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Introgression of *Allium fistulosum* into *A. cepa* mediated by *A. roylei*

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Abstract Introgression of *Allium fistulosum* into the genome of *A. cepa* using *A. roylei* as a bridging species was studied by means of genomic *in situ* hybridization (GISH). Here we demonstrate for the first time that *A. fistulosum* can be stably introgressed into *A. cepa* with a bridge-cross. The first and second bridge-cross generations were fertile, although pollen was sterile in some individuals. Only occasionally were there translocations in the second generation bridge-cross. Recombination between the three genomes was frequently seen in meiotic anaphase 1 and prophase 2 chromosomes of the first generation bridge cross and in mitotic chromosomes of the second generation bridge-cross. The number of observed recombination points in anaphase 1 and prophase 2 significantly exceeded the value expected from chiasma frequency in metaphase 1. Recombination points were randomly distributed, thus the *A. cepa* or *A. roylei* type of random distribution prevails over the *A. fistulosum* type of proximally localised chiasmata.

Key words Onion · Wild species · Multi-colour genomic *in situ* hybridisation · Chiasmata · Meiotic recombination

Introduction

Onion (*Allium cepa* L.) is one of the oldest cultivated species, and it has been in use as a food source for over 5000 years (Jones 1983). It is highly likely that during its long breeding history a number of desirable traits have been lost, a phenomenon which has also occurred in a number of other crop species, such as leek (Kik et al. 1997). Wild species often are a source of desirable traits for their related crop species. For onion, *A. fistulosum* is just such a source because it harbours many disease and pest resistance genes (Rabinowitch 1997).

The success of introgression breeding depends partly on the phylogenetic relationships between parental species. *A. cepa* and *A. fistulosum* have been classified into a single section, namely *Cepa* (Hanelt 1990). They have the same chromosome numbers (2n=2x=16) and similar karyotypes (Emsweller and Jones 1935a; Albini and Jones 1988). However the DNA content of the genome of *A. cepa* is 28% higher than that of *A. fistulosum* (Labani and Elkington 1987), and *A. cepa* chromosomes are on average 12% larger at somatic metaphase than those of *A. fistulosum* (Jones and Rees 1968).

The first attempts to introgress genes from *A. fistulosum* into *A. cepa* were reported by Emsweller and Jones (1935b). However, these were not successful, and until the present time all subsequent attempts to introgress genes from *A. fistulosum* to *A. cepa* have failed because of sterility in backcrossed generations. It has been suggested by Ulloa et al. (1995) that such sterility is due to an imbalance between the nuclear and cytoplasmic genomes. Van der Meer and De Vries (1990) and McCollum (1982) showed that *A. roylei* (2n=2x=16) crosses readily with *A. cepa* and *A. fistulosum*, respectively. From these observations the idea was born to use *A. roylei* as a bridging species between *A. fistulosum* and *A. cepa*. By using a unique multi-colour genomic *in situ* hybridisation (GISH) method, Khrustaleva and Kik (1998) showed that the three parental genomes in the first generation bridge cross [*A. cepa* × (*A. fistulosum* × *A. roylei*)] could be distinguished from each other, pointing at significant differences in repetitive DNA composition among the three species. A meiotic analysis of the first generation bridge cross revealed a high percentage of bound bivalent arms (82.6%) at metaphase 1. However, some degree of genome instability still existed because of the presence of occasional univalents in meiosis. Nonetheless, pollen fer-

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tility in the first generation bridge-cross was high. On the basis of these results it was suggested that there was a fair chance that the species barrier between onion and *A. fistulosum* could be circumvented.

However, the aforementioned results on the first generation bridge-cross were obtained from the analysis of only one bridge-cross individual. To substantiate these previous findings and to prove that *A. fistulosum* can be stably introgressed into the genome of *A. cepa* using *A. roylei* as a bridging species, we present here firstly an elaborate study on the mitosis and meiosis of a number of individuals from the first bridge-cross generation and, secondly, an analysis of the mitosis and fertility of a number of individuals of the second bridge-cross generation.

In our previous paper (Khrustaleva and Kik 1998) it was demonstrated that the centromeric region of the recombinant *A. fistulosum*/*A. roylei* chromosomes in the first bridge-cross generation originated from *A. roylei*. We hypothesised that this was due to the spatial separation of both genomes in the interspecific hybrid. The investigation described here presents in detail the crossing-over point distribution between *A. fistulosum* and *A. roylei* in the first generation bridge-cross population.

Subsequently, we studied meiotic pairing at metaphase 1 in the first generation bridge-cross. By means of GISH the recombinant chromosomes can be readily identified, and their behaviour can be monitored through meiosis (Schwarzacher et al. 1992; Parokonny et al. 1997). The recombination frequency based on the analysis of recombinant chromosomes at anaphase 1 and prophase 2 was also determined. Consequently, a comparison between the number of recombination points expected in anaphase 1 and prophase 2, on the basis of chiasma counts at metaphase 1, and the number of recombination points observed in both phases could be made. The results obtained were discussed in the light of the current debate concerning the 1:1 correspondence between chiasma and recombination frequency (Gill et al. 1995; Sybenga 1996; Moens 1996; Takahashi et al. 1997).

