Theor Appl Genet (1995) 90: 746–754

M. Ulloa-G · J. N. Corgan · M. Dunford

Evidence for nuclear-cytoplasmic incompatibility between *Allium fistulosum* and *A. Cepa*

Received: 27 June 1994 / Accepted: 28 July 1994

Abstract An F_2 population (*Allium fistulosum* × *A. cepa*) of 20 plants, 10 BC_1,[(*A. fistulosum* × *A. cepa*) × *A. cepa*], and 50 BC_2 plants,{[(*A. fistulosum* × *A. cepa*) × *A. cepa*] × *A. cepa*}were studied cytogenetically and characterized for four isozyme alleles plus various morphological characteristics. All of the progenies were in *A. fistulosum* (the bunching onion) cytoplasm. In the F_2 population we observed non-random chromosomal and allelic segregation, suppression of bulb onion allelic expression, and abnormalities in mitosis and meiosis. Most BC_2 plants resembled *A. cepa* (the bulbing onion) morphologically, but anthers, filaments, pistils, and petals were abnormal. Only 3 plants, and these were most nearly like the F_1 hybrid morphologically, produced any seeds.The data and observations support the hypothesis of nuclear-cytoplasmic incompatibility interactions between the bunching and bulb onion species.

Key words Interspecific · Introgression · Sterility

Introduction

Attempts to introgress genes from the bunching onion, *Allium fistulosum* L. into the bulb onion, *A. cepa* L. date back to 1935 (Emsweller and Jones 1935a). Despite numerous reports on interspecific hybrids and backcrosses (Bark et al. 1994; Cryder 1988; Cryder et al. 1991; Emsweller and Jones 1935a, b, 1945; Levan 1936, 1941; Maeda 1937; Peffley et al. 1985; Peffley 1986; Peffley and Currah 1988; Ulloa et al. 1994; Van Der Meer and Van Benekom 1978), no fertile bulb onion cultivars are known to possess genes from the bunching onion. In backcrosses to the bulb onion from F_1 hybrids in the bunching onion cytoplasm, Cryder et. al. 1991, reported barriers to independent segregation, and Ulloa (1993) reported infertility of similar backcrosses that had completely normal meiotic pairing. Nuclear-cytoplasmic incompatibility between the bunching and bulb onion species have been suggested (Bark et al. 1994; McCollum 1974; Ulloa 1993), but there is only minimal evidence in support of this theory.

We were able to produce populations of F_2 and BC_2 plants {(*A. fistulosum*A. *cepa*) × *A. cepa*} × *A. cepa* in bunching onion cytoplasm. This investigation presented here on the F_2 and BC_2 populations documents barriers to independent segregation, including cytogenetic abnormalities, non-random segregation of isozyme and other markers, and morphological abnormalities of the reproductive structures.

Materials and methods

The *Allium* populations

The bunching onion, cv. 'Ishikura' (source: Nickerson-Zwaan Seed Co.), was used as the seed parent, and the bulb onion selection NMSU 8020, a bolting-resistant selection from Texas Grano 502 PRR, was used as the pollen parent. The F_1 interspecific hybrid 8273 population was obtained in the field under a screen-covered isolation cage. The F_2 progeny were produced by selfing a cloned population obtained from the F_1 hybrids under a screen-covered isolation cage. The first backcrosses (BC_1) were made using 8273 as the seed parent and the bulb onion cultivarstet 'NuMex Sunlite', a selection from TG 502 PRR, as the recurrent parent. The backcross was made under field conditions. All pollen sources except the F_1 hybrid and the recurrent parent were excluded. Beehives were placed in the field to insure pollination. Ten backcross plants were selected for further studies based on chromosome count (2n=16), and morphological and

Communicated by D. R. Pring

M. Ulloa-G (✉)
SUN SEEDS Co., Research Department, El Centro, CA 92243, USA

J. N. Corgan
Department of Agronomy and Horticulture,
New Mexico State University, Las Cruces, NM 88003, USA

M. Dunford
Department of Biology, New Mexico State University
Las Cruces, NM 88003, USA

Use of trade names does not imply endorsement of the products named nor criticism of similar ones not named. This research was supported by the New Mexico Agricultural Experiment Station.

isozyme markers. Each plant was propagated asexually to produce populations of clones. The second backcrosses (BC_2) were made under a screen-covered isolation cage. The BC_1 clones were placed inside the cage in small plots as seed parents, with the bulb onion, 'NuMex Sunlite', as the pollinator.

Isozyme analysis

The plants described above were analyzed electrophoretically for the following enzyme loci: Alcohol dehydrogenase (*Adh*-1), isocitrate dehydrogenase (*Idh*-1), malate dehydrogenase (*Mdh*-1), and phosphoglucoisomerase (*Pgi*-1). The isozyme analysis was done on root tissue. The protocol described by Vallejos and Tanksley (1983) was used and the allele designations were those assigned by Peffley et. al. (1985). Allelic designations are given for the first time in *Allium* for the isozyme *Mdh*-1 (Fig. 1).

Chromosome observations

Karyotype observations and differences were based on the standard system of nomenclature (Kalkman 1984) adopted at the Fourth EUCARPIA *Allium* Symposium in 1988 (De Vries 1990). Chromosome number is based on chromosome length and centromere position. The symbol C for *cepa* follows the chromosome number, e. g. 6C for the bulb onion and 5F for the bunching onion.

Mitotic studies

Onion root tips were pretreated with colchicine (0.03%) for 3 h at 4°C, then transferred to ethanol:acetic acid (3:1). Before slide preparation the tissues were hydrolyzed in 1 *N* HCl at 60°C for 15 min, then stored in 70% ethanol until used. Individual root tips were placed on a glass slide, stained with acetocarmine, macerated, and viewed under 40 or 60× magnification.

