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

Chromosome characteristics and behavior differences in *Allium fistulosum* L., *A. cepa* L., their F_1 hybrid, and selected backcross progeny

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Abstract Mitotic and meiotic studies were performed on *Allium fistulosum*, *A. cepa*, their F_1 hybrid, and ten selected backcross (BC_1) plants [(*A. fistulosum* × *A. cepa*) × (*A. cepa*)]. Each BC_1 plant had at least one *A. cepa* isozyme allele (*Pgi*, *Idh*, or *Adh*). Chromosome morphology and behavior differed among plants. Meiocytes were observed with one, two, or three bridges and/ or fragments, indicating at least three paracentric inversions between *A. fistulosum* and *A. cepa*. Unusual crossing over and multivalent associations suggest that the 5F subtelocentric chromosome of *A. fistulosum* is involved in at least one translocation. The number of bridges and fragments and multivalent associations varied between the F_1 hybrid and BC_1 progenies. The F_1 hybrid and all BC_1 progenies were either sterile or had very little seed set. Fertility was not restored in any of the selected BC_1 plants.

Key words Cytogenetics · Inversions · Translocations

Introduction

The bulb onion (*Allium cepa* L.) is the most important *Allium* species worldwide (Lorenz and Maynard 1989). Until now genes for onion improvement have been contributed by *A. cepa*. The limited resistance of *A. cepa* to several diseases has prompted numerous attempts to incorporate resistance from other *Allium* species. *Allium fistulosum* has several characteristics of interest for improvement of the bulb onion, e.g., resistance to *Fusarium* basal rot and pink root diseases, cold hardiness, and high soluble solids (Van Der Meer and Van Benekom 1978). Several researchers are presently involved in research to introgress genes from *A. fistulosum* into *A. cepa*. Previous work includes: development of onion interspecific F_1 hybrids (Emsweller and Jones 1935a; Maeda 1937), backcross (BC) progeny (to *A. cepa*) (Cryder 1988), and addition lines (Peffley et al. 1985). The F_1 interspecific hybrid is easily produced but has low fertility. Meiotic studies have shown discrepancies in chromosome behavior (Emsweller and Jones 1935b, 1945; Levan 1936, 1941; Maeda 1937; Cochran 1950; Peffley 1986). Some hybrids were observed with a high frequency of complete bivalent pairing (Emsweller and Jones 1935b, 1945), but other hybrids had frequent multivalents (Levan 1936, 1941). In more recent research Peffley (1986) reported evidence for one translocation and a possible inversion that differentiate *A. fistulosum* and *A. cepa*. The purpose of our work was to further study the chromosome characteristics and behavior of *A. fistulosum*, *A. cepa*, their F_1 hybrid and selected backcross progenies.

Materials and methods

A. fistulosum × *A. cepa* crosses and backcross populations

The interspecific F_1 hybrid 8273 population was made in the field under a screen-covered isolation cage. *A. fistulosum* 'Ishikura' (source: Nickerson-Zwaan Seed Co.) was used as the seed parent. The *A. cepa* selection NMSU 8020 which is a bolting-resistant selection from 'Texas Early Grano' (TEG) 502 PRR was used as the pollen parent. The first backcross (BC_1) were made using 8273 as the seed parent and *A. cepa* selection NMSU 8361 ('NuMex Sunlite') as the recurrent parent. 'NuMex Sunlite' is a selection from TEG502 PRR. The backcross was made under field conditions. All pollen sources except

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M. Ulloa-G (✉)
Sunseeds 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

the F_1 hybrid and the recurrent parent were eliminated. Beehives were placed in the field to ensure pollination. Ten backcross plants were selected for further studies based on chromosome counts ($2n = 16$) and morphological and isozyme markers. Each plant was propagated asexually to produce populations of clones. The second backcrosses (BC_2) were made under screen-covered isolation cages. The ten selected BC_1 clones were placed inside cages in small plots and used as seed parents. *A. cepa* recurrent parents used in these crosses originated as selections from TEG502 PRR.

Isozyme analyses

A. fistulosum, *A. cepa*, their F_1 hybrid, and the ten backcross progenies were analyzed electrophoretically for the enzyme loci *Adh*-I, *Idh*-I, and *Pgi*-I. The protocol described by Vallejos and Tanksley (1983) was used. The allele disignations were those assigned by Peffley et al. (1985). Isozyme analyses were done on root tissue.

