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

M.J. Havey

Vegetable Crops Unit, Agricultural Research Service, U.S. Department of Agriculture, Department of Horticulture, University of Wisconsin, Madison, WI 53706

**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, a series of experiments were conducted to evaluate the possibility that other CMS sources might be suitable for replacement of the primary S cytoplasm. Male-fertility restoration of T cytoplasm, either by a dominant or a recessive nuclear gene at the nuclear locus conditioning male-fertility restoration of S cytoplasm, was evaluated in crosses of populations possessing either the S or *Allium galanthum* cytoplasm. Nuclear restorers of male fertility for S cytoplasm did not condition male fertility for the *Allium galanthum* cytoplasm, nor did nuclear restorers of male fertility for the *A. galanthum* cytoplasm condition male fertility for S cytoplasm. Male-sterile lines possessing either the S or *A. 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 *A. galanthum* cytoplasm. It is intended that these *A. galanthum*-cytoplasm 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 cytoplasm-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’. Schweigsguth (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 cytoplasms 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 cytoplasms 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-cytoplasm populations and hybrids are under continuous cultivation. This cytoplasmic uniformity is ominous (National Academy of Science, 1972) and alarming 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 cytoplasms 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.*) (Gersel, 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 *Phylldolon* (Salisb.) Prokh. contains *A. alticaum* Pall. and *A. fistulosum* L. (Vvedenskij, 1944). Hanelt (1985) placed the species of sections

Received for publication 6 Nov. 1998. Accepted for publication 21 July 1999.

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. I thank Mark Petrashek for technical assistance and Jean-Marie Boussac, Clause Seed Company, Brétigny-Sur-Orge, France, for providing the source of T cytoplasm. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

1USDA research geneticist and associate professor of horticulture.
Cepa and Phylodolon 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 cytoplasms), 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; Uloa 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 cytoplasms 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 x 5-m cages (replications) together with the same bulb numbers of male-sterile S (MSU5718A x MSU8155B and MSU611-1A x MSU611B) and T (RJ70A) cytoplasms. 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 S families selected from the open-pollinated populations ‘Brigham Yellow Globe’, ‘Mountain Danvers’, and ‘Sapporo-Ki’. The male-fertile S 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 S families were homozygous recessive (mssm) (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 cytoplasms (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 cytoplasms 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% ± 8% of planted bulbs flowered. I chose to use a diverse set of male-fertile S1 families showing varying degrees of storage ability (bulbs not shown). 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 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 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 cytoplasms to diversify the male-sterile cytoplasms used commercially to produce hybrid-onion seed.

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

| Population | Cytoplasm | Mean seed yieldx ± SE | LSDw | P |
|------------|-----------|------------------------|------|--|
| (MSU611-1A x MSU611B) | S | 8.3 ± 1.0 | | |
| (MSU5718A x MSU8155B) | S | 5.7 ± 1.0 | | |
| CMS-ga 8152A | G | 4.8 ± 1.0 | | |
| Male-fertile S, families | N | 4.0 ± 0.4 | | |
| CMS-ga 614A | G | 3.5 ± 0.4 | | |
| CMS-ga 8111A | G | 2.9 ± 0.7 | | |
| RJ70A | T | 2.1 ± 1.0 | | |

*For descriptions of populations, see Materials and Methods.

aMale-sterile cytoplasms 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.

bMean seed yield in grams per flowering bulb ± SE.

cLSD = least significant difference at P = 0.05.

dFrom 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 cytoplasms to diversify the male-sterile cytoplasms used commercially to produce hybrid-onion seed.

### Literature Cited

Bark, O.H., J.N. Corgan, and M.J. Havey. 1994. RFLP analysis of progeny from an interspecific hybrid between Allium fistulosum and Allium cepa. J. Amer. Soc. Hort. Sci. 119:1046–1049.

Barninger, E. 1965. Contribution a l’etude de la sterilité male de l’oignon (Allium cepa L.). Ann. Amélior. Plantes 15:183–199.

Boutin, V., G. Pannenbecker, W. Ecke, G. Schewe, P. Saumitou-Laprade, R. Jean, P. Vernet, and G. Michaelis. 1987. Cytoplasmic male sterility and nuclear restorer genes in a natural population of Beta maritima: Genetical and molecular aspects. Theor. Appl. Genet. 73:625–629.

Courc’el, A. de, F. Veder, and J. Boussac. 1989. DNA polymorphism in Allium cepa cytoplasms and its implications concerning the origin of onions. Theor. Appl. Genet. 77:793–798.

Emsweller, S.L. and H.A. Jones. 1935. An interspecific hybrid in Allium. Hilgardia 9:265–273.

