, the cytoplasm of *Allium galanthum* Kar. et Kir. was backcrossed for seven generations to bulb-onion populations. The flowers of galanthum-cytoplasmic populations possess upwardly curved perianth and filaments with no anthers, making identification of male-sterile plants easier than for either S- or T-cytoplasmic male-sterile onion plants. Mean seed yield per bulb of the galanthum-cytoplasmic populations was measured in cages using blue-bottle flies (*Calliphora erythrocephala* Meig.) as pollinators and was not significantly different from one of two S-cytoplasmic male-sterile F₁ lines, a T-cytoplasmic male-sterile inbred line, or N-cytoplasmic male-fertile lines. Male-sterile lines possessing either the S or galanthum cytoplasm were each crossed with populations known to be homozygous dominant and recessive at the nuclear locus conditioning male-fertility restoration of S cytoplasm and progenies were scored for male-fertility restoration. Nuclear restorers of male fertility for S cytoplasm did not condition male fertility for the galanthum-cytoplasmic populations. It is intended that these galanthum-cytoplasmic onion populations be used as an alternative male-sterile cytoplasm for the diversification of hybrid onion seed production.

Hybrid-onion (*Allium cepa*) cultivars predominate because of increased yield and uniformity (Jones and Davis, 1944; Joshi and Tandon, 1976). The onion umbel contains perfect flowers with mature pollen and receptive stigmas present at the same time. Emasculation on a large scale is not practical. Production of hybrid-onion seed is based on systems of cytoplasmic-genic male sterility (CMS). Two CMS systems have been characterized genetically and are used commercially to produce hybrid seed (Berninger, 1965; Jones and Clarke, 1943). The first source of onion CMS was discovered in the cultivar 'Italian Red' (Jones and Emsweller, 1936); male-sterile plants possess sterile (S) cytoplasm and are homozygous recessive at a single nuclear male-fertility restoration locus (*Ms*) (Jones and Clarke, 1943). A second source of CMS (T cytoplasm) was discovered by Berninger (1965) in the cultivar 'Jaune paille des Vertus'. Schweisguth (1973) identified three independently segregating loci affecting male-fertility restoration for T cytoplasm, either by a dominant allele at one locus (*A-*) or at both of two complementary loci (*B-C-*).

Except for a few hybrids in Holland and Japan generated using cytoplasm resembling T, the great majority of hybrid-onion cultivars are produced using S cytoplasm (Havey, 1994). This

source of sterile cytoplasm traces back to a single onion plant identified in Davis, California, in 1925 (Jones and Emsweller, 1936). Using polymorphic restriction-enzyme sites in the chloroplast (cp) and mitochondrial (mt) DNAs distinguishing normal (N) male-fertile and S cytoplasm of onion, we (Havey, 1993; Havey and Bark, 1994) and others (Courcel et al., 1989; Holford et al., 1991) established that S cytoplasm commonly occurs among open-pollinated (OP) populations of onion widely grown in Europe, Japan, New Zealand, and the United States. In areas producing both seed and bulbs (e.g., California and Idaho), S-cytoplasmic populations and hybrids are under continuous cultivation. This cytoplasmic uniformity is ominous (National Academy of Science, 1972) and alarmingly like the scenario that led to the epidemic of southern corn leaf blight [*Bipolaris maydis* (Nisikado & Miyake) Shoemaker] on maize (*Zea mays* L.) (Miller and Koepe, 1971). Diversification of male-sterile cytoplasm in onion is imperative to reduce this genetic uniformity.

Expression of CMS reflects a nuclear-cytoplasmic incompatibility that can occur spontaneously, appear after treatment with mutagens, or result from interspecific crosses (Hanson and Conde, 1985). Transfer of the cytoplasm from related species has produced CMS in many genera, including beet (*Beta* L.) (Boutin et al., 1987), petunia (*Petunia* Juss.) (Izhar, 1984), and tobacco (*Nicotiana* L.) (Gerstel, 1980). The cytoplasmic diversity of hybrid-onion production systems could be increased by transfer to onion of a male-sterility-inducing cytoplasm from a related species. *Allium* section *Cepa* (Mill.) Prokh. contains the bulb onion and the related species *A. galanthum*, *A. oschaninii* O. Fedtsch., *A. pskemense* B. Fedtsch., and *A. vavilovii* M. Pop. et Vved. (Vvedenskij, 1944). The closely related section *Phyllodolon* (Salisb.) Prokh. contains *A. altaicum* Pall. and *A. fistulosum* L. (Vvedenskij, 1944). Hanelt (1985) placed the species of sections

