Theor Appl Genet (2000) 101:778–782

M.J. Havey

Diversity among male-sterility-inducing and male-fertile cytoplasms of onion

Received: 27 October 1999 / Accepted: 12 February 2000

Abstract Hybrid-onion (*Allium cepa*) seed is produced using systems of cytoplasmic-genic male sterility (CMS). Two different sources of CMS (S and T cytoplasms) have been genetically characterized. Testcrosses of N-cytoplasmic maintaining and restoring genotypes to S and T cytoplasmic lines demonstrated that different alleles, or loci, restore male fertility for these two male-sterile cytoplasms. Other sources of CMS have been used or reported in Europe, Japan and India, and their relationships to S and T cytoplasms are not clear. Restriction fragment length polymorphisms were identified in the organellar genomes among commercially used male-sterile cytoplasms from Holland, Japan and India, and were compared to S and T cytoplasms. Mitochondrial DNA diversity among 58 non-S-cytoplasmic open-pollinated onion populations was also assessed. All five putative CMS lines selected from the Indian population Nasik White Globe were identical to S cytoplasm for all polymorphisms in the chloroplast genome, and always possessed the same-sized mitochondrial fragments as S cytoplasm. T cytoplasm, the male-sterile cytoplasm used to produce the Dutch hybrid Hygro F_1, and two sources of CMS from Japan, were similar and showed numbers of mitochondrial polymorphisms similar to those observed among the 58 non-S-cytoplasmic open-pollinated populations. This research demonstrates that the same, or very similar, male-sterile cytoplasms have been independently isolated and exploited for hybrid-seed production in onion.

Key words Cytoplasmic male sterility · Nuclear male fertility restoration · Organellar DNA · *Allium cepa*

Communicated by D.R. Pring

M.J. Havey
United States Department of Agriculture,
Agricultural Research Service, Department of Horticulture,
1575 Linden Drive, University of Wisconsin,
Madison, WI 53706, USA

Introduction

The production of hybrid-onion seed requires a male sterility system. Although outcrossing is encouraged by protandry (Currah and Ockendon 1978), the onion umbel is composed of hundreds of small perfect flowers that shed pollen when adjacent flowers have receptive stigmas. Large-scale emasculation is not practical. Cytoplasmic-genic male sterility (CMS) is the most economical and widely used method to produce hybrid-onion seed; although asexual propagation of individual male-sterile plants has been used on a limited scale (Jones et al. 1949; Fujieda et al. 1979; Pike and Yoo 1990). Two sources of CMS have been genetically characterized in onion. S cytoplasm is widely used by seed producers because of stable male sterility over many environments, no reduction in female fertility, and the relatively common occurrence of the recessive allele at the nuclear male fertility restoration locus (*MS*) allowing seed propagation of male-sterile lines (Jones and Clarke 1943). The second source of male-sterile cytoplasm (T) was identified by Berninger (1965), genetically characterized by Schweisguth (1973), and is used to produce hybrid-onion seed in Europe.

In the 1970s maize hybrids generated using T cytoplasm suffered an epidemic of Southern Corn Leaf Blight (Pring and Lonsdale 1989; Levings 1993), forcing recognition of the cytoplasmic uniformity and genetic vulnerability of major food crops (Anonymous 1972). Today, some of our most economically important crops are still too genetically uniform and vulnerable to major epidemics. The bulb onion (*Allium cepa* L.) is a case in point. Annually, the US onion crop is the third most-valuable commercial vegetable, averaging $ 665 000 000 through 1993–95, and following only tomato and lettuce (USDA 1995). The majority of hybrid-onion cultivars are produced using S cytoplasm (Havey 1995). This source of sterile cytoplasm traces back to a single onion plant identified in Davis, Calif., in 1925 (Jones and Emsweller 1936). Numerous research groups (Courcel et al. 1989; Holford et al. 1990; Havey 1993, 1995; Satoh

