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A putative donor of S-cytoplasm and its distribution among open-pollinated populations of onion

M. J. Havey

Vegetable Crops Unit, Agricultural Research Service, USDA, Department of Horticulture, University of Wisconsin, Madison, WI 53706 USA

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Summary. Cytoplasmic-genic male-sterility systems are used to economically produce hybrid onion seed. Previous studies have indicated that the source of cytoplasmic male sterility discovered in 1925 by Jones (S-cytoplasm) may be an alien cytoplasm. Restriction enzyme analysis of the chloroplast DNA (cpDNA) revealed five polymorphisms between S and normal (N) fertile cytoplasms. S-cytoplasm was different from the *Allium* species closely related to the bulb onion, and cladistic estimates of phylogenies supported introduction from an unknown species. S-cytoplasm was identical for all polymorphisms in the cpDNA to 'Pran', a triploid viviparous onion. 'Pran' shares morphological characteristics with 'Italian Red 13-53', the single plant source of S-cytoplasm. Densiometric scans of autoradiograms revealed that 12 of 31 open-pollinated populations of onion possessed S-cytoplasm and that introgression may have occurred since the discovery of S-cytoplasm.

Key words: Onion – Cytoplasmic male sterility – Chloroplast DNA – RFLPs

Introduction

Populations of the bulb onion (*Allium cepa* L.) have been historically maintained by open pollination after selection for bulb shape and color, day-length response, maturity, etc. Open-pollinated (OP) populations are often heterogeneous and susceptible to inbreeding depression. Jones and Davis (1944) observed that inbreeding for one to two generations often produced uniform lines. Vigor was restored by crossing between inbreds, and some hybrid combinations were higher yielding than the parental populations and more uniform for maturity and bulb size and shape (Jones and Davis 1944; Joshi and Tandon 1976). Jones and Davis (1944) concluded that uniform, high-yielding hybrids could be generated from inbreds subjected to inbreeding and selection. Significant progress towards the development of superior onion inbreds has been realized, and hybrid cultivars are grown world-wide.

Pollination control was a major obstacle to the development of hybrid onion. The onion umbel contains hundreds of perfect flowers and, although outcrossing is encouraged by protandry (Currah and Ockendon 1978), mature pollen and receptive stigmas are present at the same time. Emasculation on a large scale is not practical. The production of hybrid onion seed became economically feasible with the discovery of cytoplasmic-genic male-sterility (CMS) systems (Jones and Emsweller 1936; Berninger 1965). In 1925, Jones and colleagues were inbreeding plants of the cultivar 'Italian Red' to develop a hybrid red onion for storage. One plant (13-53) did not set seed after self pollination and was saved by virtue of bulbils in the inflorescence. Sterility in 'Italian Red 13-53' was conditioned by the interaction of the cytoplasm (S-cytoplasm) with a single nuclear restorer gene. In S-cytoplasm, fertility is restored by a dominant allele (*Ms*) at the nuclear restorer locus (Jones and Clarke 1943). Male-sterile plants possess the sterile cytoplasm and are homozygous recessive at the restorer locus (S *msms*). Male-sterile lines can be maintained by crossing the sterile line with a line possessing normal (N) cytoplasm and homozygous recessive at the restorer locus (N *msms*). When planted together in isolation, the sterile and maintainer lines are propagated by harvesting seed separately from each line. After

successive generations of backcrossing, the genotype of the sterile line approaches that of the maintainer. Hybrid seed is produced by planting the male-sterile and fertile lines and harvesting seed only from the sterile rows.

Little et al. (1944) found that 25 of 29 OP populations possessed the recessive nonrestoring allele for S-cytoplasm. Other populations, e.g. 'Australian Brown' or 'Mountain Danvers', had the dominant *Ms* allele a high frequencies, and the extraction of maintainer lines has been difficult. Little et al. (1944) also observed that two accessions of shallot (*A. cepa* var *ascalonicum*) were heterozygous at the restorer locus. Based on a sample of 367 plants, they estimated the frequency of the *ms* allele at 0.6 and concluded that little to no selection has occurred against the nonrestoring allele in natural populations. Davis (1957) evaluated OP populations from the Middle East, the proposed centre of origin of onion, and found the *ms* allele to be widespread. Little et al. (1944) suggested that the common occurrence of the recessive *ms* allele in most onion populations and shallot indicates that the mutation from *Ms* to *ms* must have occurred early in the evolution of onion or has occurred many times.