The distribution of recombination points on the chromosomes of the second generation bridge-cross population was also analysed. It is known that chiasmata of *A. fistulosum* are localised adjacent to the centromere (Levan 1933) and that the chiasmata of *A. cepa* (Emsweller and Jones 1935b) and *A. roylei* (De Vries et al. 1992a) are randomly distributed. In the species hybrid between *A. cepa* and *A. fistulosum* the chiasma distribution is most similar to *A. cepa* and localised chiasmata were not observed (Maeda 1937; Albini and Jones 1990). Khrustaleva and Kik (1998) demonstrated that in the first generation bridge-cross crossing-over events occurred predominantly in distal and interstitial regions of the recombinant *A. fistulosum/A. roylei* chromosomes.

Materials and methods

Plant material

An overview of the species, their interspecific hybrids and the first and second generation bridge-cross populations are given in Table 1. Three first generation bridge-cross populations were produced: one population originated from a cross between *A. cepa* cv 'Maxima' as a female parent and the interspecific hybrid between *A. fistulosum* and *A. roylei* as a male parent and the other two populations had a male-sterile *A. cepa* (A-line) as a female parent and the interspecific hybrid between *A. roylei* and *A. fistulosum* as a male parent. The second generation bridge-cross *A. cepa* × [*A. cepa* × *(A. roylei* × *A. fistulosum)*] was produced by using *A. cepa* cv 'Hyduro' as a female parent and the first-generation bridge-cross plant 96284–4 as a male parent. Only pairwise crosses were performed. 'Maxima', 'Hyduro' and A-line are cytoplasmic male sterile (CMS). Blow flies were used for pollination to produce the first and second generation bridge-cross populations. Plants were grown in pots in a frost-free greenhouse. The second generation bridge-cross plants were brought into a '1-year breeding cycle' (Van Kampen 1970) which allows flowering the year after sowing. However, not all plants are responsive to this treatment and in practise only a small percentage of individuals will flower.

Genomic in situ hybridization (GISH)

Genomic DNA was extracted from 4 g of young leaves using the CTAB method of Rogers and Bendich (1988). For use as a probe, total genomic DNA was sonicated to a fragment size of 3–10 kb, and 1 mg of DNA was labeled with either Dig-11-dUTP (Digoxigenin-11-2´-deoxy-uridine-5´-triphosphate) or Biotin-16-dUTP

Table 1 A description of the accessions investigated

Accession[a]	Code[b]	Parent(s)	*n*[c]	Origin
93058	CC	*A. cepa* (A line)	–	CPRO-DLO, Wageningen, The Netherlands
97001	CC	*A. cepa* cv Hyduro	–	BGS, Broek op Langedijk, The Netherlands
	CC	*A. cepa* cv Maxima	–	Advanta, Rilland, The Netherlands
84236	FF	*A. fistulosum*	–	Botanical Garden, Odessa, Ukraine
79150	RR	*A. roylei*	–	C502, Beltsville, USA
83038				
86184	FR	84236–10[d] × 79150	121	CPRO-DLO
91021	RF	84038–10 × 84236–2	35	CPRO-DLO
89447	CC×FR	Maxima × 86184	20	CPRO-DLO
96282	CC×RF	93058–4 × 91021–8	26	CPRO-DLO
96284	CC×RF	93058–6 × 91021–8	360	CPRO-DLO
97056	CC×(CC×RF)	97001 × 96284–4	55	CPRO-DLO

[a] CPRO-DLO accession number
[b] C, F and R represent one genome of *A. cepa, A. fistulosum* and *A. roylei*, respectively
[c] *n*, Number of seeds obtained
[d] A number behind a dash of an accession indicates a specific plant

(Biotin-16–2´-deoxyuridine-5´-triphosphate) by a standard nick-translation protocol (Boehringer Mannheim, Germany). Blocking DNA was autoclaved for 8 min giving a fragment size of 100–500 bp. Somatic metaphase chromosome spreads were made by squashing from actively growing root meristems (Khrustaleva and Kik 1998). For meiotic studies, anthers of a suitable size were fixed in 3:1 (v/v) ethanol-acetic acid for 1 h, rinsed four to five times in distilled water and finally incubated in 10 m*M* citrate buffer (pH 4.5) containing 0.3% (w/v) cellulase RS, 0.3% (w/v) pectolyase Y23 and 0.3% (w/v) cytohelicase for 4 h at 37°C. The macerated anthers were rinsed carefully in deionized water. The wet slides were immersed briefly in 96% ethanol and subsequently air-dried (Pijnacker et al. 1992). GISH analysis of the first generation bridge-cross was performed on meiocytes at metaphase 1, anaphase 1 and prophase 2. *In situ* hybridization, immunological detection and counterstaining procedures were the same as those previously described by Khrustaleva and Kik (1998). The stringency washing was done in 0.1×SSC for 30 min at 48°C. This washing stringency allows 78% of nucleotides to be correctly matched in the probe and target duplex (Meinkoth and Wahl 1984).