Chromosome observations

Karyotype observations and differences were based on the standard system of nomenclature (Kalkman 1984). This system was adopted in the 4th EUCARPIA *Allium* symposium in 1988 (De Vries 1990). Chromosome number is based on chromosome length and centromere position. The symbol C for the *cepa* genome is added to the number of the chromosome, e.g., chromosome 6C for *A. cepa* and 5F for *A. fistulosum*. Similar nomenclature systems for chromosomes are used in other cultivated plants (Sybenga 1983).

Pollen evaluation

Pollen viability was compared among the parents, the F_1 hybrid, and backcross plants. Florets were collected from each population at three stages of anther development: (1) before anthesis, (2) immediately after anthesis, and (3) 3–5 days after anthesis. Florets were stored in 70% ethanol. Stainability of pollen was tested with acetocarmine. A minimum of 250 pollen grains were observed per sample viewed under 20*x* magnification.

Mitotic studies

Onion root tips were collected, pretreated with colchicine (0.03%) for 3 h at 4 °C, and then transferred to a fixation solution of ethanol: acetic acid (3:1). They were hydrolyzed in 1 N HCL at 60 °C for 15 min and then stored in 70% ethanol if not used immediately. Individual root tips were placed on a glass slide, stained with acetocarmine, macerated, and viewed under 40*x* and 60*x* magnification.

Meiotic studies

Chromosome behavior at meiosis in onion pollen mother cells (PMCs) was examined. Inflorescences were collected from each population before anthesis. Floral buds were fixed in a 3:1 solution of ethanol:acetic acid and stored in 70% ethanol. Staining was with acetocarmine. Meiocytes were observed at interphase, prophase-I, metaphase-I, anaphase-I, and telophase-I.

Results and discussion

Isozyme analysis

Electrophoretic loci of *Pgi*-I, *Idh*-I, and *Adh*-I were used to detect the *A. cepa* alleles in the selected first backcross (BC_1) plants. Polymorphism among BC_1 progenies was observed at these loci. *A. cepa* alleles were present in all BC_1 plants (Table 1).

Chromosome observations

Reasonably good agreements were found with previously reported karyotypes of the two *Allium* species (Kalkman 1984; De Vries 1990). The satellite chromosome 5F of *A. fistulosum* carried the nucleolar organizer region (NOR) at a subtelocentric position, while in *A. cepa* nucleolar attachment was to chromosome 6C, suggesting that the NOR may be at a telocentric position. A secondary constriction was not observed at mitosis in the *A. cepa* chromosome. Both NORs were capable of forming a nucleolus in each species. However, in the interspecific F_1 hybrid only one nucleolus was observed at prophase-I. Like many other metabolic events, the capacity of the NOR to form a nucleolus is under genetic control. In the F_1 hybrid the only formed nucleolus was attached to the 5F *A. fistulosum* chromosome (Fig. 1A and B).

Table 1 Meiotic analysis of pollen mother cells, seed production, and isozyme segregation for the F_1 hybrid and five selected backcross plants (*Allium fistulosum* × *A. cepa*) × *A. cepa*

Observation	F_1 hybrid	Backcross progeny				
		3	4	6	9[a]	10
% Bivalent pairing	42	100	37	80	–	47
% Meiotic figures with bridges[b] #1	5.3	0	4	3	–	4
#3	0.4	0	0	0	–	0
% Cells with micronuclei[c]	51	6	22	18	–	23
Stainable pollen (%)	9	0	10	27	–	20
Viable seed/umbel (no.)	0.25	4.4	1.2	3.3	6.1	0.3
Seedlings produced (no.)	23	3	5	3	50	1
Isozyme segregation[d] *Pgi*I	1/2	1/1	1/2	1/1	1/2	1/2
*Idh*I	1/2	1/1	1/1	1/1	1/1	1/2
*Adh*I	1/2	1/2	1/2	1/2	1/2	1/2

[a] BC_1 plant #9 did not produce anthers

[b] Percent of meiotic figures with one or three bridges

[c] Percent of cells with at least one micronucleus (100 cells counted)

[d] Isozyme segregation, 1 = any allele from *A. cepa*, 2 = any allele from *A. fistulosum*