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Cepa and *Phyllodolon* into a single Section *Cepa*. Interspecific progenies are rare between bulb onion and the wild species *A. oschaninii* and *A. pskemense* (McCollum, 1974; Raamsdonk et al., 1992). Maternal phylogenies estimated using polymorphisms in the chloroplast DNA placed *A. oschaninii* and *A. pskemense* outside of a well supported clade containing *A. altaicum*, *A. cepa* (both N and S cytoplasm), *A. fistulosum*, *A. galanthum*, and *A. vavilovii*. Within this well supported maternal clade, the cytoplasm of *A. vavilovii* is closely related to and may be the donor of N cytoplasm of onion (Havey, 1997). Interspecific crosses between onion and *A. fistulosum* are highly sterile (Emsweller and Jones, 1935; Valk et al., 1991) and show deleterious nuclear-cytoplasmic interactions (Bark et al., 1994; Ulloa et al., 1995). Interspecific hybrids have been generated between bulb onion and the wild species *A. galanthum* (McCollum, 1980; Raamsdonk et al., 1992) and *A. roylei* Stearn (Meer and Vries, 1990) making possible the transfer of these cytoplasm to bulb onion. McCollum (1971, 1980) observed both male and female sterility in *A. galanthum* by *A. cepa* interspecific hybrids and generated amphidiploids to seed propagate the hybrids. In this research, seed yield, floral morphology, and male-fertility restoration of cytoplasmic male-sterile onion populations possessing the cytoplasm of *A. galanthum* are described.

MATERIALS AND METHODS

Plant introduction (PI) 280091 of the U.S. Department of Agriculture (USDA) was used as the source of the *A. galanthum* cytoplasm. The initial interspecific hybrid and four generations of backcrosses to bulb-onion populations were generated by G. McCollum, USDA Agricultural Research Service, Beltsville, Md. In 1989, the author received the BC4 populations CMS-ga 614, 2215, 8111, 8152, 8154, 8155, 8540, and 8543 from G. McCollum. Plants were grown in the field and, after storage over the winter at 6 °C, planted with N-cytoplasmic male-fertile plants of long-day storage germplasm. Male fertility was scored in each generation by visual examination of anthers and only male-sterile plants were used in backcrosses. Umbels of individual male-sterile and male-fertile parents were covered with cotton-mesh cages and crossed using house (*Musca domestica* Linn.) or blue-bottle flies (Pike, 1986). Three additional backcrosses to *A. cepa* were completed.

Seed yield and male-fertility restoration of galanthum-cytoplasmic lines were evaluated. In 1996 and 1997, at least 10 bulbs of three BC7 families (CMS-ga 614, 8111, and 8152) were planted in two mesh 4 × 5-m cages (replications) together with the same bulb numbers of male-sterile S (MSU5718A × MSU8155B and MSU611-1A × MSU611B) and T (RJ70A) cytoplasm. The S-cytoplasmic male-sterile lines are used commercially to produce the hybrids 'Sweet Sandwich' (Peterson et al., 1986) and 'Spartan Banner 80', respectively. Male-fertile populations in cages were N-cytoplasmic S₁ families selected from the open-pollinated populations 'Brigham Yellow Globe', 'Mountain Danvers', and 'Sapporo-Ki'. The male-fertile S₁ families in one cage were known to be homozygous dominant (*MsMs*) at the nuclear male-fertility restoration locus for S cytoplasm; in the second cage, the S₁ families were homozygous recessive (*msms*) (Havey, unpublished). The male fertility or sterility of all caged plants was scored individually. Ten bulbs of each of at least four male-fertile populations were planted in each cage to ensure that copious pollen was available. Blue-bottle flies were used as pollinators. Umbels from each entry in the cages were harvested

separately, total seed yield measured, and seed yield per flowering bulb calculated. Male-fertility restoration of the S- and galanthum-cytoplasmic populations was scored in 1998 over the entire life of the umbels for bulbs grown from the 1996 cage-produced seed.

DNA was isolated from the galanthum-cytoplasmic populations (Havey, 1991). Previously characterized polymorphisms in the chloroplast (cp) genome that distinguish among the species of *Allium* section *Cepa* (cpDNA-1, -2, -3, -4, -5, -8, -9, -11, -15, -17, -18, -19, -21, -24, -25, -26, -27, -28, -29, -30, -31, -32, -35, -36, -37, -38, -39, -40, and -41) were evaluated to confirm cytoplasm (Havey, 1992, 1993). DNAs from B1750A (male-sterile S-cytoplasmic inbred), B1750B (male-fertile N-cytoplasmic maintainer of B1750A), and *A. galanthum* PI 280091 were used as controls.

Results and Discussion

The interspecific hybrid and BC4 families generated by G. McCollum had *A. galanthum* PI 280091 as the female parent.

Fig. 1. Umbels of (A) galanthum-cytoplasmic and (B) nuclear male-fertility restored S-cytoplasmic onion. Note curved perianth and lack of anthers in galanthum-cytoplasmic flowers.