Photographs were taken on Fujicolor 400 ASA colour negative film using an epifluorescence Axiophot microscope (Zeiss) and an appropriate filter. Photographs were printed from Adobe Photoshop using brightness/contrast and colour adjustment functions.

Pollen fertility

Pollen morphology and viability were studied in malachite green-acid fuchsin-orange G-stained preparations of microspores (Alexander 1969).

Recombination point localisation

The relative location of a recombination point was calculated as the ratio of the distance between the recombination point and the centromere and the total length of a chromosome arm. The location of a recombination point was considered proximal, interstitial or distal when this ratio was in between 0–0.33, 0.34–0.66 and 0.67–1.00, respectively.

Karyotype analysis

Idiogrammes of the karyotypes were constructed according to the standard onion nomenclature system proposed by Kalkman (1984) and confirmed by the Fourth Eucarpia *Allium* Symposium (De Vries 1990).

Results

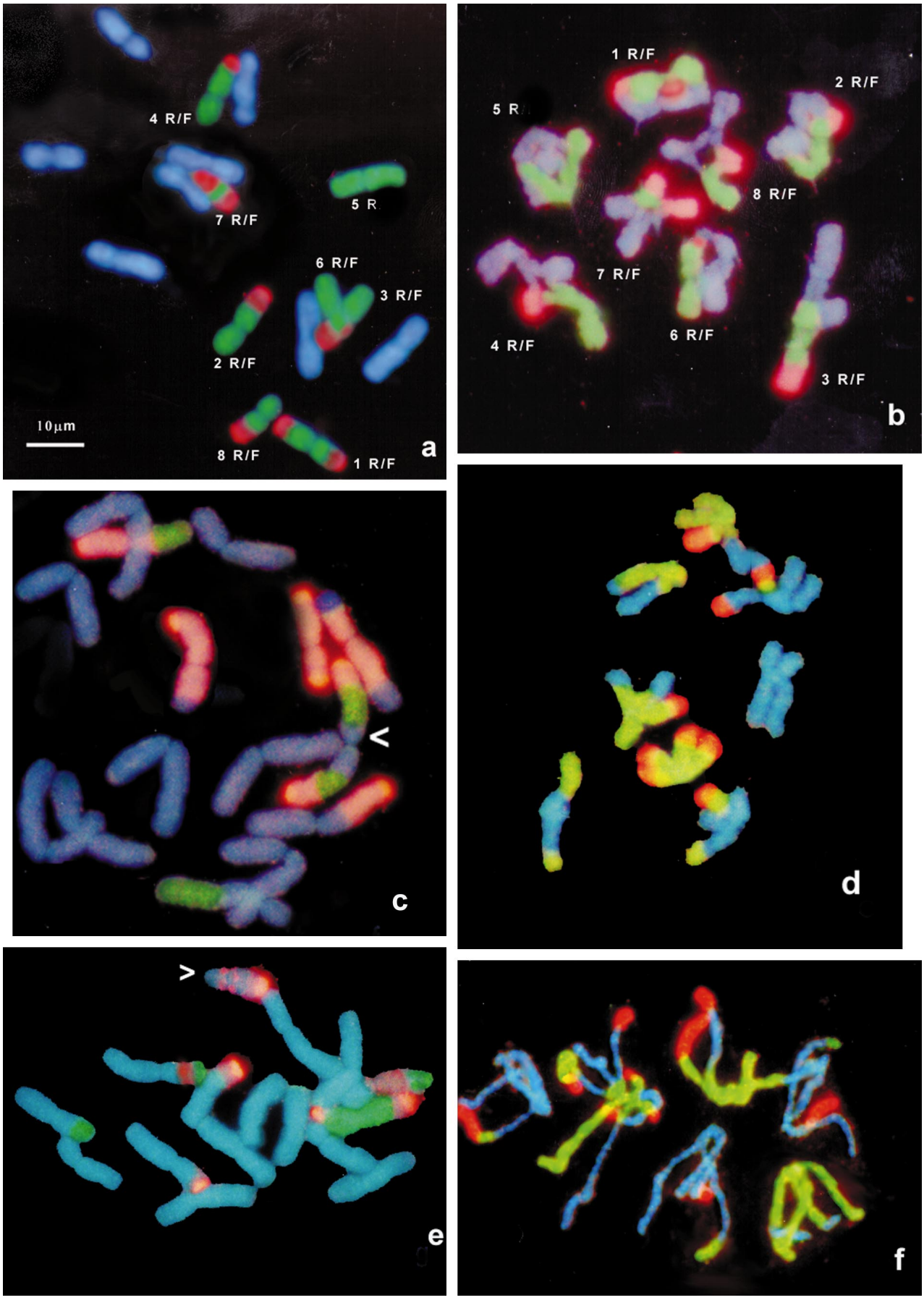
Mitosis in the first generation bridge-cross

