Toward the Identification of Cytoplasmic Male Sterility in Leek: Evaluation of Organellar DNA Diversity among Cultivated Accessions of *Allium ampeloprasum*

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ABSTRACT. Hybrid leek is more uniform and higher yielding than open-pollinated cultivars and is presently produced by asexual propagation of a genic male-sterile plant. A cheaper method to produce hybrid leek seed would be a system of cytoplasmic-genic male sterility (CMS). Restriction fragment length polymorphisms (RFLPs) in the organellar genomes have correlated with CMS in many crops. We undertook gel-blot analyses of the chloroplast and mitochondrial DNAs to assess cytoplasmic diversity among 62 accessions of the major cultivated forms of *Allium ampeloprasum* L. (leek, kurrat, and great-headed garlic). No polymorphisms were detected in the chloroplast genome of leek and kurrat. Three accessions of leek and one of kurrat possessed one or two of seven polymorphic mitochondrial probe–enzyme combinations. Great-headed garlic differed from leek and kurrat for six polymorphisms in the chloroplast genome and for many mitochondrial probe–enzyme combinations. Our analyses revealed few organellar polymorphisms among accessions of leek and kurrat, reducing the probability that selection of polymorphic cytoplasm will reveal CMS in leek.

Populations of the cultivated seed-propagated alliums [bulb (*Allium cepa* L.) or bunching onion (*A. fistulosum* L.), chive (*A. schoenoprasum* L.), or leek (*A. ampeloprasum* L. var. *porrum*)] have been maintained historically by open pollination after selection for shape of the bulb or pseudostem, uniformity, maturity, and disease resistances. These alliums are outcrossing diploids ($2n = 2x = 16$) or tetraploids ($2n = 4x = 32$) that show severe inbreeding depression (Jones and Davis, 1944; Smith and Crowther, 1995). Hybrid alliums offer numerous advantages to growers, including significant heterosis over the inbred parent or open-pollinated source of the inbred, greater uniformity, and the possibility to combine dominantly inherited disease resistances (Currah, 1986; Jones and Davis, 1944). However, pollination control is a major obstacle. The 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. Large-scale emasculation is not practical.

Cytoplasmic male sterility (CMS) is known in many crops and commonly used to produce hybrid seed (Hanson and Conde, 1985). Among the cultivated alliums, CMS has been described in bulb onion (Berninger, 1965; Jones and Clarke, 1943), bunching onion (Inden and Asahira, 1990), and chive (Tatlioglu, 1982). Hybrid leek is more uniform and significantly higher yielding than open-pollinated populations (Gagnebin and Bonnet, 1979; Kampe, 1980; Schweisguth, 1970; Smith and Crowther, 1995). Male sterility has been reported in leek; however, genetic studies revealed a genic male-sterility system that requires roguing of male-

fertile segregates in hybrid production fields (Schweisguth, 1970). Although asexual propagation of a genic male-sterile plant may be used to produce hybrid-leek seed (Smith and Crowther, 1995), CMS would be a cheaper alternative. Toward this goal, Peterka et al. (1997) generated an interspecific hybrid between S-cytoplasmic onion and leek as an initial step to transfer CMS from onion to leek.

Restriction-enzyme analyses have revealed differences in the mitochondrial DNAs among normal fertile and sterile cytoplasm in many crops (Hanson and Conde, 1985). The cytoplasmic male-sterile and normal male-fertile cytoplasm of chive (Potz and Tatlioglu, 1993) and onion (Havey, 1995) showed many polymorphisms in the mitochondrial DNA. Polymorphisms in the chloroplast DNA distinguishing sterile and normal fertile cytoplasm have been described for bulb onion (Havey, 1993) and sorghum (Chen et al., 1990). For these CMS systems, male-sterile plants were isolated, maternal transmission established, and organellar polymorphisms identified. Because a genic male-sterility system is known in leek, we undertook restriction-enzyme analysis of the leek organellar genomes to identify divergent cytoplasm that may correlate with CMS. Our goal was to identify individual plants possessing unique cytoplasm and, over the long term, evaluate for male sterility among these plants and their self-pollinated and testcross progenies. Kik et al. (1997) purified mitochondrial DNA from leek populations and, after digesting with restriction enzymes, identified two mitochondrial types. In this research, we used DNA blots to evaluate for divergent cytoplasm and observed few polymorphisms in the organellar genomes of leek and kurrat.