et al. 1993; Sato 1998) identified restriction fragment length polymorphisms (RFLPs) in the organellar genomes distinguishing normal (N) male-fertile and S cytoplasms of onion. These RFLPs have been used to demonstrate that many open-pollinated (OP) populations of onion also possess S cytoplasm (Courcel et al. 1989; Havey 1993; Satoh et al. 1993; Havey and Bark 1994), including ones grown widely in the USA (NuMex Sunlite and Texas Grano 1015Y), Japan (Sapporo-Ki), and New Zealand (Pukekohe Longkeeper). Often bulb and seed production occurs in the same areas, e.g., the Central Valley of California or the Treasure Valley of Idaho, resulting in year-round cultivation of onion and an undesirable state of genetic uniformity.

Additional sources of CMS may exist in onion. A putative CMS was recently extracted from the Indian population 'Nasik White Globe' (Pathak and Gowda 1993); however, genetic studies on this putative CMS have not appeared in print. Other potentially unique sources of CMS have been isolated from Japanese populations (Mr. Toyoshi Iwata, Shippo Seed Company, Japan, personal communication). Dutch seed-companies use CMS of uncertain origin to produce Rijnsburger-type hybrids. The relationships among S, T, and these other male-sterile cytoplasms are not known. The objective of the present study was to evaluate for RFLPs among S, T, and commercially used male-sterile cytoplasms from Holland, India and Japan, and to determine if different male-sterile cytoplasms are used commercially to produce hybrid-onion seed. Preliminary results of this study were published in a non-refereed format (Havey 1994). I also compared the mitochondrial polymorphisms among these CMS sources with the diversity observed among non-S-cytoplasmic open-pollinated onion populations (Havey 1997). To study the nuclear restoration of male fertility among S and T cytoplasms, I testcrossed maintainer and restorer lines of S cytoplasm and a maintainer of T cytoplasm to S- and T-cytoplasmic male-sterile lines and scored male fertility restoration.

Materials and methods

Genomic DNA was isolated (Havey 1991) from inbred populations possessing normal (N) male-fertile, S, T, and uncharacterized male-sterile cytoplasms (Table 1). I included DNA from 45 USDA plant introductions (PIs) of onion previously demonstrated to not possess S cytoplasm (Havey 1997): 164807, 168960, 168961, 168967, 174019, 174021, 175571, 177247, 179167, 181929, 207456, 210994, 218059, 222228, 222698, 222764, 223327, 233186, 233187, 235353, 236025, 239633, 243008, 251021, 251509, 262915, 262919, 262920, 262924, 262925, 262928, 264140, 269415, 269417, 269418, 269423, 271309, 271312, 273626, 274781, 274782, 280554, 288274, 288275 and 288692. These PIs were from the former USSR, the Indian subcontinent, the Middle East, or the Mediterranean region and were introduced into the USDA system prior to 1964. The following OP populations from Asia were provided by Dr. C. Pathak [Asian Vegetable Research and Development Center (AVRDC), Taiwan] and were included in the chloroplast and mitochondrial evaluations: Agri Found Dark Red (India), Agri Found Light Red (India), Akta Niketan (India), Kalpatiya (Sri Lanka), N-53 (India), Nasik Red (India), Phule Sayed (India), Poona Red (India), Pusa Madhvi (India), Pusa Red (India), Pusa White (India), Red Bombay (Tanzania), Red Pinoy (Philippines) and Tahirpuri (Bangladesh). Restriction-enzyme digestions, electrophoresis, alkaline transfer to filters, and hybridization conditions have been previously described (Havey 1991, 1993). The DNAs listed in Table 1 and the OP populations from AVRDC were evaluated for 29 polymorphisms (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) in the chloroplast DNA that distinguish between *Allium* species closely related to onion (Havey 1992, 1993). All DNAs were evaluated for polymorphisms at or between restriction-enzyme sites by hybridizing with the mitochondrial clones *cox1* (Isaac et al. 1985), *cox2* (Fox and Leaver 1981), *cox3* (Hiesel et al. 1987), *cob* (Dawson et al. 1984), *atp6* (Dewey et al. 1985) and *atpα* (Morikami and Nakamura 1987), to blots carrying single restriction-enzyme digests of *Bam*HI, *Bst*EII, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Sac*I, *Xba*I or *Xho*I. Polymorphisms were scored as band-size differences on autoradiograms. Fragment sizes were estimated by comparison to *Hin*dIII-digested λ DNA (Schaffer and Sederoff 1981).