A GISH study was carried out on six individual plants of the first generation bridge-cross. The first generation bridge-cross plants were produced using *A. cepa* as a female parent and the interspecific hybrid between *A. fistulosum* and *A. roylei* as a male parent. Two types of crosses were carried out: in one cross (accession 89447, 3 plants analysed) the interspecific hybrid between *A. fistulosum* and *A. roylei* was used and in the other two crosses the reciprocal interspecific hybrid was used (accession 96282 and 96284, 1 and 2 plants analysed, respectively). The three parental genomes could be clearly identified in each individual bridge-cross plant by multi-colour GISH (Fig. 1a). Idiogrammes of the karyotypes of the six first generation bridge-cross plants were constructed (Fig. 2a–f). The centromeric regions of the eight chromosomes in each genotype of the bridge-cross individuals originated from either *A. roylei* or *A. fistulosum* except in accession 89447–1 (Fig. 2d), which possessed only *A. roylei* centromeric regions. The median number of recombination points per chromosome ranged from 0.5–2.0 (Table 2). In general the recombination points were randomly distributed over the chromosomes, although a higher number of recombination points were observed in the interstitial regions of the chromosomes (Table 2; χ^2_2=5.69, 0.05<*P*<0.10). Also, we observed the tendency for a higher number of recombination points in the distal area and a lower number in the proximal area of a chromosome arm when the male parent of the bridge-cross population possessed the cytoplasm of *A. roylei* (in case of the interspecific hybrid between *A. roylei* and *A. fistulosum*: RF) compared to the case in which the male parent carried the *A. fistulosum* (FR) cytoplasm (Table 2; Fisher exact probability test: 0.05<*P*<0.10, two-tailed). In both types of interspecific hybrids about 50% of the crossing-overs occurred in the interstitial region.

Meiosis in the first generation bridge-cross

Karyotype analysis of the first generation bridge-cross mitotic metaphase chromosomes allowed a positive identification of all eight chromosomes, and this greatly assisted in the analysis of chromosome behaviour in meiosis. The *A. cepa* chromosomes originating from the female parent paired well with their homoeologous recombinant chromosomes originating from the interspecific hybrid between *A. fistulosum* and *A. roylei* which was used as a male parent (Fig. 1b). In all observed PMCs, for chromosome 1, the *A. cepa* (1 C) and *A. fistulosum/A. roylei* (1F/R) chromosome pair formed mostly a ring bivalent. The 2C-2F/R pair always formed ring bivalents with two or occasionally three chiasmata. The 3C-3F/R pair formed a ring or open bivalent and often had two chiasmata in the long arm. For the 4C-4F/R pair an open bivalent with one chiasma was observed. The 5C-5R pair frequently formed a ring bivalent, and the 6C-6F/R pair formed a ring or open bivalent. The pairing of recombinant chromosome 7F/R was unpredictable. This recombinant chromosome consisted predominantly of *A. fistulosum*. The 7C-7F/R pair formed in 30% of the cases a cross open bivalent; in 20%, a univalent pair; in another 20%, an open rod bivalent; and finally in the last 30%, a ring bivalent. The 8C-8F/R pair formed in 70% of the cases an open bivalent and in 30%, a univalent pair.

Analysis of the PMCs in anaphase 1 and prophase 2 showed a high frequency of recombination between the three genomes (Fig. 1d, f). Of the chromosomes 73% proved to be recombinant (data not shown). Cross-over points were best observed in prophase 2, where the chromosomes were less condensed (Fig. 1f). This allowed us


4 R/F
7 R/F
5 R
6 R/F
3 R/F
2 R/F
10μm
8 R/F
1 R/F
a
1 R/F
2 R/F
5 R
8 R/F
7 R/F
4 R/F
6 R/F
3 R/F
b
c
d
e
f

to determine the number of recombination points per chromosome very accurately and made a comparison with chiasma frequency at metaphase 1 possible. Chiasma frequency was determined using a sample of ten GISH-metaphases. A comparison of the results from this analysis with those of a previously carried out elaborate study on 681 Fe-acetocarmine stained metaphases (Khrustaleva and Kik 1998) did not show a significant difference (Table 3; χ^2_3=2.15, P=0.54). Therefore, the pooled data were used for the calculation of the expected recombination point frequency. In classical genetics chiasmata are understood to be a direct result of crossing-over. Crossing-over at any single chiasma occurs only between two of the four chromatids in a bivalent. The calculation of expected recombination point frequency from the chiasma data was performed by assuming random involvement of chromatids and lack of chiasma interference (Schulz-Schaeffer 1980; Takahashi et al. 1997). The prediction was that the percentage of chromosomes without recombination would 44.1%, but we found only 26.7% (Table 4). The number of chromosomes with three recombination points was also higher than expected: 6.7% observed versus 0.4% expected. Moreover, we found a chromosome which had four recombination points. However, a bivalent with more than three chiasmata was never observed at metaphase 1. A significant difference was found between the observed frequency of recombination points per chromosome at anaphase 1 and prophase 2 and the expected number based on the analysis of chiasma frequency at metaphase 1 (Table 4; χ^2_2=8.45, P=0.01).