Materials and methods

Pooled total genomic DNA was isolated (Havey, 1991) from at least 50 seedlings of 41 accessions of open-pollinated leek populations from the USDA Plant Introduction (PI) Station, Pullman, Wash. (204673, 206937, 206938, 249532, 254535, 256050, 357191, 357192, 357193, 357194, 357195, 357196, 357197, 357198, 357199, 357201, 357203, 357204, 357205, 357206, 357208, 357209, 368335, 368336, 368337, 368338, 368339, 368340, 368341, 368342, 368343, 368345, 368346, 368348, 368349,

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Table 1. Polymorphisms in the mitochondrial genome among accessions of leek and kurrat.

Polymorphic accessions ^z	Probe ^z	Enzyme revealing polymorphism	Wild-type fragment ^y	Unique fragment ^y
Kurrat	<i>atp9</i>	<i>Dra</i> I	5.0	2.6
357201	<i>atp9</i>	<i>Bam</i> HI	4.6, 2.3	3.2
	<i>atp9</i>	<i>Hind</i> III	2.6, 0.9	2.8
368337	<i>cob</i>	<i>Dra</i> I	3.0, 1.6	1.3
	<i>cob</i>	<i>Sac</i> I	4.0	2.5
368341	<i>atp9</i>	<i>Bgl</i> II	5.8, 4.8	3.3
	<i>atp9</i>	<i>Eco</i> RI	4.0, 1.5	3.0

^zFor description of accessions and probes, see Materials and Methods.

^yFragment sizes in kilobases. For all accessions, both the wild-type and unique fragments were visible on autoradiograms.

368350, 368351, 368352, 368353, 370334, 370335) and 19 commercial leek populations from the seed companies Rijk Zwaan (RZ-1 through 6), De Lier, Holland; S&G/Sandoz (SG-1 through 5), Enkhuizen, Holland; Carl Sperling (CS-1 through 4), Lüneburg, Germany; and Bejo (B-1 through 4), Warmerhuizen, Holland. We included two other cultivated types, kurrat (*A. ampeloprasum* var. *kurrat*) (Carl Sperling), and great-headed garlic (*A. ampeloprasum* var. *holmense*) (Freida, Los Angeles, Calif.). DNA was digested at 10 units/μg with *Bam*HI, *Bgl*II, *Bst*EII, *Dra*I, *Eco*RI, *Eco*RV, *Hind*III, *Kpn*I, *Pst*I, *Sac*I, *Xba*I, or *Xho*I as recommended by manufacturer (Gibco-BRL, Gaithersburg, Md.), subjected to electrophoresis through 0.8% agarose gels in 1× TBE (Sambrook et al., 1989), and transferred to Zetaprobe (Biorad, Richmond, Calif.) filters using 0.4 M NaOH and 0.6 M NaCl (Havey, 1991). Membranes were baked at 80 °C for at least 1 h, washed for 1 h at 60 °C in 0.1× SSC and 0.1% (w/v) SDS, and prehybridized at 60 °C for 4 h in 6× SSC, 5× Denhardt's reagent (1 g·L⁻¹ each of Ficoll, polyvinyl-pyrrolidone, bovine serum albumin), 0.5% (w/v) SDS, and 100 mg·mL⁻¹ sheared herring sperm DNA (Sambrook et al., 1989). Whole recombinant plasmids were nick-translated (Sambrook et al., 1989), heat-denatured, and hybridized overnight to filters in the prehybridization solution. Filters were rinsed at room temperature in 2× SSC (Sambrook et al., 1989) and washed for 30 min at 50 °C in 0.1% SDS and 0.1× SSC and twice for 15 min at 60 °C in 0.1% SDS and 0.1× SSC. Filters were wrapped in plastic film and placed on X-ray film (Kodak XAR-5) with intensifying screens at -80 °C. RFLPs among accessions were scored and fragment sizes estimated (Schaffer and Sederoff, 1981). The most common fragments among all accessions were considered as wild type.

We hybridized a complete set of clones from the orchid (*Oncidium excavatum* Lindley) chloroplast genome (Chase and Palmer, 1989) and evaluated for 42 previously described chloroplast poly-

morphisms (Havey, 1992, 1993) distributed over the entire chloroplast DNA molecule. For the mitochondrial genome, we hybridized clones of the major mitochondrially encoded enzymatic subunits of apocytocrome b [*cob* (Dawson et al., 1984)], cytochrome oxidase (*cox*) subunits 1 (Isaac et al., 1985), 2 (Fox and Leaver, 1981), and 3 (Hiesel et al., 1987), and *atp6* and *atp9* (Dewey et al., 1985) to the same filters used for the chloroplast DNA analysis.