Gerstel, D.U. 1980. Cytoplasmic male sterility in Nicotiana: A review. N.C. Agr. Expt. Sta. Tech. Bul. 263:1–31.

Hanfelt, P. 1983. On taxonomy, chorology and ecology of the wild species of Allium sect. Cepa (Mill.) Prokh. Flora 176:99–116.

Hanson, M. and M. Conde. 1985. Functioning and variation of cytoplasmic genomes: Lessons from cytoplasmic–nuclear interactions affecting male fertility in plants. Int. Rev. Cytol. 94:213–267.

Havey, M.J. 1991. Phylogenetic relationships among cultivated Allium species from restriction enzyme analysis of the chloroplast genome. Theor. Appl. Genet. 81:752–757.

Havey, M.J. 1992. Restriction enzyme analysis of the chloroplast and nuclear 45s ribosomal DNA of Allium sections Cepa and Phylldolont. Plant Systematics Evol. 183:17–31.

Havey, M.J. 1993. A putative donor of S cytoplasm and its distribution among open-pollinated populations of onion. Theor. Appl. Genet. 86:128–134.

Havey, M.J. 1994. The cytoplasms of sterile lines used to produce commercial hybrid-onion seed. Allium Improvement Nws1. 4:25–27.

Havey, M.J. 1997. On the origin and distribution of normal cytoplasm of commercial hybrid-onion seed. J. Amer. Soc. Hort. Sci. 122:90–93.

Holford, P., J. Croft, and H. Newbury. 1991. Differences between, and possible origins of, the cytoplasms found in fertile and male-sterile onions (Allium cepa L.). Theor. Appl. Genet. 82:737–744.
Izhar, S. 1984. Male sterility in Petunia, p. 77–91. In: K.C. Sink (ed.). Monographs on theoretical and applied genetics: Petunia. Springer Verlag, Heidelberg, Germany.

Jones, H. and A. Clarke. 1943. Inheritance of male sterility in the onion and the production of hybrid seed. Proc. Amer. Soc. Hort. Sci. 43:189–194.

Jones, H. and G. Davis. 1944. Inbreeding and heterosis and their relation to the development of new varieties of onions. USDA Tech. Bul. 874.

Jones, H. and S. Emsweller. 1936. A male sterile onion. Proc. Amer. Soc. Hort. Sci. 34:582–585.

Joshi, H. and J. Tandon. 1976. Heterosis for yield and its genetic basis in the onion. Indian J. Agr. Sci. 46:88–92.

McCollum, G. 1971. Sterility of some interspecific Allium hybrids. J. Amer. Soc. Hort. Sci. 96:359–362.

McCollum, G. 1974. Chromosome behavior and sterility of hybrids between the common onion, Allium cepa, and the related wild A. oschaninii. Euphytica 23:699–709.

McCollum, G. 1980. Development of the amphidiploid of Allium galanthum x A. cepa. J. Hered. 71:445–447.

Meer, Q. van der and J. de Vries. 1990. An interspecific cross between Allium roylei Stearn and Allium cepa L. and its backcross to A. cepa. Euphytica 47:29–31.

Miller, R. and D. Koepppe. 1971. Southern corn leaf blight: Susceptible and resistant mitochondria. Science 173:67–69.

National Academy of Science. 1972. Genetic vulnerability of major crops. Natl. Acad. Sci. (USA), Wash., DC.

Peterson, C.E., P.W. Simon, and L.A. Ellerbrook. 1986. ‘Sweet Sandwich’ onion. HortScience 21:1466–1468.

Pike, L.M. 1986. Onion breeding, p. 357–394. In: M.J. Bassett (ed.). Breeding vegetable crops. AVI Publishing, Westport, Conn.

Raamsdonk, L. van, W. Wietzma, and J. de Vries. 1992. Crossing experiments in Allium L. section Cepa. Bot. J. Linn. Soc. 109: 293–303.

Schweisguth, B. 1973. Etude d’un nouveau type de sterilite male chez l’oignon, Allium cepa L. Ann. Amélior. Plantes 23:221–233.

Ulloa, M., J.N. Corgan, and M. Dunford. 1995. Evidence for nuclear–cytoplasmic incompatibility between Allium fistulosum and Allium cepa. Theor. Appl. Genet. 90:746–754.

Valk, P. van der, S. de Vries, J. Everink, F. Verstappen, and J. de Vries. 1991. Pre- and post-fertilization barriers to backcrossing the interspecific hybrid between Allium fistulosum L. and A. cepa L. with A. cepa. Euphytica 53:201–209.

Vvedenskij, A. 1944. The genus Allium in the USSR. Herbertia 11:65–218.