A second source of CMS in the bulb onion has been characterized genetically. Berninger (1965) discovered sterility (T-cytoplasm) in the French cultivar 'Jaune paille des Vertus'. Schweisguth (1973) characterized this source of CMS and identified three independently segregating restorer loci. Fertility is restored by a dominant allele at one locus (*A*-) or at both of two complementary loci (*B*-*C*-). The complex inheritance and common occurrence of restorers make T-cytoplasm more difficut to use. Although male sterility has been observed in other onion populations, e.g., 'Dorata di Parma', 'Pukekohe Longkeeper', 'Red Wethersfield', 'Scott County Globe', 'Stuttgarter Riesen', and 'Zittauer Glebe', it has not been characterized, and the relationships to S- or T-cytoplasms are unclear (Peterson 1953; Courcel et al. 1989).

CMS indicates a nuclear-cytoplasmic incompatibility that can occur spontaneously, appear after treatment with mutagens, or result from interspecific crosses (Hanson and Conde 1985). Restriction enzyme (RE) analyses of the chloroplast (cpDNA) (Brown et al. 1986; Chen et al. 1990) and/or mitochondrial DNAs (mtDNA) (Levings and Pring 1976; Kemble et al. 1980) of fertile and sterile cytoplasms have shown fragment size differences. CMS in maize and sorghum is attributed to differences in the mitochondrial genome (Pring et al. 1979). Chen et al. (1990) observed differences in the bands produced by RE digestions of cpDNA of male-fertile and male-sterile lines of sorghum and proposed that the cpDNA may be involved with the expression of CMS. An alternate explanation would be that the differences at RE sites in the cpDNA are not directly involved in the expression of CMS, but indicate an alloplasmic origin.

Courcel et al. (1989) and Holford et al. (1991) used RE analysis of the mitochondrial and chloroplast genomes to demonstrate differences among N-, S-, and T-cytoplasms in onion. Courcel et al. (1989) proposed that two main groups of cytoplasms exist, of which M-cytoplasm was the most common. *Bam*HI digests of the mtDNA thereof distinguished four subgroups (M_1–M_4) and included N- (M_2) and T-cytoplasms (M_4). S-cytoplasm could be distinguished from the M-cytoplasm group in both its mtDNA and cpDNA. Courcel et al. (1989) identified S-cytoplasm in Italian cultivars and postulated that onion may possess two centres of origin, the Mediterranean and Near East for S- and M-cytoplasms, respectively. Holford et al. (1991) were able to distinguish among N-, S-, and T-cytoplasms with *Bam*HI and *Hin*dIII digests of mtDNA or hybridizations of mitochondrial clones. Differences between N- and S-cytoplasms have also been reported for *Eco*RI, *Hin*dIII, and *Xba*I digests of cpDNA.

The chloroplast genome of onion is maternally inherited (Corriveau and Coleman 1988). Mutations at RE sites in the cpDNA have been identified and used to estimate the phylogenetic relationships among the bulb onion and other *Allium* species (Havey 1991a, 1992). I undertook the RE analysis of the cpDNA of S-cytoplasm that is reported here in order to estimate its origin and determine its prevalence among OP populations of onion.

Materials and methods

YB986A and B3350A (S *msms*, S-cytoplasm backcrossed to selections out of 'Yellow Bermuda' and 'Downing Yellow Globe', respectively) and B3350B (N *msms*) were surveyed for polymorphisms in their cpDNA. Leaf tissue from at least 20 seedlings of each line was harvested in bulk and lyophilized. Procedures for isolation of pooled DNA from the seedlings, digestions with each of 15 restriction enzymes, electrophoresis, blotting, nick translation and hybridization of clones from the chloroplast genome of the orchid *Oncidium excavatum* (Chase and Palmer 1989), and autoradiography have been reported (Havey 1991a, 1992). S-cytoplasm was compared to representative *Allium* species in sections *Cepa* and *Phyllodolon*, *A. altaicum* Pall. (TAX33), *A. fistulosum* L. (PI223853), *A. galanthum* Kar. et Kir. (TAX698), *A.* oschaninii O. Fedtsch. (TAX978), *A. pskemense* (TAX514) B. Fedtsch., and *A. roylei* Stearn (PI243009), and to 'Pran', a triploid viviparous onion [*Allium* × *proliferum* (Moench) Schrad., syn. *Allium cepa* L. var. *viviparum* (Metzger) Alefeld]. *Allium ampeloprasum* L. (PI256051) was used as the outgroup. The origins of the *Allium* accessions have been reported (Havey 1991b, 1992). The phylogenetic relationships among N-cytoplasm, S-cytoplasm, and *Allium* species were estimated using Phylogenetic Analysis Using Parsimony (PAUP 3.Or, D. Swofford, State Natural History Survey Division, Champaign, Ill. USA) by cladistic