Results and Discussion

We detected no RFLPs in the chloroplast DNA among the 60 accessions of leek and one of kurrat. Three accessions of leek (PIs 357201, 368337, and 368341) and kurrat possessed both the wild-type fragments and uniquely sized fragments not present in the mitochondrial genome of the other 57 leek accessions (Table 1). All elite populations provided by the four seed companies were monomorphic for all mitochondrial probe-enzyme combinations. The uniformity of the leek mitochondrial genome was also reported by Kik et al. (1997), who observed two mitochondrial types. Our evaluation of greater numbers of accessions revealed little additional variability in the mitochondrial genome of leek. Importantly, *atp9* revealed mitochondrial polymorphisms in three accessions (Table 1); chimeric coding regions involving subunits of the F₁-ATPase complex have been associated with CMS in *Petunia* (Young and Hanson, 1987) and maize (Dewey et al., 1986).

Great-headed garlic differed from leek and kurrat for six polymorphisms in the chloroplast genome (Table 2). Although the chloroplast genome is highly conserved and polymorphisms are rarely observed among closely related plants (Palmer, 1990), RFLPs in the chloroplast DNA have been described among male-fertile and sterile cytoplasms of onion and sorghum, possibly due to introgression of alien cytoplasms. The mitochondrial genome of

Table 2. Polymorphisms in the chloroplast genome between great-headed garlic (*Allium ampeloprasum* var. *holmense*) and leek (*A. ampeloprasum* var. *porrum*).

Character ^z	Leek ^y	Great-headed garlic ^y	Probe or enzyme ^x	Change ^w
7	1	0	13-16/ <i>Xho</i> I	6.0 = 4.0 + 2.0
9	0	1	17/ <i>Bgl</i> II	2.0 = 1.6 + 0.4
13	1	0	11-12a/ <i>Dra</i> I	3.0 = 1.6 + 1.4
22	0	1	11-12a/ <i>Eco</i> RI	6.0 = 4.1 + 1.9
43	0	1	13-16/ <i>Bam</i> HI	3.5 = 2.4 + (1.1)
44	0	1	19a-b/ <i>Bgl</i> II	Presence of 1.5-kb band

^zCharacters 7, 9, 13, and 22 were reported previously (Havey, 1992) and are included here for convenience only. Characters 43 and 44 are new polymorphisms in the *Allium* chloroplast genome.

^y(0) Absence and (1) presence of restriction-enzyme sites.

^xProbes and restriction enzymes are described in Materials and Methods.

^wFragment sizes in kilobases. Parenthesis indicates that fragment did not appear on autoradiograms.

great-headed garlic differed from leek and kurrat for many probe-enzyme combinations: *atp6* (*Bgl*III, *Dra*I, *Eco*RI, *Kpn*I, *Hind*III, *Pst*I, *Sac*I), *atp9* (*Dra*I, *Eco*RI, *Hind*III, *Kpn*I), *cob* (*Bst*EII, *Dra*I, *Eco*RI), *cox1* (*Bst*EII, *Dra*I, *Hind*III, *Pst*I, *Sac*I, *Xho*I), *cox2* (*Bgl*II, *Bst*EII, *Dra*I, *Eco*RI, *Hind*III), and *cox3* (*Bst*EII, *Dra*I, *Eco*RI, *Hind*III, *Kpn*I, *Sac*I, *Xba*I, and *Xho*I). Kik et al. (1997) also observed significant variability between the mitochondrial genomes of great-headed garlic and leek. Differences in the chloroplast and mitochondrial genomes between great-headed garlic and leek were similar in number to those revealed between N and S cytoplasms of onion (Havey, 1993).

CMS may result from introgression of an alien cytoplasm (Gerstel, 1980; Havey, 1993; Izhar, 1984) or be conditioned by chimeric mitochondrial open-reading frames (Dewey et al., 1986; Young and Hanson, 1987). For CMS conditioned by an alien cytoplasm, the mitochondrial genome may possess many polymorphisms. If a rearrangement of the mitochondrial genome produced a chimeric open-reading frame conditioning CMS, relatively few polymorphisms may be detected in the mitochondrial DNA (Hanson, 1991). We undertook restriction-enzyme analysis of the organellar genomes to identify polymorphic organellar genomes and reduced the number of candidate plants to evaluate for a putative CMS system. Although we evaluated more accessions of leek, our results agree with those of Kik et al. (1997) demonstrating few polymorphisms in the organellar genomes of leek and kurrat, reducing the probability that a CMS system will be identified by this approach. We isolated individual leek plants possessing mitochondrial polymorphisms (Table 1) and have initiated a long-term study to evaluate for male sterility among self-pollinated and testcross progenies. Because of the few mitochondrial polymorphisms observed among accessions of leek and kurrat, a better approach to generate a CMS system may be transfer of an alien cytoplasm to leek by backcrossing, such as that undertaken by Kik et al. (1997) or Peterka et al. (1997).

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