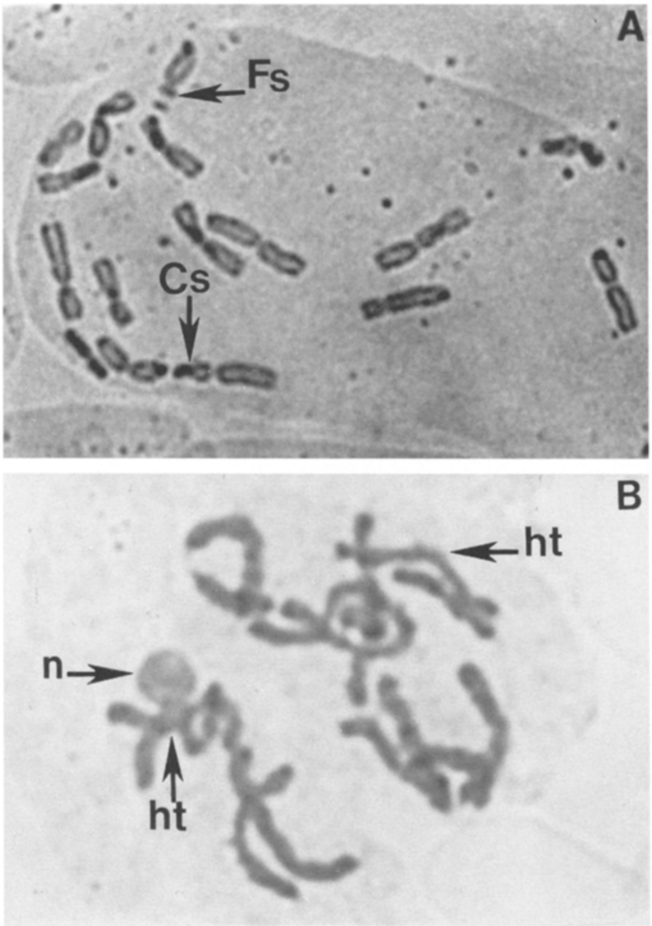

Fig. 1A, B **A** Chromosomes at metaphase (mitosis): *A. fistulosum* (5Fs) × *A. cepa* (*6Cs*) interspecific F_1 hybrid. **B** Chromosomes at diakinesis (prophase-I) showing nucleolus variation (*n*): interspecific F_1 hybrid with two trivalents (*ht*), one involving the NOR chromosome

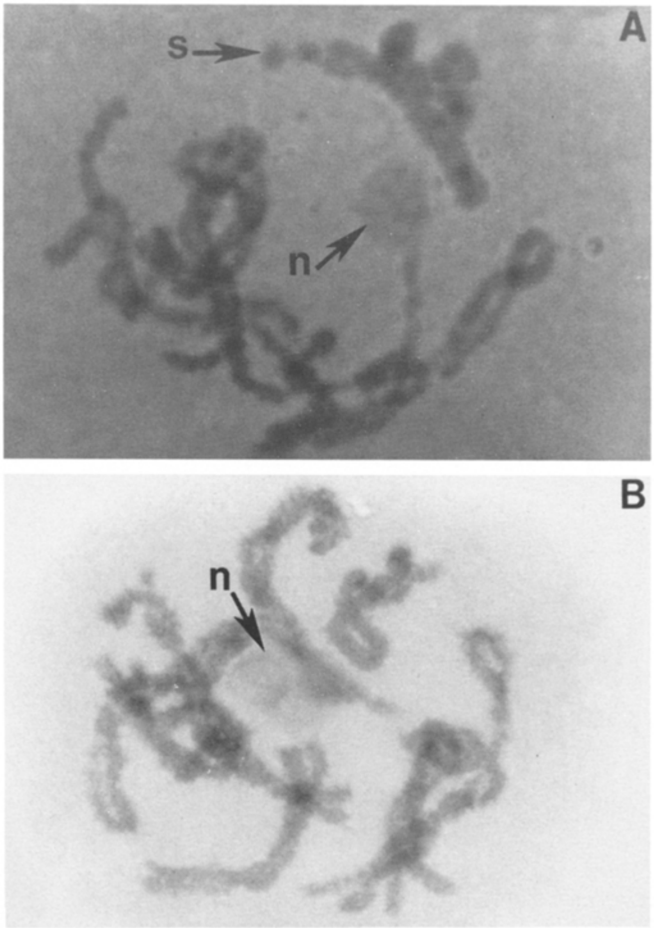

Fig. 2A, B Chromosomes at diakinesis (prophase-I) showing nucleolus variation. **A** Backcross progeny #2 with two satellites (*s*) in similar recovered *A. cepa* homologous chromosomes. **B** Backcross progeny #7 with two nucleoli (*n*)