At least ten bulbs of two S-cytoplasmic F_1 male-sterile lines (MSU5718A × MSU8155B and MSU611–1A × MSU611B) and a T-cytoplasmic male-sterile inbred line (RJ70 A) were planted in three 4 × 5 m mesh cages. In the first cage at least ten bulbs of RJ70B, the male-fertile maintainer of T cytoplasm, were planted as the source of pollen. In the second cage, at least ten bulbs of BYG15–23B, a male-fertile maintainer of S cytoplasm, were planted. In the third cage, the sources of pollen were five N-cytoplasmic S_1 families selected from the open-pollinated populations Brigham Yellow Globe, Mountain Danvers and Sapporo-Ki, known to be homozygous dominant (*MsMs*) at the nuclear male fertility restoration locus for S cytoplasm (Havey, unpublished). After establishing the male fertility or sterility of all caged plants, honey bees or blue-bottle flies were introduced as pollinators. Umbels from each entry in the cages were separately harvested, seed cleaned, and bulbs produced, vernalized, and flowered. At least 25 bulbs were planted and male fertility restoration of the S- and T-cytoplasmic populations were scored over at least 2 years.

Table 1 Sources and cytoplasmic types of commercially used onion populations evaluated for restriction fragment length polymorphisms in their chloroplast and mitochondrial DNAs

Population	Type	Source
B1750 A	USDA S-cytoplasmic inbred (Jones and Clarke 1943)	USA
B1750 B	N-cytoplasmic inbred maintainer of B1750 A	USA
RJ70 A	T-cytoplasmic male-sterile inbred from Clause Seed Company (Schweisguth 1973)	France
RJ70 B	N-cytoplasmic inbred maintainer of RJ70 A	France
Hygro F_1	Rijnsburger-type hybrid from Bejo Seed Company	Holland
57–3 A	Male-sterile inbred from Shippo Seed Company	Japan
57–3 B	N-cytoplasmic inbred maintainer of the 57–3 A	Japan
Imai Wa-Se A	Male-sterile inbred from Shippo Seed Company	Japan
OMI13, 7, 5, 8 and M11I1	Male-sterile populations from Nasik White Globe (Pathak and Gowda 1993)	India

Results and discussion

Cytoplasms of OP populations from AVRDC

Chloroplast polymorphisms demonstrated that the Asian populations Agri Found Light Red, Akta Niketan, Kalpatiya, N-53, Nasik Red, Phule Sayed, Pusa Madhvi, Pusa Red, Pusa White, Red Bombay, Red Pinoy and Tahirpuri do not possess S cytoplasm at levels revealed by DNA gel-blot analyses (Havey 1993). The population Poona Red possessed a mixture of N and S cytoplasms, with N cytoplasm predominating (autoradiograms not shown).