This PI was identical to the galanthum-cytoplasmic populations for all evaluated cp DNA polymorphisms (autoradiograms not shown). Both PI 280091 and the galanthum-cytoplasmic backcross populations possessed character 29, an autapomorphic gain of an *EcoRI* site unique to the cytoplasm of *A. galanthum* (Havey, 1992).

BC4 seed received from G. McCollum produced white, red, and yellow bulbs of poor quality (i.e., doubled and cracked bulbs, poor outside skins, and short storage ability). An additional three generations of backcrossing to yellow long-day storage germplasm was completed for CMS-ga 614, 8111, and 8152; backcrossing with the other *A. galanthum* populations was abandoned because of poor storage ability or poor female fertility. The BC7 generations produced commercially acceptable yellow bulbs showing varying degrees of storage ability (bulbs not shown). Bulbs, leaves, and scapes were morphologically indistinguishable from *A. cepa*. However, the male-sterile flowers of the galanthum-cytoplasmic populations were easily distinguished from male-fertile flowers. Stamens possessed filaments with no anthers and the perianth was reduced in size and tended to rotate upwards (Fig. 1). This obvious morphological distinction would be advantageous to workers removing male-fertile plants from seed-production fields. Because blue-bottle flies were used as pollinators, I did not determine if bee visitations, and therefore seed yield, would be reduced by the lack of anthers or the curved perianth restricting bee access to the nectar.

In addition to conditioning male sterility, alien cytoplasmic can reduce female fertility (McCollum, 1971; Valk et al., 1991). Mean seed yields per flowering bulb of each of the three galanthum-cytoplasmic populations with that of S- and T-cytoplasmic male-sterile lines and N-cytoplasmic male parents were compared. Averaged across all populations, 87% $\pm$ 8% of planted bulbs flowered. I chose to use a diverse set of male-fertile *S₁* families from 'Brigham Yellow Globe', 'Mountain Danvers', and 'Sapporo-ki' to ensure that copious amounts of pollen were available when the male-sterile populations were receptive as females. Mean seed yields of the three galanthum-cytoplasmic lines were not significantly different from those of the male-fertile N-cytoplasmic populations, the T-cytoplasmic male-sterile inbred line, and the MSU5718A x MSU8155B *F₁* female line (Table 1). The *F₁* female line, MSU611-1A x MSU611B, yielded significantly greater seed than all other populations except MSU5718A x MSU8155B (Table 1).

Restoration of male fertility in the galanthum-cytoplasmic lines was evaluated using the nuclear allele (*Ms*) known to restore male fertility in S cytoplasm (Jones and Clarke, 1943). For all three galanthum-cytoplasmic populations, all plants were male sterile regardless whether the pollinizing population was homozygous dominant or recessive at the *Ms* locus. As expected, fertility restoration of the two S-cytoplasmic populations was 100% when pollinated by the homozygous-dominant pollinizer populations and 0% when pollinated by the homozygous-recessive populations. Absence of male-fertility restoration indicates known restorers of S cytoplasm, such as 'Pukekohe Longkeeper' (Havey, 1993) or 'Texas Early Grano 502 PRR' (Havey and Bark, 1994), can be used as maintainers for the galanthum-cytoplasmic male-sterile lines.

This research demonstrates that galanthum-cytoplasmic onion populations are a potentially useful source of CMS. The complete absence of anthers makes the galanthum CMS system extremely easy to rogue in the field (Fig. 1). Female fertility, as determined using cages and flies, was not significantly different

Table 1. Mean seed yield of cytoplasmic male-sterile and pollinizer populations of onion (*Allium cepa*).

Population ^z	Cytoplasm ^y	Mean seed yield ^a
(MSU611-1A x MSU611B)	S	8.3 $\pm$ 1.0
(MSU5718A x MSU8155B)	S	5.7 $\pm$ 1.0
CMS-ga 8152A	G	4.8 $\pm$ 1.0
Male-fertile <i>S₁</i> families	N	4.0 $\pm$ 0.4
CMS-ga 614A	G	3.5 $\pm$ 0.4
CMS-ga 8111A	G	2.9 $\pm$ 0.7
RJ70A	T	2.1 $\pm$ 1.0
LSD ^w		2.7

^zFor descriptions of populations, see Materials and Methods.

^yMale-sterile cytoplasmic are S (Jones and Clarke, 1943), T (Schweisguth, 1973), and G (*Allium galanthum*) as described in the text. N = the normal male-fertile population of the bulb onion.

^aMean seed yield in grams per flowering bulb $\pm$ SE.

^wLSD = least significant difference at *P* = 0.05.

from that of S-, T-, and N-cytoplasmic populations (Table 1). It is intended that these galanthum-cytoplasmic onion populations be used with S and T cytoplasmic to diversify the male-sterile cytoplasmic used commercially to produce hybrid-onion seed.

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