Heteromorphic bivalents were frequently observed (Table 1), suggesting that recombination of chromosome segments may occur. Exchange of homologous sequences of homoeologous chromosomes appears to occur between the two species. The 5F chromosome may cross-over with two different chromosomes of the *A. cepa* genome, a submetacentric 1C (or 2C) and 6C in one or two different events (Figs. 2A and 3A, B). In one plant (Fig. 2A) the NOR (*n*) was associated with a non-satellite chromosome, and one chromosome had two satellites (*s*). This arrangement cannot be explained by a cross-over event. Schubert and Wobus (1985) proposed a mobile NOR hypothesis, based on work with a ^{125}I-rDNA clone from *Vicia faba* against *A. cepa* and three strains of topset onion. The NORs of both species were able to move between telomeric heterochromatic blocks of different *Allium* chromosomes in the parental species, *A. cepa*, as well as in the topset onion. Our results support the hypothesis of the interchromosomal mobility of NORs in *Allium* genomes by means of recombination of homologous sequences of the homoeologous chromosomes. However, this study does not reject the possibility of movement of preferential chromosomal sites by means of adjacent transposable elements (Fig. 2A and B). These results support a genetic dominance expression for the nucleolus of the NOR.

With respect to chromosomal configurations these results agree with previous reports (Maeda 1937; Emsweller and Jones 1945). Only random chiasmata were observed in *A. cepa* and occasional non-localized bivalents in *A. fistulosum*. In the F_1 hybrid and selected backcross progenies (BC_1) random chiasmata were also observed. In *A. fistulosum* no ring bivalents were seen at metaphase-I and cruciform bivalents normally possessed only one chiasma near the centromere region.

Structural differences between chromosomes of the two species were evident in the F_1 hybrid and BC_1 progenies. As many as three paracentric inversions which did not include the centromere regions were observed in the interspecific F_1 hybrid (Fig. 4A). These inversions would produce changes in gene order. Repeated observations of unusual cross-overs and

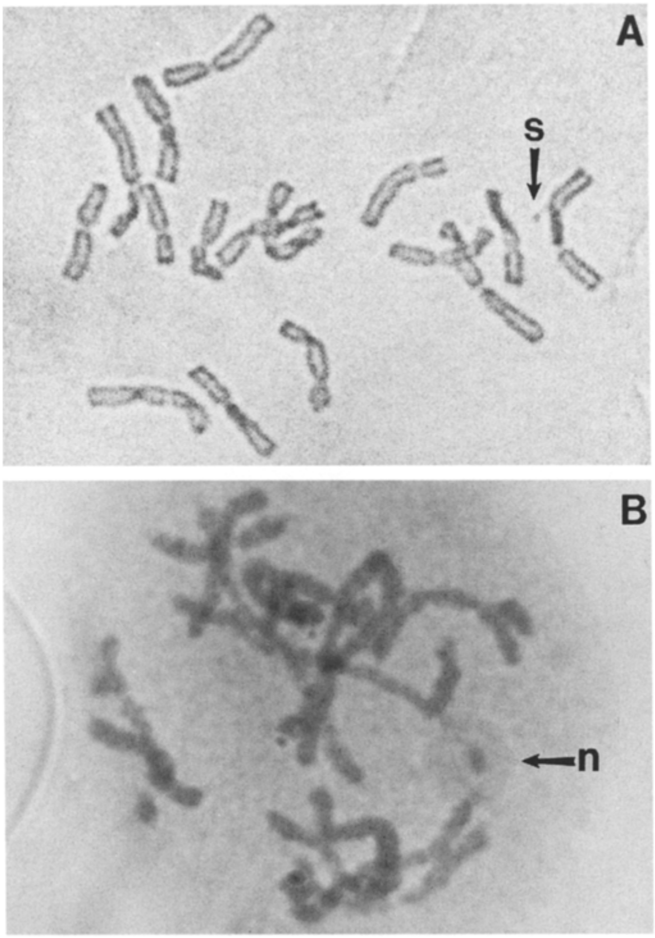

Fig. 3 A, B Unusual crossing-over. **A** Backcross #7 chromosome at mitosis showing unusual position of satellite (*s*). **B** Interspecific F_1 hybrid, with variation in nucleolus position and degree of attachment (*n*)