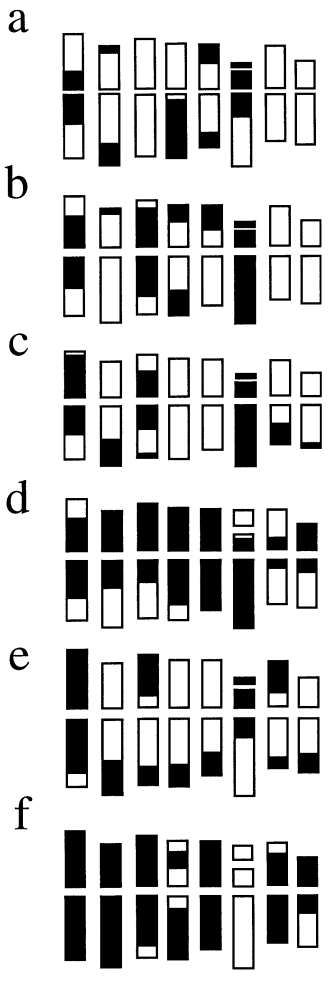


Fig. 2a–f Idiogram of GISH karyotypes in the first generation bridge-cross. Accession: **a** 89447–8, **b** 96284–4 (the male parent of the second generation bridge-cross population), **c** 96282–3, **d** 89447–1, **e** 96284–12, **f** 89447–9. □ *A. fistulosum*, ■ *A. roylei*

Mitosis in the second generation bridge-cross

The second generation bridge-cross population (accession 97056) was produced by using *A. cepa* as the female parent and a first generation bridge-cross plant as the male parent. A GISH study was carried out on eight individual plants which were randomly chosen from this second generation bridge-cross population, and idiogrammes of karyotypes of these eight individuals were constructed (Fig. 3a-h). The identification of chromosomes in each individual was assisted by the knowledge of the distribution and size of the segments originating from *A. roylei* or *A. fistulosum* on the chromosomes of the male parent accession 96284 (Fig. 2b).

◀ **Fig. 1a–f** Multi-colour GISH in the first and second generation bridge-cross populations. *A. fistulosum* (Biotin, CY3) – red fluorescence, *A. roylei* (FITC) – *green fluorescence*, *A. cepa* (block, DAPI) – *blue fluorescence*. **a** Mitotic metaphase of the first generation bridge-cross individual 89447–1, **b** metaphase 1 in a PMC of 89447–1, **c** mitotic metaphase of the second generation bridge-cross individual 97056–7 (*arrow* indicates chromosome with segments from all three parental species on both arms), **d** anaphase 1 in a PMC of 89447–1, **e** mitotic metaphase of the second generation bridge-cross individual 97056–12 (*arrow* indicates a chromosome with five recombination points), **f** prophase 2 in a PMC of 89447–1

Table 5 summarises the frequency and distribution of recombination points along the eight individual chromosomes. The recombination points between *A. cepa* and *A. fistulosum* segments and between *A. cepa* and *A. roylei* segments were only determined because the recombination points between *A. fistulosum* and *A. roylei* resulted from a previous crossing-over. The mean recombination frequency per haploid genome was 7.88. Almost every *A. cepa* chromosome originating from the male parent possessed a segment from *A. fistulosum* and/or *A. roylei*. For instance, chromosome 1 C/F/R of accession 97056–7 even possessed segments from all three parental species on both arms (Fig. 1c). The median number of recombination points per individual chromosome ranged from 0.5 to 2.0. Three recombination points between *A. cepa* and *A. roylei* were observed on the longest chromosome: 1 C/F/R of accession 97056–3 (Fig. 3g). Five recombination points between *A. fistulosum* and *A. cepa* were found on the shortest chromosome 8 C/F of accession 97056–12 (Figs. 1e; 3h). Thus, the number of crossing-over events did not depend on chromosome size. Recombination occurred randomly in distal, interstitial and proximal chromosome regions (Table 5; χ^2_2=1.24, 0.5<P<0.7). However, preferential localisation of recombination was found for some chromosomes. For exam-

Table 2 Frequency and distribution of recombination points in six genotypes from the first generation bridge-cross populations