Table 1. Onion inbreds or populations evaluated for S-cytoplasm

Inbred or population[a]	Source[b]	Lot[c]	Location
B986 A	SunSeeds	–	El Centro, Calif., USA
B3350 A	USDA	909-1	Madison, Wis., USA
B3350 B	USDA	909-2	
RJ70 A	Clause	880190	Brétigny-Sur-Orge, France
RJ70 B	Clause	880193	
Imai Wase A	Shippo	418	Kagawa, Japan
Australian Brown	SunSeeds	833-34/15	Brooks, Ore., USA
Brigham Yellow Globe	Aristogenes	92051	Parma, Idaho, USA
California Early Red	NSSL	28165.01	Ft. Collins, Colo., USA
Crystal Wax	SunSeeds	–	El Centro, Calif., USA
Downing Yellow Globe	SunSeeds	2324/15	Brooks, Ore., USA
Early Yellow Globe	SunSeeds	2393/27	Brooks, Ore., USA
Italian Red	NSSL	5578.01	Ft. Collins, Colo., USA
Mountain Danvers	Waldow	–	Olathe, Colo., USA
Oregon Danvers	Crookham	83N70	Caldwell, Idaho, USA
Pukekohe Longkeeper	SunSeeds	3311/15	Brooks, Ore., USA
Red Creole	PETO	–	Payette, Idaho, USA
Red Wethersfield	Crookham	53309	
Rijnsburger	SunSeeds	1819/2	Brooks, Ore., USA
Sapporo-Ki	Shippo	–	
Sensyu-Ki	Shippo	–	
Southport Red Globe	Crookham	55389	
Southport White Globe	SunSeeds	9192/2	Brooks, Ore., USA
Southport Yellow Globe	NSSL	3915.03	
Stuttgarter Reisen	SunSeeds	4148/12	Brooks, Ore., USA
Strigonowskij	SunSeeds	9214/12	Brooks, Ore., USA
Sweet Spanish Colorado #6	SunSeeds	806/15	Brooks, Ore., USA
Sweet Spanish Peckham	SunSeeds	915-17/15	Brooks, Ore., USA
Sweet Spanish Winegar	SunSeeds	1824/2	Brooks, Ore., USA
Texas Early Grano 502	Asgrow	SPN118	Arvin, Calif., USA
White Creole	SunSeeds	81-323032	El Centro, Calif., USA
White Ebenezer	Crookham	53306	
White Knight	Asgrow	–	
White Portugal	SunSeeds	2103/1-5	El Centro, Calif., USA
Yellow Bermuda	Bonanza	251026-320.8	Yuba City, Calif., USA
Yellow Ebenezer	SunSeeds	829/1-5	Brooks, Ore., USA
Yellow Globe Danvers	Ohio Seed	8530	West Jefferson, Ohio., USA

[a] Inbreds are S-cytoplasm (YB986 A and B3350 A) and its maintainer (B3350 B), T-cytoplasm (RJ70 A) and its maintainer (RJ70 B), and an uncharacterized Japanese source of sterile cytoplasm ('Imai Wase A')
[b] USDA, United States Department of Agricuture, Vegetable Crops Unit, NSSL, USDA National Seed Storage Laboratory, Ft. Collins, Colo., USA. Other names are companies producing onion seed
[c] Seed lot, when known

analysis based on Wagner and Dollo parsimony. Felsenstein's (1985) bootstrap method was assumed to estimate the confidence of nodes.

A source of T-cytoplasm (RJ70 A), its maintainer (RJ70 B), and an uncharacterized source of CMS from the Japanese cultivar 'Imai Wase' (Table 1) were scored for polymorphisms in the cpDNA that distinguish N- and S-cytoplasms.