Meiotic studies

Chromosome behavior at meiosis in pollen mother cells (PMCs) was examined. Floral buds were collected before anthesis and fixed in 3:1 ethanol:acetic acid. Staining was with acetocarmine. Meiocytes were observed at interphase, prophase I, metaphase I, anaphase I, telophase I, prophase II, metaphase II, and anaphase II.

Pollen evaluation

Pollen production was evaluated by field anther collection and observation for presence of pollen. Observations were made every 2 days on F_2 and BC_2 plants during the flowering period.

Morphological characters

Only traits that are distinctly different between the bunching and bulb onion were chosen. The bunching onion 'Ishikura' is white and the bulb onion parents are yellow. The F_1 hybrid is yellow. The pattern of anthesis is regular in the bunching onion from the top to the bottom of the umbel, while in the bulb onion cymes flower at random throughout the umbel. In the F_1, the pattern of anthesis is random, like that of the bulb onion. The bunching onion and the F_1 hybrid have highly branched root systems, while in the bulb onion root branching is minimal. The diameter of the main roots of the bunching onion and the F_1 is relatively large in comparison to that of the bulb onion, and the root diameter of the F_1 is like that of the bunching onion. The bunching onion has green tepals, the bulb onion has white tepals, and in the F_1 the tepals are intermediate in color and distinctive from either parent.

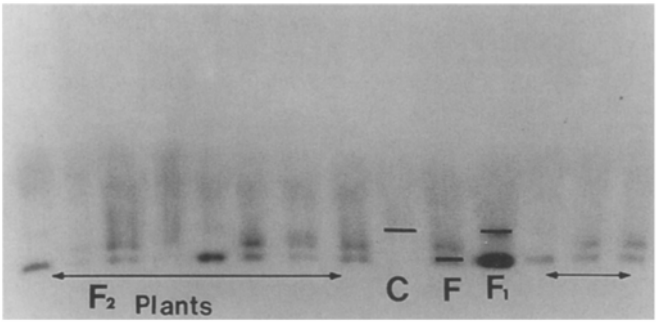

Fig. 1 *Mdh* (E.C. 1.1.1.37). Profile in TRIS citrate (TC) buffer using starch gel. *C A. cepa* with a fast allele, *Mdh-1*^{1}, *F A. fistulosum* with slow allele *Mdh-1*^{2}. F_1 *A. fistulosum* × *A. cepa* F_1 hybrid with a dimorphic locus. *Arrows* indicate different F_2 plants

Screening for pink root resistance

Pyrenochaeta terrestris inoculum was produced by a technique of Netzer et. al. (1985). A New Mexico isolate designated 'Franzoy' was grown on PDA agar in a 15-ml petri dish for 20 days. Inoculum was further increased by placing six, 1-cm^2 pieces of colonized agar into 100 g of sterile, pesticide free wheat seed in 100 ml distilled water; this was maintained at 24°±1°C for 15 days. Cultures were agitated daily to ensure even growth. Cultures were then dried at room temperature for 48 h. The inoculum was blended with 1.5 l distilled water and added to 60 l of growth medium, followed by thorough mixing.

Plants for screening were dug from field plots during the winter and transplanted into the medium. They were placed outdoors in a cool environment for 11 days to permit root development, then moved into a warm greenhouse. Soil temperatures were maintained at 26°±1°C for 20 days The plants were then dug out, and each plant was screened for level of pink root infection, based on an interaction scale as follows: 1=excellent root system with no symptoms of infection, 3=extensive root system with less than 25% of roots infected, 5=average root system with 26–50% of roots infected, 7=poor root system with 51–75% of roots infected, and 9=poor or nonexistent root system, with more than 75% of roots infected.

For F_2 and BC_1 plants, one division of each clone plus an *A. cepa* control were randomly arranged in each of five flats for a randomized complete block design, with five replications.

Statistical methods

Segregation of isozyme alleles was based on the alleles of the two species and their F_1 hybrid. Analysis for color segregation was based on the inheritance of color in the bulb onion. The expected ratio in the F_2 is based on the assumption that genes for color are allelic between the two species. Since color expression was dominant in the F_1, our assumption is that neither parent had the dominant I (color inhibitor) gene, and therefore the expected ratio in the F_2 is 3 colored to 1 white. Segregation ratios were tested by χ^2 analysis for goodness-of-fit to expected Mendelian ratios for isozyme and bulb color segregation.

Results and discussion

Cytogenetics

Meiotic studies were done on 16 diploid F_2 plants that flowered (Table 1). The percentage of bivalent pairing was generally higher in the F_2 plants than in the F_1, and several were highly fertile. Only 2 F_2 plants had more than 2n=16

Table 1 Meiotic analysis of pollen mother cells and seed and pollen production in *A. fistulosum*, *A. cepa*, their F_1 hybrid and F_2

Entries	% bivalent[a]	% cells with micronuclei[b]	%cells with bridges		Seeds per umbel	Pollen production
			1	2[c]		
A. fistulosum	100	0	0	0	–	Yes
A. cepa	100	0	0	0	–	Yes
F_1 hybrid	42	51	5.0	0	4	No
F_2–3	82	9	10.0	1.0	4	No
–4	92	0	0	0	18	Yes
–5	79	24	0	0	8	Yes
–6	98	0	0	0	120	Yes
–8	80	17	0.5	0	52	Yes
–9	85	19	1.0	0	94	No
–10	62	37	11.0	4.0	0	No
–12	69	13	0	0	255	No
–15	90	0	0	0	108	Yes
–17	91	7	1.0	0.4	14	No
–18	90	2	0	0	62	Yes
–19	93	5	0.5	0	61	Yes
–21	80	21	1.5	0	38	Yes
–22	82	47	2.0	0	55	Yes
–23	87	3	0.2	0	52	Yes

[a] Observed heteromorphic bivalents at metaphase I
[b] Percent cells with at least one micronucleus
[c] 1or 2 bridges per cell

chromosomes. However, in some plants, mitotic cells had chromosome fragments that resembled supernumary or 'B' chromosomes. In 4 of the F_2 plants fission-refusion at the centromere region was observed in nearly every chromosome (Fig. 2A, B). Most structural chromosome or chromatid changes involved both breakage and reunion. Breakage-reunion is thought to occur spontaneously in response to stress or as a result of mutagenesis (Stadler 1931; Sax 1938).