Chromosome	Recombination points												
	Number/chromosome			Location (over six genotypes)									
	Median	Maximum	Minimum	Proximal			Interstitial			Distal			Total
				Σ	RF[a]	FR[b]	Σ	RF	FR	Σ	RF	FR	
1	2	2	0	2	1	1	6	3	3	1	1	0	9
2	1	2	0	1	0	1	4	2	2	1	1	0	6
3	1.5	3	0	2	1	1	4	3	1	3	3	0	9
4	1	3	0	3	0	3	4	2	2	1	1	0	8
5	0.5	2	0	0	0	0	3	2	1	1	0	1	4
6	0.5	1	0	2	1	1	0	0	0	1	0	1	3
7	1	2	0	3	1	2	1	1	0	2	1	1	6
8	1	1	0	1	0	1	2	1	1	1	1	0	4
Total				14	4	10	24	14	10	11	8	3	

[a] *A. roylei* cytoplasm
[b] *A. fistulosum* cytoplasm

Table 3 Frequency of chiasmata in bivalents in PMCs of the first generation bridge-cross

Accession	A[a]	N[b]	Number of bivalents with:			
			0 chiasma	1 chiasma	2 chiasmata	3 chiasmata
Fe-acetocarmine stained						
89447–1 1996	15	681	900 17.1%	1428 27.1%	2796 53.0%	154 2.9%
GISH						
89447–1 1997	5	10	10 12.8%	25 32.1%	40 51.3%	3 3.85%

[a] A, Number of anthers examined
[b] N, Total na of PMCs examined

Table 4 Frequency of crossover points in PMCs at anaphase 1 and prophase 2 of the first generation bridge-cross

Accession	Number of chromosomes with *n* recombination points				
	n=0	*n*=1	*n*=2	*n*=3	*n*=4
Observed (GISH)					
89447–1	20 26.7%	38 50.7%	11 14.7%	5 6.7%	1 1.3%
Expected (chiasma analysis)					
89447–1	44.1%	41.1%	14.4%	0.4%	0

ple, on chromosome 8 recombination points were mostly proximally located whereas on chromosome 7 recombination took place mostly in the distal part of the chromosome. On the nucleolar organising region (NOR)-bearing chromosome six recombination points were mainly localised in the interstitial and distal region. The NOR of this chromosome was inherited in five genotypes from *A. cepa* and in three genotypes from *A. roylei*.

Translocated chromosomes in two genotypes were found. Chromosome 4 of accession 97056–3 revealed an unexpected GISH image on the short arm. Instead of a large *A. roylei* segment in this terminal part of chromosome, which is present in the parental chromosome, only *A. fistulosum* chromatin was found (Fig. 3g). Chromosome 3 of accession 97056–8 possessed an unexpected *A. roylei* segment in the terminal region of the long arm (Fig. 3b), unexpected since this segment was absent in the parental chromosome (Fig. 2b). In accession 97056–5 a chromosome with a deficiency was found; on chromosome 2 C/F the loss of a terminal *A. roylei* segment on the short arm was observed (Fig. 3c). A comparison of this chromosome with its parental chromosome showed the absence of a short *A. roylei* segment in the terminal area (Fig. 2b).

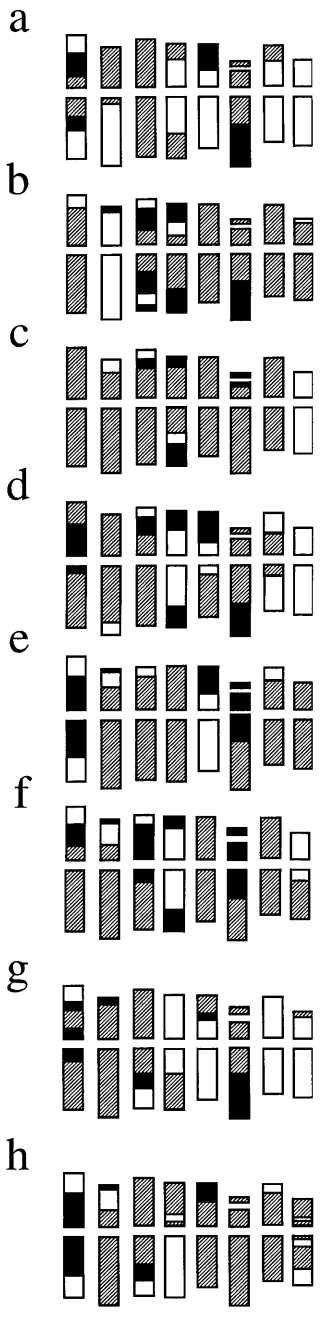


Fig. 3a–h Idiograms of GISH karyotypes in the second generation bridge-cross. Accession: **a** 97056–7, **b** 97056–8, **c** 97056–5, **d** 97056–11, **e** 97056–4, **f** 97056–6, **g** 97056–3, **h** 96056–12. □ *A. fistulosum*, ▨ *A. cepa*, ■ *A. roylei*

Pollen fertility and seed set in the second generation bridge-cross

Four of the eight GISH-analysed second generation bridge-cross individuals produced flowers. One proved to be fully fertile (93.5%), two produced sterile pollen and one produced no pollen at all. The fertile plant was selfed and seeds were obtained.