Polymorphisms were used to estimate the prevelence of S-cytoplasm in OP onion populations. Firstly, total genomic DNA from B3350A and B3350B was mixed in ratios of 0:1, 1:99, 5:95, 10:90, 20:80, 30:70, 40:60, 50:50, 75:25, and 1:0, respectively, based on spectrophotometric determinations of concentrations, and evaluated for cpDNA-1 and -4 (Havey 1991a). The relative intensities of bands within single lanes of autoradiograms were compared by densiometric scanning using a Shimadzu CS900U densiometer. Genomic DNAs from 31 OP populations of onion (Table 1) were isolated from bulked leaf tissue of at least 30 seedlings and evaluated for characters cpDNA-1 and -2 (Havey 1991a), as described above. The proportion of S-cytoplasm in each population was then estimated by densiometric scanning, assuming that individual plants with N-cytoplasm or S-cytoplasm contributed equal amounts of cpDNA to the total pooled sample.

Results

Five polymorphisms (cpDNA-1, -2, -4, -41 and -42) in the cpDNA differentiated N- and S-cytoplasm (Table 2). Characters cpDNA-1, -2, and -4 have been previously described (Havey 1991a). Character 41 appeared as 5.0-kilobase (kb) (N-cytoplasm) versus 4.0-kb (S-cytoplasm) bands on *Bam*HI digests hybridized with orchid clones 19 a and b. This polymorphism

Table 2. Presence or absence of polymorphisms at or between restriction enzyme sites in the chloroplast DNA of fertile and sterile cytoplasms in onion and *Allium* × *proliferum* cv 'Pran'

cpDNA character[a]	Orchid clones	Enzyme	Polymorphism	Progenitor state[c]	B986A[d]	B3350A	Pran	B3350B
1	3, 4, and 6a	*Bgl*II	4.0 = 3.0 + 1.0	+	+	+	+	−
2	12b c	*Bgl*II	4.0 = 3.5 + 0.5	+	+	+	+	−
4	12b c	*Eco*RV	7.0 = 4.0 + 3.0	−	−	−	−	+
41	19a b	*Bam*HI	5.0 = 4.0 + (1.0)[b]	−	+	+	+	−
42	17	*Eco*RI, *Bgl*II	Insertion	−	−	−	−	+

[a] Characters 1, 2, and 4 have been previously reported (Havey 1991a) and are listed here for convenience only. S-cytoplasm was identical to *A. cepa* for 37 other polymorphisms described by Havey (1991, 1992). Characters 41 and 42 are described in text. +, presence of state; −, absence of state

[b] Dubious polymorphic restriction enzyme site, smaller fragment not observed

[c] Proposed progenitor state of polymorphisms as estimated from outgroup

[d] For origin of accessions, see Table 1

was not likely an insertion or deletion because equally sized differences were not observed with other enzymes. However, the 1.0-kb fragment (5.0 = 4.0 + 1.0) was not observed on autoradiograms, and this polymorphism may be due to a rearrangement. Character 42 was scored as a 0.1-kb insertion in N-cytoplasm (3.1 versus 3.0-kb bands for N-cytoplasm and S-cytoplasm, respectively, visible with *Bgl*II and *Eco*RI and orchid clone 17). The smaller fragment was observed in divergent *Allium* species (e.g., *A. ampeloprasum*, *A. fistulosum*, *A. galanthum*, and *A. oschaninii*) and considered to be the progenitor state. Three (cpDNA-4, 41, and 42) of the polymorphisms are likely to be the same ones as those reported by Courcel et al. (1989); however, they did not report two polymorphisms (cpDNA-1 and 2) at *Bgl*II sites in the cpDNA. Holford et al. (1991) detected polymorphisms in the cpDNA with *Eco*RI, *Hin*dIII, and *Xba*I; however, I detected no differences at RE sites with these enzymes, and the polymorphisms may be the result of the putative structural changes. A problem with scoring band-size differences on gels is that a single structural change can be scored as a polymorphism for more than one enzyme.