Mitotic abnormalities also occurred in BC_2 plants. In 1 progeny, one-third of the cells had three nuclei surrounded by a single cell wall (Fig. 3A), and cell chromosome counts varied between 8, 16, or 24 (Fig. 3B–D). It is known that nuclear division is not always followed by breakdown of the nuclear membrane; this phenomenon has been referred to as endomitosis (Schulz-Schaffer 1980).

In the PMCs of F_1 hybrids and BC_1 progeny, spindle formation sometimes showed parallel or divergent fibers instead of those converging at the two poles. Those plants with a tripolar orientation had chromosomes which formed three separate nuclei (Figs. 4A; 5). The spindle at the second meiotic division may again be divergent, producing more than four microspores. A similar divergent spindle was reported in barley, and the phenomenon was called 'cytomitoxis' (Smith 1942; Gates 1911), in which the fusion of some chromosomes are thought to occur prior to meiosis and cell walls are absent.

Spindle metabolism depends on the close control of a complex intra-cellular environment. Any interference with meiosis-specific proteins regulating crossing-over could conceivably result in synapses or premature disynapses, nondisjunction, and ultimately aneuploidy (Backer and Allen 1987). A recent model supports a hypothesis called chromosome displacement (Ford 1987). Displacement is strongly influenced by the size of the chromosomes and does not reflect spatial ordering at metaphase (I or II). The size of the chromosomes of the bunching onion is smaller than that for the bulb onion (Jones and Rees 1968; Peffley

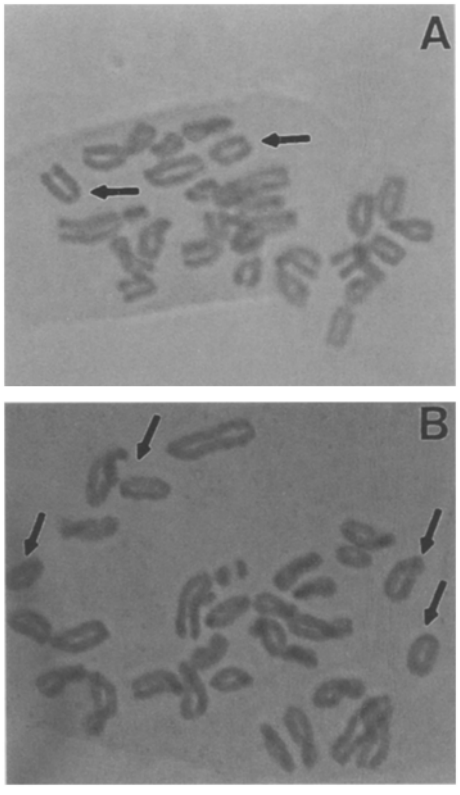

Fig. 2A, B Chromosomes at mitosis in *A. fistulosum* × *A. cepa* F_2 progeny. F_2 progeny no. 2 (**A**) and F_2 progeny no. 3 (**B**) showing fission-refusion of chromosomes at the centromere region (*arrows*)

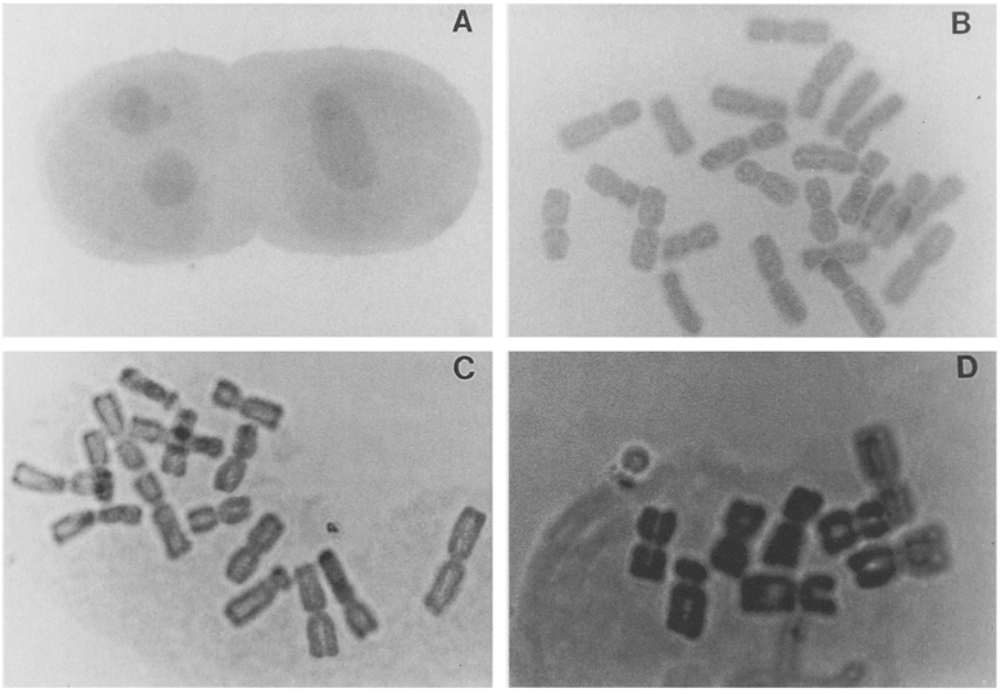

Fig. 3A–D Chromosomal variation at mitosis in BC progeny. **A** BC_2 progeny no. 1 with three nuclei surrounded by a single cell wall. **B, C, D** BC_2 progeny no. 6-1 with 24, 16, or 8 chromosomes in the same root tip, respectively

and Currah 1988; Vosa 1976). Chromosome size could affect placement and spindle fiber attachment, in some cases resulting in mis-division. Mal-orientated kinetochores may occur due to a failure in the synthesis of specific protein-regulating events. It is possible that the larger the chromosome the greater the driving force by cytosol metabolism to move chromosomes to the poles.