Mitochondrial DNA diversity among non-S-cytoplasmic OP populations

I previously used polymorphisms in the chloroplast genome to demonstrate that most open-pollinated onion populations introduced into the USDA plant germplasm system prior to 1964 do not possess S cytoplasm (Havey 1997). However, these chloroplast polymorphisms also do not distinguish between normal (N) male-fertile and T cytoplasmic populations (Havey 1993). I evaluated mitochondrial DNA diversity among 45 non-S-cytoplasmic OP populations (Havey 1997) and the 13 non-S-cytoplasmic Asian populations from AVRDC described above, scoring the most-commonly occurring fragments as wild-type. The majority of mitochondrial probe-enzyme combinations did not reveal RFLPs among these non-S-cytoplasmic OP populations. When RFLPs were present, polymorphic fragments were often shared among several populations (Fig. 1 A). Four non-S-cytoplasmic OP populations were distinguished from the other populations for numerous mitochondrial probe-enzyme combinations (autoradiograms not shown): Pusa Red (*cob*/*Eco*RI, *cox2*/*Eco*RI, *cox3*/*Eco*RV, *atp6*/*Eco*RI, *atp6*/*Eco*RV and *atpα*/*Hin*dIII); N-53 (*cox2*/*Eco*RI); Red Pinoy (*atp6*/*Eco*RV, *cox1*/*Bam*HI, and *cox2*/*Eco*RI); and PI273626 (*cox2*/*Eco*RV and *cob*/*Eco*RI). The occurrence of RFLPs for numerous mitochondrial probe-enzyme combinations indicates that these populations may possess a relatively unique cytoplasm, as compared to the other populations in this sample of onion germplasm.

Mitochondrial DNA diversity among commercially used sources of CMS

The most-widely used source of CMS is S cytoplasm (Jones and Clarke 1943). This CMS source is most likely an alien cytoplasm (Havey 1993) and differs from N cytoplasm for many polymorphisms in the chloroplast and mitochondrial genomes (Courcel et al. 1989; Holford et al. 1991; Havey 1993, 1995; Satoh et al. 1993; Sato 1998). All five putative CMS lines from Nasik White Globe were identical to S cytoplasm for all polymorphisms in the chloroplast genome (autoradiograms not shown), and always possessed the S-cytoplasmic fragments for all mitochondrial probe-enzyme combinations (Fig. 1B). These results indicate that the putative CMS sources from Nasik White are identical, or very similar, to S cytoplasm.

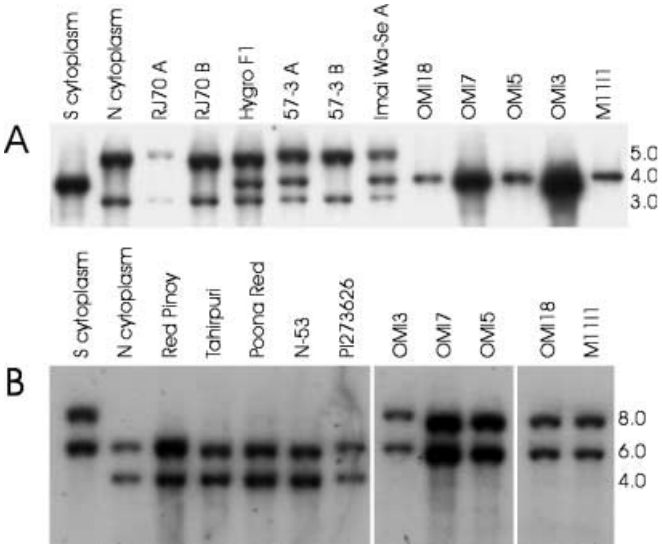

Fig. 1 Representative restriction fragment length polymorphisms in the mitochondrial genome among male-sterile (*S*) cytoplasm and its maintainer (*N*), T cytoplasm (*RJ70 A*) and its maintainer (*RJ70B*), N cytoplasmic populations (*Red Pinoy, Tahirpuri*, *Poona Red, N-53*, and plant introduction *273626*), other M-cytoplasmic sources of CMS (*Hygro F1, 53–7 A*, and *Imai Wa-Se-A*) and a maintainer (*57–3B*), and the Nasik-White (*OMI3, OMI5, OMI7, OMI18*, and *M11I1*) sources of putative cytoplasmic male sterility. For descriptions of populations, see Materials and methods and Table 1. Probe-enzyme combinations usedare *cox1* with *Xba*I (**A**) and *cox2* with *Eco*RV (**B**). Probes are described in the Materials and methods. Approximate fragment sizes in kilobases are shown on the right.