Discussion

Introgression of *A. fistulosum* into *A. cepa* mediated by *A. roylei*

This is the first paper that reports on the stable introgression of *A. fistulosum* into the genome of *A. cepa*. The approach that was followed was to first cross *A. fistulosum* with *A. roylei* and then to cross this interspecific hybrid with *A. cepa*: the so-called bridge-cross approach. The introgression process was followed in detail using multicolour GISH. It was found that recombination between the three genomes in the second generation bridge-cross was frequent and that recombination points were randomly distributed along the chromosomes. Spatial separation of parental genomes was not observed in the interspecific hybrid between *A. fistulosum* and *A. roylei* because the centromeric regions of five genotypes of the first generation bridge-cross originated from either parent. Only in accession 89447–1 did the centromeric region of all eight chromosomes belong to *A. roylei*. This phenomenon occurring in accession 89447–1 had been observed earlier by Khrustaleva and Kik (1998). Furthermore, a large number of seeds were produced on some plants of the first and second generation bridge-cross plants. Not all plants were equally fertile. This was probably due to the use of CMS onion plants as female parents: only bridge-cross plants which possessed the restorer gene(s) for the CMS-T cytoplasm present in *A. roylei* (De Vries and Wietsma 1992) were fertile. However there were also indications that the lack of other *A.*

Table 5 Frequency and distribution of recombination points in eight genotypes of the second generation bridge-cross *A. cepa* × [(*A. cepa* × (*A. roylei* × *A. fistulosum*)]

Chromosome	Recombination points						
	Number/chromosome			Location (over eight genotypes)			
	Median	Maximum	Minimum	Proximal	Interstitial	Distal	Total
1	1.5	3	0	6	4	1	11
2	1	1	0	1	3	3	7
3	1	3	0	2	3	4	9
4	2	2	0	3	5	3	11
5	0.5	1	0	1	3	1	5
6	1	1	1	1	5	2	8
7	0.5	2	0	1	1	3	5
8	0.5	5	0	5	1	1	7
Total				20	25	18	63

roylei chromosome segments in the bridge-cross can lead to sterility because in one plant no pollen was produced at all instead of male-sterile or fully fertile pollen. In this case *A. roylei* genes involved in male sexual reproduction were probably lacking. It is possible that the presence of these genes circumvent or restore the nucleo-cytoplasmic imbalance that leads to sterility in the *A. cepa* × (*A. cepa* × *A. fistulosum*) backcross (Ulloa et al. 1995). Analysis of a larger second generation bridge-cross population from which the pollen fertility and the karyotype are known per plant will allow the determination of the location of the restorer gene(s) and the genes involved in the male sexual reproductive system.

In the first and second generation bridge-cross some genome instability was observed. Khrustaleva and Kik (1998) reported that univalents were present in first generation bridge-cross, and in this study univalents and chromosome aberrations were found in the metaphase of the second generation bridge-cross. Fortunately, the frequency of genomic imbalance was very low and, therefore, we expect that the observed cytogenetic abnormalities will not interfere too much with the introgression process.

By means of this bridge-cross not only genes from *A. fistulosum* can be introgressed into *A. cepa* but simultaneously also genes from *A. roylei*. In *A. fistulosum* resistance genes are present against *Botrytis squamosa* (Currah and Maude 1984), *Pyrenochaeta terrestris* (Netzer et al. 1984), *Colletotrichum gloeosporioides* (Galvan et al. 1997), *Urocystis cepulea* and OYDV (Rabinowitch 1997), whereas in *A. roylei* resistances are present against *Peronospora destructor* (Kofoet et al. 1990) and *Botrytis squamosa* (De Vries et al. 1992b). It will be clear that via the bridge-cross approach unique populations can be developed in which these resistance genes can segregate. The challenge for the future will be to map these genes and to introgress them into the onion germplasm. The use of molecular markers as well as GISH will greatly facilitate this process.