Of 20 F_2 plants 19 had a chromosome that resembled the 5F chromosome of the bunching onion, being easily identifiable by its satellite and nucleolus attachement. Ulloa-G et al. (1994) reported that in the F_1 hybrid the 5F chromosome from the bunching onion carried the nuclear organizer (NOR) at a subtelocentric position. The NOR is responsible for expression of the nucleolus, which contains rRNA, but ribosomal genes become transcriptionally inactive during the terminal stages of pollen maturation (Mascarenhas 1990). In the F_1 hybrid, microspores carrying the 5F chromosome could have a maturity advantage, resulting in preferential segregation of the 5F chromosome (Fig. 4D).

The tendency for genome reorganization described above may occur when a genome is under stress (McClintock 1984). These phenomena have been described as 'genome shock' (Graves 1988; McClintock 1984). In the case described here the stress or shock is most likely a result of nuclear-cytoplasmic incompatibility.

Segregation of markers

In the F_2 plants the segregation of enzymes loci *Adh-1*, *Mdh-1*, and *Pgi-1* deviated significantly from the expected ratios (Table 2). The segregation of *Idh*-1 and bulb color fits the expected ratio, assuming that color genes in the bunching onion are allelic to those in the bulb onion. The deviations from the expected ratios for the other three loci are statistically significant. Even with the relatively small populations the data indicate a definite preferential segregation for bunching onion alleles.

Inheritance characteristics of the other morphological markers are not known, except as they are expressed in the F_1 hybrid. In Table 3, cc indicates that the bulb onion characteristic is dominant, ff that the bunching onion characteristic is dominant, and fc that the expression is intermediate between the two species, in the F_1 hybrid. With normal segregation, we expected that for the umbel opening characteristic, for which bulb onion genes are dominant, more than half of the F_2 plants would exhibit bulb onion morphology. In fact, segregation was strongly in favor of bunching onion expression. Tepal color, which in the F_1 is intermediate between the species, also segregated strongly in favor of the bunching onion character. For pink root resistance, 3 of 16 F_2 plants were scored as susceptible (Table 3), but the F_2 population had a mean pink root score nearly the same as that of the bunching onion parent (Table 4).

Bark et. al. (1994) reported that segregation of DNA restriction fragment length polymorphism fragments in 2 of 3 BC_1 plants employed in this study showed significant deviations from expected ratios. There seems little doubt that in the F_1 hybrid between bunching and bulb onion, in bunching onion cytoplasm, gamete selection occurs at some level, and that segregation of certain bunching onion alleles is favored.