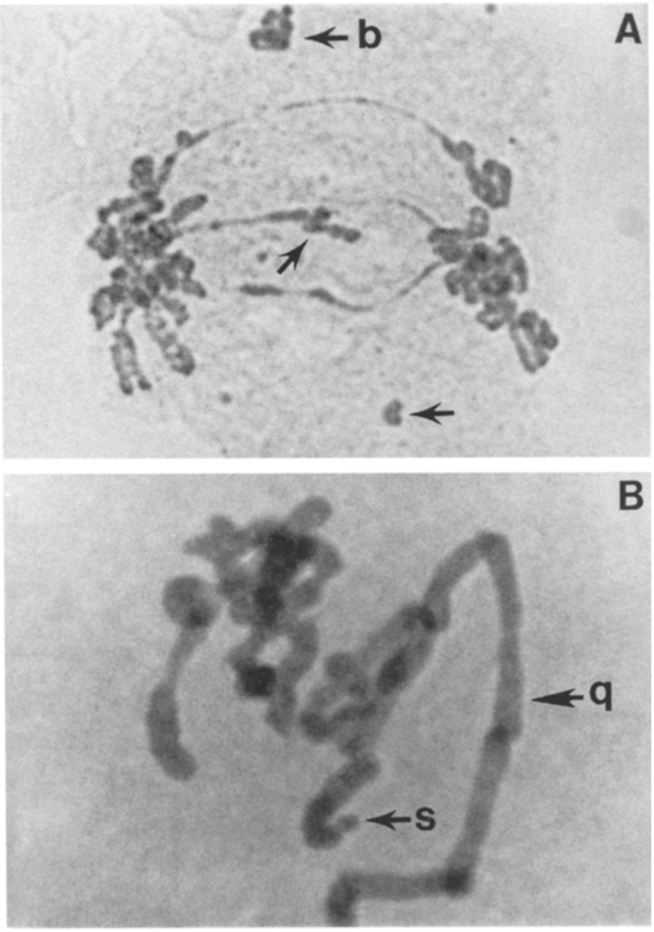

Fig. 4 A, B **A** Bridges observed at anaphase-I: An *A. fistulosum* × *A. cepa* F_1 hybrid with three bridges with chromosome fragments (*arrows*) and a non-disjunction bivalent (*b*). **B** Chromosome associations (*q*) in backcross progeny #3 with an association of chromosomes involving the satellite chromosome (*s*)

multivalent associations suggest that the chromosome bearing the NOR (5F) is involved in at least one translocation (Figs. 1 B and 4B). Evidence for one translocation and a possible inversion were previously reported by Peffley (1985). The present results further document abnormality in chromosome behavior in F_1 hybrids and BC_1 plants between *A. fistulosum* and *A. cepa.*

Fertility in interspecific plants is essential for success in interspecific gene introgression. Using *A. fistulosum* as a seed parent, interspecific F_1 hybrids are easily produced by intercrossing plants in screen-covered isolation cages. Heteromorphic pairing occurs readily in the F_1 hybrid and BC_1 progenies. BC_1 3 and 6 progenies had a high percentage of normal chromosome pairing (Table 1), suggesting that these progenies should be partially or normally fertile, but the number of BC_2 plants from these progenies was almost nil. In addition, pollen stainability in BC_1 populations was not greatly different from that of the F_1 hybrid. Our data suggest that difficulties other than those associated with chromosome pairing may contribute to the sterility of the BC_1 plants studied (Table 1). Van der Valk et al. (1991) found difficulties in pollen-tube growth in the F_1 hybrid. It is also possible that nucleocytoplasmic incompatibility interactions may occur between the two species. If so, gene introgression from *A. fistulosum* into *A. cepa* may be difficult to accomplish when *A. fistulosum* is used as the seed parent.

Conclusions

Three paracentric inversions were detected in the *A. fistulosum* × *A. cepa* F_1 hybrid. Repeated observations of unusual cross-overs and multivalent associations suggest that the 5F chromosome is involved in at least one translocation.

Random chiasmata were observed in *A. cepa* and occasional non-localized bivalents in *A. fistulosum.* The only formed nucleolus was attached to the *A. fistulosum* 5F chromosome in the interpecific F_1 hybrid, suggesting genetic dominance for nucleolus expression.

Chromosome pairing occurred readily in the F_1 hybrid and BC_1 progenies. The BC_1 plants were sterile or else produced very few seeds. The fertility of BC_1 plants was not related to the extent of normal chromosome pairing at meiosis, suggesting the possibility of nucleocytoplasmic incompatibility.

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