Recombination point distribution

The difference in DNA amount and chiasma distribution between the three parental species did not prevent chromosome pairing and subsequent crossing-over in the male meiosis of the first generation bridge-cross. On the contrary, a high recombination frequency was observed between the three parental genomes in the second generation bridge-cross. The most widely accepted model for meiotic recombination is the homology-dependent double-strand break-repair model, and it is thought that recombination occurs predominantly between DNA stretches which have sufficient homology (Szostak et al. 1983). This means there is still a considerable amount of homology between the three evolutionary closely related *Allium* species involved in the bridge cross. In order to determine the likelihood of successful gene transfer from *A. fistulosum* into onion mediated via *A. roylei*, we must establish the frequency and distribution of recombination points between the parental genomes. The location of the recombination points along the recombinant *A. fistulosum*/*A. roylei* chromosomes in the first generation bridge-cross proved to be randomly distributed, and this was also the case in the second generation bridge-cross. For some chromosomes preferential localisation of the recombination points was observed. For example, on chromosome 8, which consists only of *A. fistulosum* (Fig. 2b; Table 5), the recombination points were mostly proximally located. This means that on this chromosome an *A. fistulosum* type of recombination took place. However this was not due to the percentage of *A. fistulosum* on the chromosome, because on chromosome 7, which also consisted completely of *A. fistulosum*, mostly interstitial localisation of the recombination points was observed. Such a preferential localisation of recombination points is most likely characteristic for specific chromosomes. In backcross populations between *A. fistulosum* and *A. cepa*, Maeda (1937) found that bivalents with *A. fistulosum*-type chiasmata were always the same size, indicating a definite pair. However, Emsweller and Jones (1945) found no chromosome specificity for chiasma localisation.

Furthermore, in the first generation bridge-cross populations there were indications that there was a cytoplasmic effect on the localisation of chiasma distribution: in *A. fistulosum* cytoplasm there were more proximal chiasmata and in *A. roylei* cytoplasm, more distal chiasmata. Nothing is known about nucleo-cytoplasmic interactions in the interspecific hybrid between these two species. Only De Vries et al. (1992c) reported that in reciprocal crosses between both species the number of univalents was 2.5 times higher in *A. roylei* cytoplasm than in *A. fistulosum* cytoplasm.

It can be concluded that the genetic control of the chiasma localisation is complex in *Allium*, as has also been put forward by Jones (1983) when describing chiasma localisation in the interspecific hybrid between *A. cepa* and *A. fistulosum* in comparison with its parental species. It is clear that the distribution of chiasmata in *Allium* warrants further cytogenetic research.

The 1:1 correspondence between chiasma and recombination frequency

The GISH study of meiosis in first generation bridge-crosses demonstrated that chiasma counts at metaphase 1 are lower than the frequency of recombination points observed at anaphase 1 and prophase 2. Recently, the 1:1 correspondence of chiasmata and genetic exchanges has been questioned by Takahashi et al. (1997). They used GISH for the analysis of recombinant chromosomes and showed that the frequency of recombination points on mitotic chromosomes in a subsequent generation was higher than expected from the analysis of orcein-stained meiotic chromosomes from the parent. One of their explanations for the observed discrepancy between chias-

mata and recombination frequencies was that gametes of the parent which contained non-recombinant chromosomes were less viable and did not reach the progeny. All populations of 'future' gametes were examined by us just after cross-over, and a similar result was obtained. In the light of this observation the question arises whether all exchanges can be recovered as chiasmata. A main reason for exceeding the expected recombination frequencies can be the existence of methodological errors. For instance, this could happen when two closely spaced crossovers are misidentified as a single chiasma, or when some crossovers are not manifested as chiasmata (Maguire 1982; Nilsson et al. 1993). However, Tease and Jones (1995) demonstrated via BrdU incorporation that chiasma analysis can provide accurate data on recombination patterns. This probably only holds true for complementary chiasmata when different chromatids are involved in both chiasmata but not in the case of compensating chiasmata which takes place when synaptonemal complex (SC) interruption occurs (Sybenga 1996). According to Moens (1996) the amount of sister chromatid cohesion in the latter case is not enough to withstand chromosome repulsion and, consequently, no chiasma instead of two chiasmata are observed. Armstrong et al. (1998) added to this discussion that two closely adjacent crossovers are supported by chiasmata, however they can not be resolved by eye and appear as a single chiasma. In the framework of this discussion, it would be of great interest to know how physically close two chiasmata can be formed and what the strength of sister chromatid cohesion must be to oppose the chromosome repulsion force in order to stabilise chiasmata. Normally the distance between two chiasmata is sufficient due to the interference effect, and two chiasmata are observed. However, when irregularities occur in the SC this could result in the occurrence of crossovers which are very close to each other. Disturbance of synapsis has been reported to take place in interspecific hybrids between *A. fistulosum* and *A. cepa* (Albini and Jones 1990). The presence of a chromosome with five recombination points was shown in a second generation bridge-cross individual. This observation clearly supports the occurrence of interference suppression. It could be excluded that rearrangements in somatic and pre-meiotic (or meiotic) cells play a significant role because we observed occassional translocations and we did not find a mosaic of recombinant chromosomes in plant cells as was found Pasakinskiene et al. (1997) when analysing interspecific hybrids between *Lolium multiflorum* and *Festuca arundinaceae*.

Taking all together, most probably the shortage of chiasma in the *Allium* bridge cross can be explained by SC irregularities. This results in locally suppressed interference and, subsequently, closely adjacent exchange between the same two chromatids. The chiasmata in this case occur so close together that they can not be resolved by eye and appear as one chiasma; however, it is also possible that they do not form at all.

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