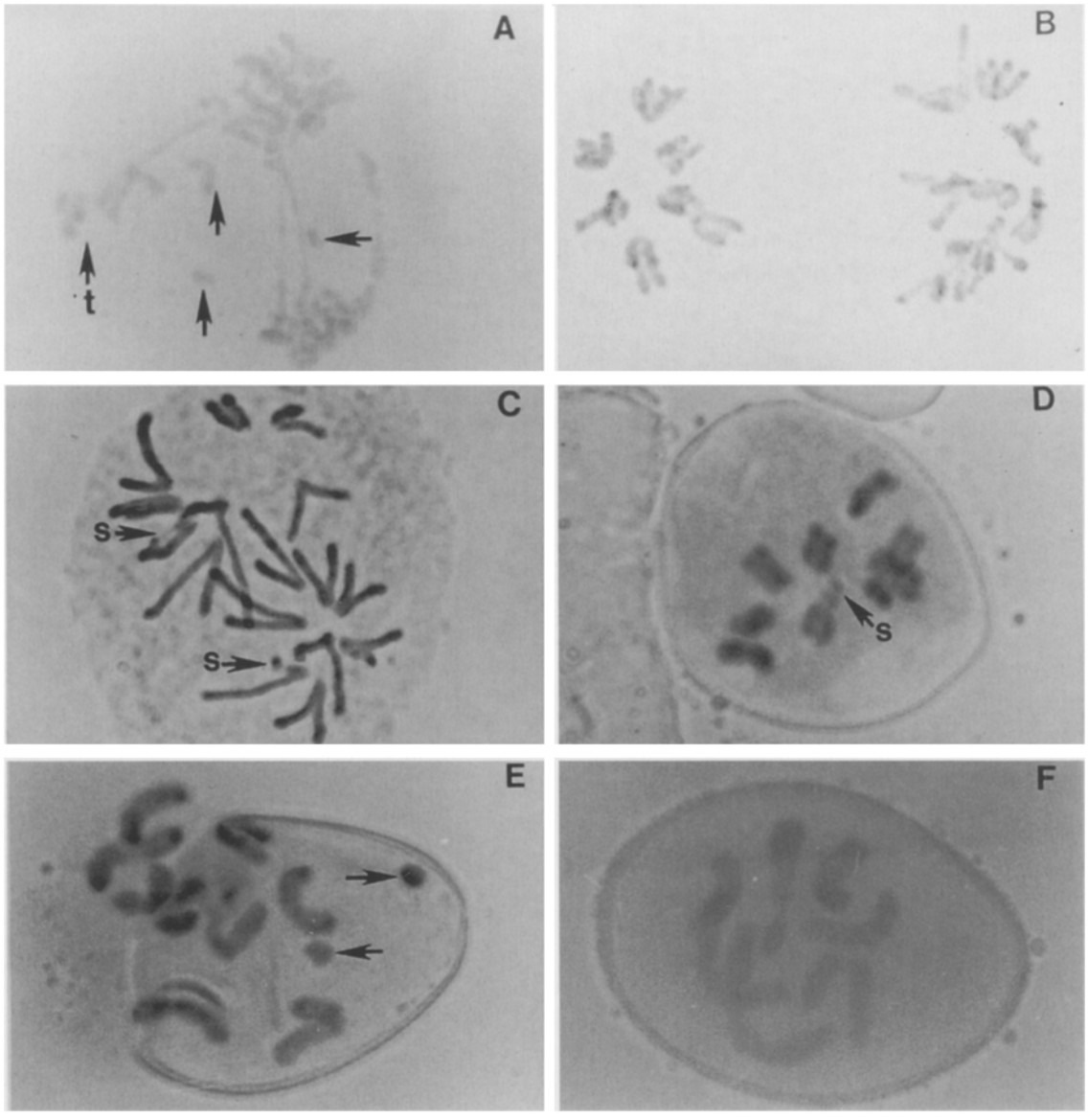

Fig. 4A–F Observations of pollen mother cells (PMCs) in *A. fistulosum* × *A. cepa* F_1 hybrid. **A** Three bridges with fragments (*arrows*) and a possible tripolar orientation of chromosomes (*t*), **B** uneven segregation of bivalents in a bipolar orientation, **C** subtelocentric satellite chromosomes (SSCs) 5F and 6C segregated to opposite poles at anaphase II, **D** microspore with a normal chromosome number (8) at pollen mitosis with a chromosome morphologically similar to 5F SSC from *A. fistulosum* (*s*), **E** abnormal microspore at pollen mitosis with nine chromosomes and two fragments or small chromosomes, **F** abnormal microspore with seven chromosomes

Morphological abnormalities

All of the BC_1 plants were male-sterile (no pollen could be detected in anthers at anthesis). All except 1 had normal flower morphology in that pistils and anthers were present. In 1 plant no anthers were present at anthesis. None of the 50 BC_2 plants had anthers at anthesis . Other anomalies included very short petals, abnormally small pistils, and shortened filaments (Figs. 6, 7). Most BC_2 plants resembled the bulb onion in their foliage and bulbing characteristics, and were completely sterile.

Our observations suggest that when recurrent backcrosses are made from an F_1 hybrid in bunching onion cytoplasm to the bulb onion, the end result is complete sterility. These observations plus the evidence of cytogenetic anomalies and preferential segregation indicate a high level of nuclear-cytoplasmic incompatibility between the two species. If other sources of the bunching onion cytoplasm can be found in which this incompatibility is less severe, it may be possible to develop new sources of cytoplasmic male sterility for hybrid seed production. However, that does not appear possible with the source that we used, the cultivar 'Ishikura'.

Attempts to introgress genes from bunching onion into the bulb onion by using the bunching onion as the pistil-