A second genetically characterized source of CMS is T cytoplasm (Berninger 1965; Schweisguth 1973). Courcel et al. (1989) assigned both N and T cytoplasms to the M cytoplasmic group, which was clearly distinguished from S cytoplasm (Courcel et al. 1989; Havey 1995). No chloroplast polymorphisms were observed among RJ70 A, RJ70 B, 57–3 A, 57–3 B, Imai Wa-Se A, and the male-sterile cytoplasm used to produce Hygro F_1, indicating that they are all members of the M-cytoplasmic class (Courcel et al. 1989). Although some mitochondrial polymorphisms were observed among these cytoplasms, none were consistent across all male-sterile cytoplasms (Table 2). I previously reported that T cytoplasm and its N-cytoplasmic maintainer can be distinguished by *cob* and *Xba*I (Havey 1995); however, it is not likely that this polymorphism is associated with the expression of CMS because the N-cytoplasmic fragment was present in Hygro F_1 (Table 2). These results imply that the T-cytoplasmic source of CMS, or closely related types, have been independently isolated from diverse onion populations. In spite of their different geographical origins, it is possible that the same mitochondrial lesion(s) conditions male sterility in Imai Wa-Se A, RJ70 A, Hygro F_1 and 57–3 A.

Table 2 Approximate sizes of fragments in kilobases revealed by polymorphic mitochondrial probe-enzyme combinations among non-S-cytoplasmic sources of male-sterile cytoplasms and their maintainers (when available)

Line[a]	Fragment sizes in kb			
	atp6/*Hin*dIII	*atpα*/*Xba*I	*cob*/*Xba*I[b]	*cox1*/*Xba*I
RJ70 A	9.0	>12.0[c]	8.0	np[d]
RJ70 B	9.0	5.0	6.0	np
Hygro F1	9.0	5.0	6.0	4.0
57–3 A	9.0	>12.0	8.0	4.0
57–3 B	9.0	>12.0	8.0	np
Imai Wa-Se A	>12.0	>12.0	8.0	4.0

[a] For origins of onion lines, see Table 1. RJ70 A and B are T cytoplasm and its maintainer, respectively. 57–3 A and B are a source of Japanese male-sterile cytoplasm and its maintainer, respectively. Maintainers for Imai Wa-Se A and the male-sterile line used to produce the hybrid Hygro F_1 were not available

[b] Polymorphism previously reported by Havey (1995)

[c] Fragment sizes listed as >12.0 were too large to be resolved confidently on 0.8%-agarose gels

[d] np = fragment not present

Male fertility for plants possessing S cytoplasm is restored by a dominant allele at a single locus (Jones and Clarke 1943). Male fertility restoration for T cytoplasm is more complex and is conditioned by at least three independently segregating loci (Schweisguth 1973). Although the genetics of male fertility restoration are different, the relationships among these male fertility restoration loci for S and T cytoplasms are unclear. I test-crossed known maintainers of S and T cytoplasm to S and T cytoplasmic male-sterile inbreds. Maintainers of S cytoplasm did not restore male fertility in the T-cytoplasmic male-sterile line RJ70 A. A maintainer of T cytoplasm (RJ70B) did not restore male fertility in S cytoplasm and was scored as homozygous recessive at the *Ms* locus. As expected, all crosses of maintainers to their male-sterile counterparts [(MSU5718A × MSU8155B) × BYG15–23B, (MSU611–1A × MSU611B) × BYG15–23B, and RJ70A × RJ70B] produced only male-sterile testcross progenies. The testcrosses of known restorers of S cytoplasms to the T-cytoplasmic inbred RJ70 A did not show male fertility restoration. Although I did not have a known restorer of T cytoplasm to complete all possible testcrosses, these results clearly demonstrate that a maintainer of T cytoplasm did not restore male fertility for S cytoplasm and a restorer of male fertility for S cytoplasm was a maintainer for T cytoplasm. Therefore, different loci or different alleles at the same locus or loci must condition the restoration of male fertility for S and T cytoplasm.