T-cytoplasm (RJ70 A), its maintainer (RJ70 B), and the CMS line from 'Imai Wase' were identical to N-cytoplasm (B3350B) for the five polymorphisms distinguishing N- and S-cytoplasm; both Courcel et al. (1989) and Holford et al. (1991) did not detect polymorphisms in the cpDNA between T- and N-cytoplasms. The S-cytoplasm was not identical to any of the cultivated or wild *Allium* species previously studied (Havey 1991a, 1992). Cladistic analysis of 37 polymorphisms in the cpDNA (cpDNA-14, 16, 20, 33, and 37 were monomorphic) resulted in eight equally parsimonious Wagner trees and four Dollo trees of 41 steps (trees not shown). Bootstrap analyses using Wagner and Dollo parsimony placed S-cytoplasm into an unresolved group of species closely related to the bulb onion, including N-cytoplasm, *A. altaicum*, *A. fistulosum*, *A. galanthum*, and *A. roylei* (Fig. 1). N- and S-cytoplasm comprised a monophyletic group in only 32% and 35% of the bootstrapped Wagner and Dollo trees, respectively. This phylogenetic estimate supports the hypothesis of Courcel et al. (1989) and Holford et al. (1991) that S-cytoplasm is of alloplasmic origin.

S-cytoplasm was identical for all cpDNA polymorphisms to 'Pran', a triploid viviparous onion cultivated by the Muslim population of Northwest India (Singh et al. 1967; Koul and Gohil 1971). Of the five characters distinguishing N- and S-cytoplasm, four were shared by S-cytoplasm and other *Allium* species (Havey 1992). One polymorphism (cpDNA-41) was observed only in 'Pran' and S-cytoplasm.

Densiometric scans of autoradiograms estimated the prevalence of S-cytoplasm in OP onion populations.

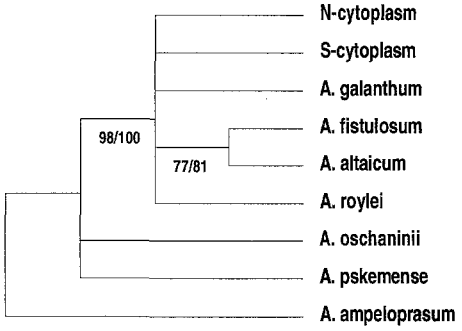

Fig. 1. Strict consensus of the most-parsimonious Wagner and Dollo trees estimating the phylogenetic relationships among normal (*N*) and sterile (*S*) cytoplasms of onion and closely related *Allium* species. Number below lines represent the frequency (> 75%) at which the nodes were present in 100 independently generated most-parsimonious Wagner and Dollo trees, respectively. The relationships among the *Allium* species have been previously reported (Havey 1992) and are included here for comparison

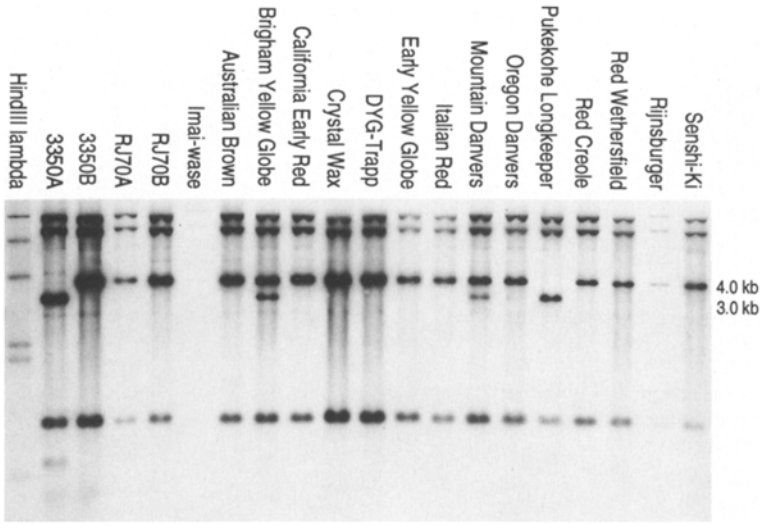

Fig. 2. S-cytoplasm in open-pollinated cultivars of onion as demonstrated by the presence of the 3.0-kb band. Lanes contain *Bgl*II-digested DNA hybridized with the orchid chloroplast clones 3, 4, and 6a

Table 3. Estimated frequencies of S-cytoplasm in open-pollinated populations of onion