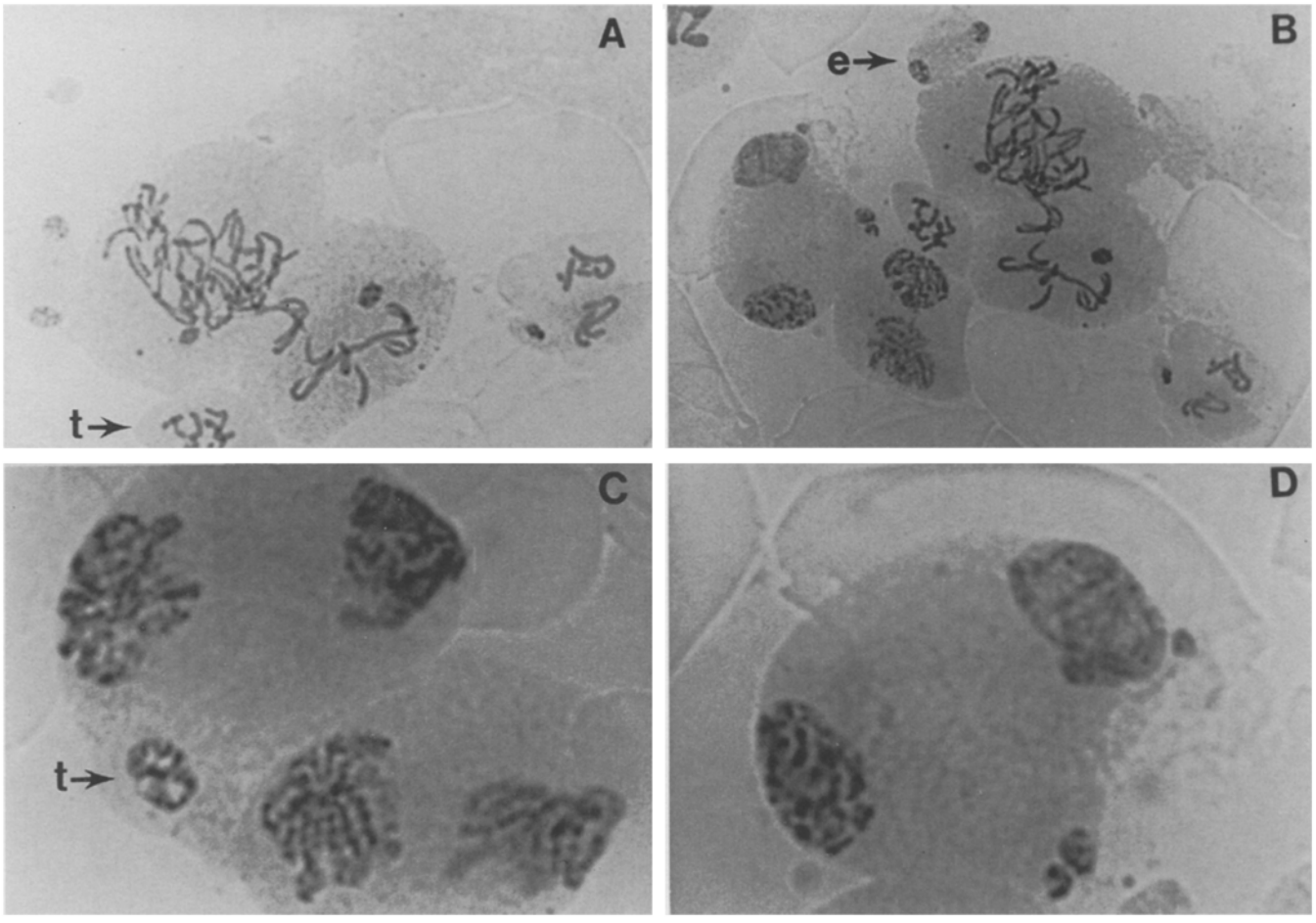

Fig. 5A–D Chromosomal variation at meiosis in BC_1 progeny no. 8. Tripolar orientation of chromosomes (*t*) results in three separate nuclei

Table 2 Segregation for isozyme and color alleles in an *A. fistulosum* × *A. cepa* F_2 population

Locus	Number of plants	F_2 expected ratios	F_2 observed ratios	χ^2
		fist:het:cepa[a]	fist:het:cepa[a]	
Adh-1	18	1:2:1	9:9:0	9.0*
Idh-1	21	1:2:1	7:10:4	0.9ns
Mdh-1	18	1:2:1	10:8:0	11.3**
Pgi-1	21	1:2:1	13:5:3	15.2**
		Yellow:white	Yellow-white	
Bulb color	20	3:1	15:5	0ns

*,** Significant at $P<0.05$ and $P<0.01$, respectively
[a] fist, Homozygous for bunching onion alleles; het, heterozygous; cepa, homozygous fo bulbing onion alleles

Table 3 Phenotypic expression for pink root resistance plus several morphological characteristics in an *A. fistulosum* × *A. cepa* F_2 population

Morphological character	Number of plants	F_1 hybrid expression[a]	F_2 expression[a]
			ff:cc
Umbel opening	17	cc	13:4
Root branching	20	ff	13:7
Root diameter	20	ff	15:5
Pink root resistance[b]	16	ff	13:3
			ff:fc:cc
Tepal color	17	fc	12:4:1

[a] ff, Expression is the same as that of the bunching onion parent; fc, expression intermediate between bunching and bulb onion; cc, expression is the same as the bulb onion parent
[b] Pink root infection was scored on an interaction scale in which 1 = no symptoms to 9 = all roots infected. Plants which scored 3 or less were considered to carry resistance genes from the bunching onion

late parent will likely be unsuccessful. Van der Valk et. al. (1991) demonstrated disturbed pollen tube growth in the styles of F_1 interspecific plants that were pollinated with *A. cepa* pollen, but the effect was similar for reciprocal crosses suggesting that this barrier to backcrossing was not strictly a cytoplasmic effect. Although hybrids are made most readily with the bunching onion as the pistillate parent, reciprocal crosses have been made. We are presently evaluating a group of BC_1 plants from a hybrid in bulb onion cytoplasm.