Vries and Wietsma (1992) generated interspecific hybrids between Hygro F_1 as the male-sterile female parent and *Allium roylei* Stearn. They reported that a hybrid plant was male-fertile, indicating that *A. roylei* possessed nuclear factors restoring male fertility for an onion male-sterile cytoplasm. My study demonstrates that Hygro F_1 possesses a T-cytoplasmic-like CMS system and that the male fertility restoration locus, or loci, in *A. roylei* may therefore not be the same as the *Ms* locus restoring male fertility for S cytoplasm.

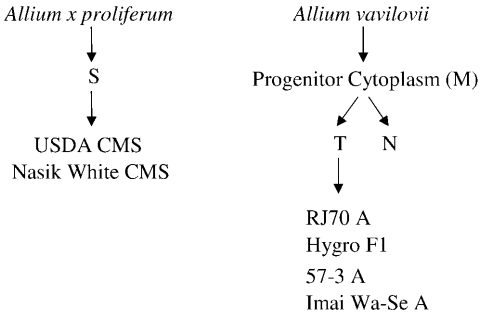

Fig. 2 Origins and relationships among normal (*N*) male-fertile and sterile (*S and T*) cytoplasms of the bulb onion as estimated from restriction enzyme analyses of the chloroplast and mitochondrial DNAs. This diagram expands upon the relationships first proposed by Courcel et al. (1989) using results presented in this and earlier (Havey 1993, 1995, 1997) publications

These experiments establish that the same, or very similar, male-sterile cytoplasms have been independently isolated and exploited for hybrid-seed production in onion. Using these results and those from earlier publications (Havey 1993, 1995, 1997), I can expand upon the relationships among CMS sources previously proposed by Courcel and co-workers (1989) (Fig. 2). S cytoplasm is most likely an alien cytoplasm transferred to onion via *Allium* x *proliferum* (Moench) Schrad. (Havey 1993). S cytoplasmic-like sources of CMS have been independently extracted from Italian Red (Jones and Emsweller 1936) and Nasik White Globe (Pathak and Gowda 1993). N and T cytoplasms most likely originated from a progenitor M cytoplasm (Courcel et al. 1989), which I proposed came from the wild species *Allium vavilovii* M. Pop. et Vved. (Havey 1997). T-cytoplasmic-like sources of CMS may have been extracted from Japanese (57–3 A and Imai Wa-Se A) and Dutch (cytoplasm used to produce Hygro F1) populations. Because most of the world's hybrid-onion seed is produced using S or T cytoplasms, it would be prudent for seed companies to establish which source(s) of CMS is (are) in use and to consider diversifying cytoplasms to reduce an undesirable state of genetic uniformity.

Acknowledgements Names are necessary to report factually on available data; however, the 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 help. I gratefully acknowledge sources of male-sterile cytoplasms from Dr. Jean-Marie Boussac, the Clause Seed Company (T cytoplasm), Dr. C.S. Pathak, AVRDC (Sources of Nasik-White cytoplasm), and Mr. Toyoshi Iwata, the Shippo Seed Company (57–3 A and Imai Wa-Se A). I also thank Dr. Pathak for providing seed of the Asian open-pollinated populations. This project was partially funded by USDA/OICD project TW18.

References

Anonymous (1972) Genetic vulnerability of major crops. National Academy of Science USA, Washington, D.C.