Population	Frequency[a]
Australian Brown	0.00
Brigham Yellow Globe	0.27 ± 0.06
California Early Red	Trace[b]
Crystal Wax	0.00
Downing Yellow Globe	0.00
Early Yellow Globe	0.00
Italian Red	0.00
Mountain Danvers	0.20 ± 0.03
Oregon Danvers	Trace
Pukekohe Longkeeper	1.00
Red Creole	0.00
Red Wethersfield	Trace
Rijnsburger	0.00
Sapporo-Ki	0.40 ± 0.13
Sensyu-Ki	0.00
Southport Red Globe	0.00
Southport White Globe	0.00
Southport Yellow Globe	0.00
Strigonowskij	0.00
Stuttgarter Reisen	0.00
Sweet Spanish Colorado #6	0.03 ± 0.02
Sweet Spanish Peckham	0.00
Sweet Spanish Winegar	0.00
Texas Early 502 Grano	0.00
White Creole	0.00
White Ebenezer	0.02 ± 0.02
White Knight	0.00
White Portugal	Trace
Yellow Bermuda	0.07 ± 0.02
Yellow Ebenezer	0.09 ± 0.04
Yellow Globe Danvers	0.06 ± 0.04

[a] Mean frequency of S-cytoplasm ± standard deviation estimated from densiometric scans of autoradiograms for cpDNA-1 and -2. For description of characters, see Table 2

[b] Trace, light bands visible but not detected by all densiometric scans

Mixtures of known amounts of genomic DNA from plants with N- or S-cytoplasm were initially evaluated for characters cpDNA-1 and -4, which represent a gain and a loss of a RE site, respectively, for S-cytoplasm, and the relative intensities of closely sized bands within a single lane of autoradiograms were compared. Light bands at 7.0 kb for cpDNA-4 indicated that the presence of S-cytoplasm could not be distinguished from incomplete digestion of the cpDNA from N-cytoplasm. For cpDNA-1, a significant regression ($P < 0.01$, $r^2 = 0.974$) indicated that the relative band intensities accurately reflected the amount of each cytoplasm in the mixture. To avoid scoring partial digestions as the presence of S-cytoplasm, polymorphisms resulting from the gain of a RE site were used (cpDNA-1 and -2). S-cytoplasm was detected in 12 of the 31 OP populations (Fig. 2, Table 3). Populations with the dominant restorer allele at a high frequency, e.g., 'Australian Brown' and 'Texas Early Grano 502' (Little et al. 1944; Davis 1957), were in N-cytoplasm (Table 3). Interestingly, the strain of 'Pukekohe Longkeeper' used in this study was exclusively in S-cytoplasm.

Discussion

S-cytoplasm was not identical to any of the Alliums closely related to the bulb onion. Of the five characters distinguishing N- and S-cytoplasms, four (cpDNA-1, -2, -4, and -42) were present in other divergent *Allium* species and likely represent the progenitor state. However, S-cytoplasm was identical to 'Pran' for all 42 polymorphisms in the cpDNA, and this viviparous

interspecific hybrid may be the origin of Jones' source of sterile cytoplasm. One of the parents of 'Pran' was *A. cepa*, as evidenced by RE sites in the 45s nuclear rDNA (Havey 1991b). Two other reports have documented sexually derived triploids in *Allium*; Schweisguth (1984) successfully generated triploid plants by crossing an *A. galanthum* by *A. cepa* amphidiploid with *A. cepa*, and Perkins et al. (1958) developed 'Delta Giant' shallot by backcrossing an *A. cepa* by *A. fistulosum* amphidiploid to *A. cepa*.

Pran shares morphological similarities with 'Italian Red 13-53', the single plant source of S-cytoplasm (Jones and Emsweller 1936); both plants produced spindle-shaped red bulbs and top-sets mixed with flowers that shed no pollen. However, there are also differences between the accession of 'Pran' in my possession and descriptions of 'Italian Red 13-53'. The bulb of 'Italian Red 13-53' drawn by Jones and Clarke (1947) depicts a shape typical of 'Italian Red'. Grown under day lengths in Madison, Wis., USA (46 parallel), 'Pran' produced bulbs that are smaller and firmer than those of 'Italian Red' (Magruder et al. 1941). Jones and Clarke (1947) reported that 'Italian Red 13-53' produced 136 top-sets on five seed stems; the plants of 'Pran' in my possession produced only 5-10 top-sets per umbel. Jones described 'Italian Red 13-53' as having excellent female fertility in crosses with the bulb onion (Jones and Clarke 1947). In 1991, I grew 'Pran' in a nursery with onion and closely related *Allium* species, and no seed was harvested in spite of excellent bee activity. Given the morphological similarities between 'Pran' and cv 'Italian Red', it is reasonable to imagine a chance hybridization occurring in an unknown garden introducing the alien cytoplasm of 'Pran' into onion. However, two additional scenarios come to mind. The first is that Jones may have mixed transplants of 'Italian Red' with plants grown from top-sets of 'Pran'. This scenario is unlikely given Jones' description of 'Italian Red 13-53' as having excellent female fertility and a bulb shape typical of 'Italian Red'. Jones and Clarke (1947) stated that 13-53 was the only one of the sterile Italian Red plants to produce top-sets, indicating that this trait was not commonly encountered. Finally, Jones and Mann (1963) make no mention of a triploid viviparous onion, indicating that they may not have known of this plant. A second possibility is that 'Pran' and the strain of 'Italian Red' grown by Jones originated from an ancestral interspecific hybrid. 'Italian Red' would have been seed propagated and selected for bulb type and color, day-length response, etc., while 'Pran' was asexually propagated. In this case, the presence of N-cytoplasm in 'Italian Red' must have been introduced and became prominent. No evidence of S-cytoplasm was detected in the population of 'Italian Red' used in this study (Fig. 2, Table 3).