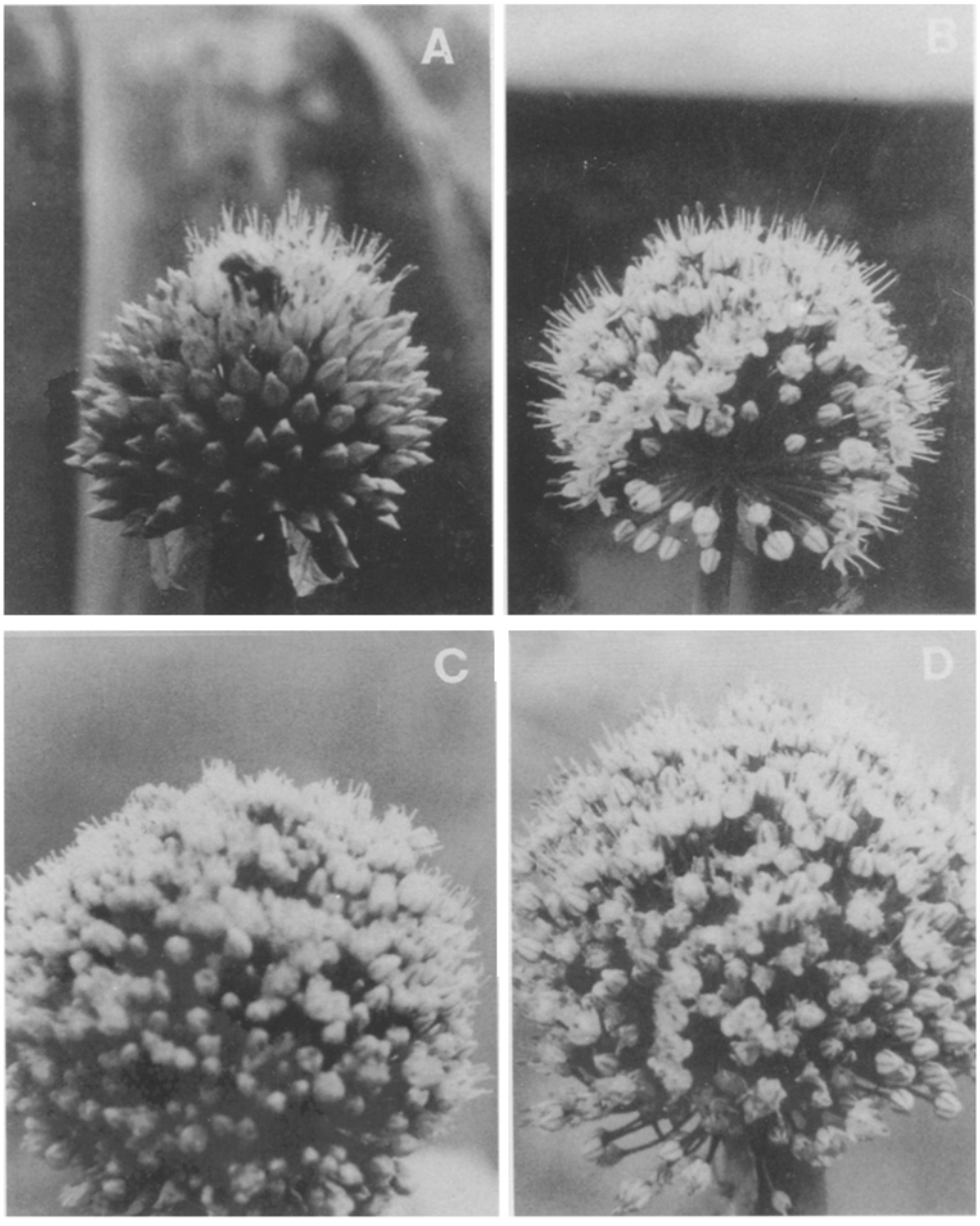

Fig. 6A–D Umbel and flower differences. **A** *A. fistulosum*, **B** *A. cepa*, **C** BC_2 progeny no. 1, **D** BC_1 progeny no. 6

Table 4 Scores for pink root infection in *A. fistulosum, A. cepa, A. fistulosum* × *A. cepa* F_1 hybrids, F_2s, and BC_1 to *A. cepa*

Source	Number of plants scored	PRR score[a]		
		Mean	SD	Range
A. fist., Ishikura	5	1.4	0.8	1–3
A. cepa, Sunlite	5	5.0	0	–
F_1 hybrid	5	1.0	0	–
F_2 population	20	1.8	1.3	1–4
BC_1 population	10	1.8	1.2	1–5

[a] Pink root infection was scored on an interaction scale for each plant or division in which 1=extensive root system and no symptoms of infection to 9 = poor root system and more than 75% of roots infected

Conclusions

The F_2 plants from a bunching onion × bulb onion hybrid, in bunching onion cytoplasm, showed preferential segregation of certain bunching onion alleles. Cytogenetic anomalies suggested a genome under stress. BC_2 plants that were morphologically similar to the bulb onion were completely sterile and had various flower anomalies. The evidence suggests strong nuclear-cytoplasmic incompatibility between the bulb onion nucleus and bunching onion cytoplasm.

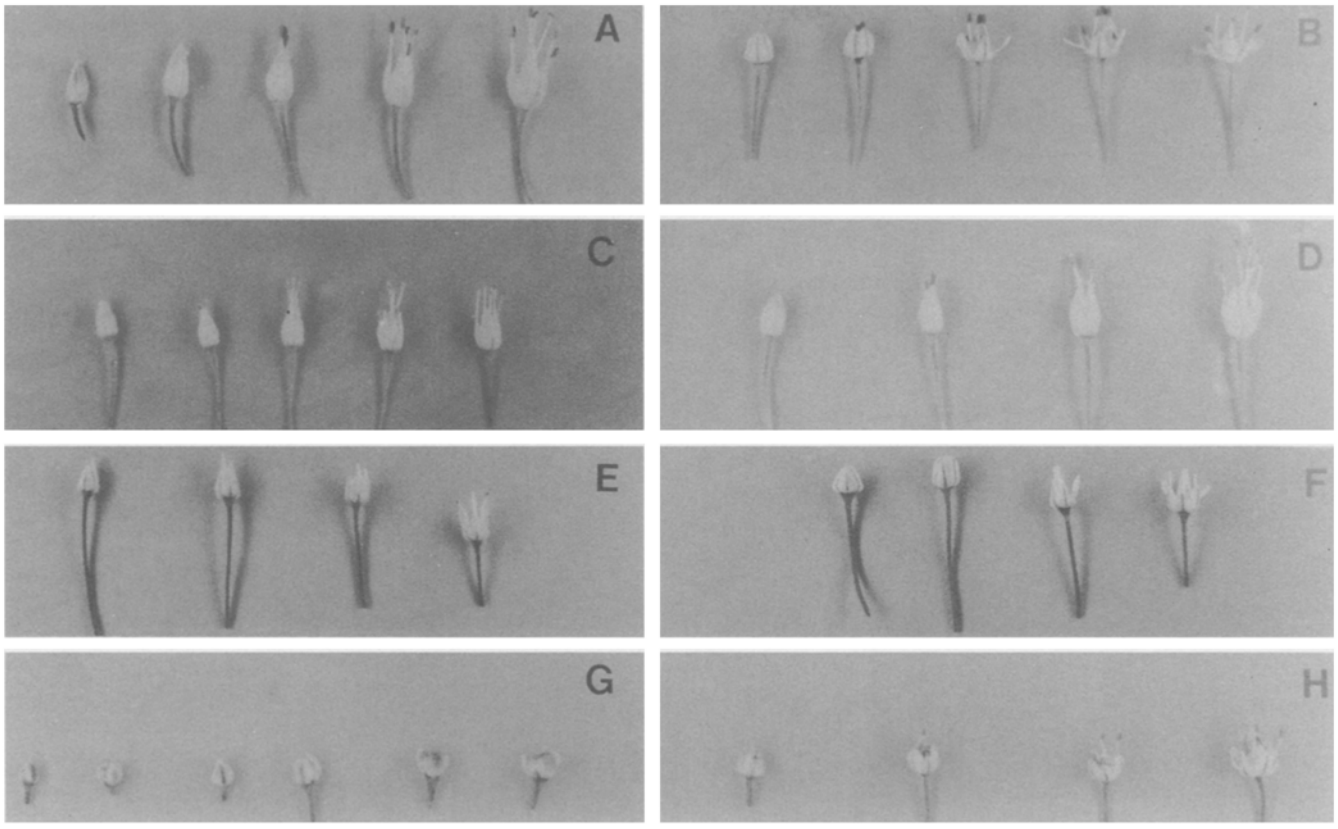

Fig. 7A–H Flower morphology and its anomalies in BC progenies. **A** *A. fistulosum*, **B** *A. cepa*, **C** *A. fistulosum* × *A. cepa* F_1 hybrid, **D** F_2 plant, **E** BC_2 progeny no. 6, **F, G** BC_2 progeny no. 1, **H** BC_1 progeny no. 6