Berninger E (1965) Contribution a l'etude de la sterilite male de l'oignon (*Allium cepa* L.). Ann Amélior Plant 15:183–199

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

Currah L, Ockendon D (1978) Protandry and the sequence of flower opening in the onion. New Phytol 81:419–428

Dawson A, Jones V, Leaver C (1984) The apocytochrome b gene in maize mitochondria does not contain introns and is preceded by a potential ribosome binding site. EMBO J 3:2107–2113

Dewey R, Levings C III, Timothy D (1985) Nucleotide sequence of the ATPase subunit 6 gene of maize mitochondria. Plant Physiol 79:914–919

Fox T, Leaver C (1981) The *Zea mays* mitochondrial gene coding cytochrome oxidase subunit II has an intervening sequence and does not contain TGA codons. Cell 28:315–323

Fujieda K, Matsuoka N, Fujieda Y (1979) Vegetative multiplication of onion, *Allium cepa* L., through tissue culture. J Jpn Soc Hort Sci 48:186–194

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

Havey M (1992) Restriction enzyme analysis of the chloroplast and nuclear 45 s ribosomal DNA of *Allium* sections *Cepa* and *Phyllodolon*. Plant Syst Evol 183:17–31

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

Havey M (1994) The cytoplasms of sterile lines used to produce commercial hybrid-onion seed. *Allium* Improv Newslett 4: 25–27

Havey M (1995) Cytoplasmic determinations using the polymerase chain reaction to aid in the extraction of maintainer lines from open-pollinated populations of onion. Theor Appl Genet 90:263–268

Havey M (1997) On the origin and distribution of normal cytoplasm of onion. Genetic Resour Crop Evol 44:307–313

Havey M, Bark O (1994) Molecular confirmation that sterile cytoplasm has been introduced into open-pollinated populations of Grano-type onion. J Am Soc Hort Sci 119:90–93

Hiesel R, Schobel W, Schuster W, Brennicke A (1987) The cytochrome oxidase subunit I and III genes in *Oenothera* mitochondria are transcribed from identical promoter sequences. EMBO J 6:29–34

Holford P, Croft J, Newbury H (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

Isaac P, Jones V, Leaver C (1985) The maize cytochrome c oxidase subunit I gene: sequence, expression, and rearrangement in cytoplasmic male-sterile plants. EMBO J 4:1617–1623

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

Jones H, Emsweller S (1936) A male-sterile onion. Proc Am Soc Hort Sci 34:582–585

Jones H, Perry B, Edmundson W (1949) Vegetative propagation of short-day varieties of onions as an aid in a breeding program. J Am Soc Hort Sci 53:367–370

Levings CS III (1993) Thoughts on cytoplasmic male sterility in cms-T maize. Plant Cell 5:1285–1290

Morikami A, Nakamura K (1987) Structure and expression of the pea mitochondrial F_1-ATPase α-subunit gene and its pseudogene involved in homologous recombination. J Biochem 101:967–976

Pathak C, Gowda R (1993) Breeding for the development of onion hybrids in India: problems and prospects. Acta Hort 358: 239–242

Pike L, Yoo K (1990) A tissue culture technique for the clonal propagation of onion using immature flower buds. Sci Hort 45:31–36

Pring D, Lonsdale M (1989) Cytoplasmic male sterility and maternal inheritance of disease susceptibility in maize. Annu Rev Phytopathol 27:483–502

Schaffer H, Sederoff R (1981) Improved estimation of DNA fragment lengths from agarose gels. Anal Biochem 115:113–122

Sato Y (1998) PCR amplification of CMS-specific mitochondrial nucleotide sequences to identify cytoplasmic genotypes of onion (*Allium cepa* L.). Theor Appl Genet 96:367–370

Satoh Y, Nagai M, Mikami T, Kinoshita T (1993) The use of mitochondrial DNA polymorphism in the classification of individual plants by cytoplasmic genotypes. Theor Appl Genet 86:345–348

Schweisguth B (1973) Etude d'un nouveau type de sterilite male chez l'oignon, *Allium cepa* L. Ann Amélior Plant 23:221–233

USDA (1995) Agricultural statistics. Washington, D.C.

Vries J de, Wietsma W (1992) *Allium roylei* Stearn restores cytoplasmic male sterility of Rijnsburger onion (*A. cepa* L.). J Genet Breed 46:379—382