The exclusive use of S-cytoplasm in hybrid seed production in the USA has raised concerns of genetic vulnerability (Anonymous 1972). Cladistic analyses of differences at or between RE sites in the chloroplast genome confidently placed S-cytoplasm among *Allium* species closely related to the bulb onion (Fig. 1) and supported proposals of an alloplasmic origin (Courcel et al. 1989; Holford et al. 1991). Although generally sterile, interspecific hybrids between onion and closely related *Allium* species may generate new sources of CMS. Another alloplasmic source of male sterility exists; in 1988, the USDA released a CMS line developed by backcrossing USDA plant introduction 280091 of *A. galanthum* to the bulb onion.

Davis (1957) and Little et al. (1944) documented the presence of the non-restoring *ms* allele in various OP populations of onion. They concluded that the allele was not under negative selection pressure, assuming that the OP populations were in N-cytoplasm. However, the dominant restorer allele (*Ms*) would condition fertility if the cultivar were in S-cytoplasm or a mixture of cytoplasms. S-cytoplasm was found in 12 of 31 OP populations of onion (Table 3). Courcel et al. (1989) presented evidence that S-cytoplasm exists in the Italian cultivar 'Dorata di Parma' and an unknown cultivar from Rovigo, Italy, and concluded that Italian cultivars must possess S-cytoplasm. However, S-cytoplasm was not observed in the samples of 'Italian Red' studied here. This discrepancy could be due to the numbers of plants used to perpetuate the cultivar. If S-cytoplasm were relatively rare in a population, it could increase or decrease in prevalence by the random sample of bulbs used to produce seed each generation. Courcel et al. (1989) suggested that the presence of S-cytoplasm in Italian cultivars may reflect a unique Mediterranean center of origin for onion, in addition to that of the Near East (S- and M-cytoplasms, respectively). S-cytoplasm was discovered in 'Italian Red', and there is no reason to doubt that this onion originated from Italy. My results indicate that a separate Mediterranean center of origin for onion, as proposed by Courcel et al. (1989), may not exist. The presence of S-cytoplasm in onion populations of today may be due to inadvertant introgression. For example, S-cytoplasm was found in 'Brigham Yellow Globe' (BYG) and not 'Southport Yellow Globe' (SYG), even though BYG was selected directly out of SYG (Magruder et al. 1941). If the *Ms* allele were prevalent, S-cytoplasm could be introduced into onion populations with no obvious expression of male sterility. The possibility clearly exists that S-cytoplasm has been inadvertently introduced into onion populations since Jones' discovery of 'Italian Red 13-53'.

Mutations in the cpDNA differentiating N- and S-cytoplasms have practical uses to companies produc-

ing hybrid onion seed. Holford et al. (1988) pointed out that RFLPs in the mitochondrial genome could be used to assure that hybrid seed is in sterile cytoplasm. Estimates of the relative amount of N-cytoplasm in hybrid cultivars would reflect the degree of contamination by the pollinating line, assuming that the pollinator is not in restored S-cytoplasm. When autoradiograms are used to estimate the presence of N- or S-cytoplasm, it is important to evaluate for gains of RE sites to avoid confusing partial digestions with a contaminating cytoplasm. The presence of N- or S-cytoplasms in mixtures can be estimated using cpDNA-4 or cpDNA-1, -2, and -41, respectively. However, these chloroplast markers are most useful in establishing the cytoplasm of individual plants to avoid wasting resources attempting to develop maintainer lines from plants in S-cytoplasm.

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