References

Backer LC, Allen JW (1987) Meiotic origin of aneuploidy: an overview. In: Aneuploidy, part A: incidence and etiology. Alan R Liss, New York, pp 219–232

Bark OH, Havey MJ, Corgan JN (1994) Restriction fragment length polymorphism analysis of progeny from an *Allium fistulosum* by *Allium cepa*. Hybrid J Amer Soc Hortic Sci 119:1046–1049

Cryder CM (1988) A study of the associations of heritable traits in progeny from the interspecific backcross of (*Allium fistulosum* L. × *Allium cepa* L.) × *A. cepa* L. PhD thesis, New Mexico State University, Las Cruces, N.M.

Cryder CM, Corgan JN, Urquhart NS, Clason D (1991) Isozyme analysis of progeny derived from (*Alliumfistulosum* × *Allium cepa*)×*Allium cepa*. Theor Appl Genet 82:337–345

De Vries JN (1990) Onion chromosome nomenclature and homoeology relationships workshop report. Euphytica 49:1–3

Emsweller SL, Jones HA (1935a) An interspecific hybrid in *Allium*. Hilgardia. 9:265–273

Emsweller SL, Jones HA (1935b) Meiosis in *Allium fistulosum, Allium cepa*, and their hybrid. Hilgardia 9:277–288

Emsweller SL, Jones HA (1945) Further studies on the chiasmata of the *Allium cepa* × *A. fistulosum* hybrid and its derivatives. Am J Bot 32:370–379

Ford JH (1987) Chromosome displacement hypothesis. In: Aneuploidy, part A: incidence and etiology. Alan R Liss, New York, pp 237–247

Gates RR (1911) Pollen formation in *Oenothera gigas*. Ann Bot 25:909–974

Graves JAM (1988) Genome instability in interspecific cell hybrids I. Unstable expression of genes in divergent cell hybrids. J Genet 67:9–22

Jones RN, Rees H (1968) Nuclear DNA variations in *Allium*. Heredity 23:591–605

Kalkman ER (1984) Analysis of the C-banded karyotype of *Allium cepa* L. standard system of nomenclature and polymorphism. Genetica 65:141–148

Levan A (1936) Die zytologie von *Allium cepa* × *A. fistulosum*. Hereditas 21:195–214

Levan A (1941) The cytology of the species hybrid *Allium cepa* × *A. fistulosum* and its polyploid derivatives. Hereditas 27:253–272

Maeda T (1937) Chiasma studies in *Allium fistulosum, Allium cepa* and their F_1, F_2, and backcross hybrids. Jpn J Genet 13:146–159

Mascarenhas JP (1990) Gene activity during pollen development. Annu Rev Plant Physiol Plant Mol Biol 41:317–338

McClintock B (1984) The significance of responses of the genome to challenge. Science 226:792–801

McCollum G (1974) Hybrid origin of top onion, *Allium cepa*, var *'viviparum'*. Z. Pflanzenzucht 71:222–232

Netzer D, Rabinowitch HD, Weintal CH (1985) Greenhouse technique to evaluate onion resistance to pink-root. Euphytica 34: 385–391

Peffley EB (1986) Evidence for chromosomal differentiation of *Allium fistulosum* and *A. cepa*. J Am Soc Hortic Sci 111:126–129

Peffley EB, Currah L (1988) The chromosomal locations of enzyme-coding genes in *Adh-1* and *Pgm-1* in *Allium fistulosum*. Theor Appl Genet 75:945–949

Peffley EB, Corgan JN, Horak HG, Tanksley SD (1985) Electrophoretic analysis of *Allium* alien addition lines. Theor Appl Genet 71:176–184

Sax K (1938) Chromosome aberrations induced by X-rays. Genetics 23:494–516

Schulz-Schaeffer J (1980) Cytogenetics, plants-animals-humans. Springer, Berlin Heidelberg New York

Smith L (1942) Cytogenetics of a factor for multiploid sporocytes in barley. Amer J Bot 29:451–456

Stadler LJ (1931) The experimental modification of heredity in crop plants I. Induced chromosome irregularities. Sci Agric 11: 557–572

Ulloa-G M (1993) A cytogenetic, isozyme and morphological study on interspecific progenies from *Allium fistulosum* × *Allium cepa* crosses. Ph.D. Thesis, New Mexico State University, Las Cruces, NM

Ulloa-G M, Corgan JN, Dunford M (1994) Chromosome characteristics and behavior differences in *Allium fistulosum* L., *A. cepa* L., their F_1 hybrid, and selected backcross progeny. Theor Appl Genet 89:567–571

Van der Meer QP, van Benekom JL (1978) Improving the onion crop (*Allium cepa* L.) by transfer of characters from *Allium fistulosum* L. Bull Warzywhiczy XXII, Instytut Warzywhictwa Skierniewice

Van der Valk P, de Vries SE, Everink JT, Verstappen F, de Vries JN (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

Vallejos CE, Tanksley SD (1983) Segregation of isozyme markers and cold tolerance in an interspecific backcross of tomato. Theor Appl Genet. 66:241–247

Vosa CG (1976) Heterochromatin patterns in *Allium*. I The relationship between the species of the cepa group and its allies. Heredity 36:383–392