Genomic *in situ* hybridization analysis of intergeneric hybrids between *Brassica* species and *Orychophragmus violaceus* and detection of rDNA loci in *O. violaceus*

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ABSTRACT

The chromosome complements of intergeneric hybrids between the cultivated *Brassica* species and *Orychophragmus violaceus* (2n=24) and of their progenies were studied, at mitosis and meiosis, by genomic *in situ* hybridization. The mixoploids from the cross with *B. juncea* (2n=36) were divided into three groups according to their morphology and chromosome constitutions. In the mixoploids from the cross with *B. carinata* (2n=34), the majority of PMCs (2n=34) had only *B. carinata* chromosomes, but some PMCs in certain plants had 1-3 pairs of *O. violaceus* chromosomes. Plants with partial *B. nigra* complement and additional *O. violaceus* chromosomes were observed in the progenies of hybrids with *B. nigra* (2n=16). Finally, four chromosome pairs were found to carry ribosomal cistrons in the *O. violaceus* complement. The above results were briefly discussed in relation to the events that could have affected the chromosome constitution of the intergeneric hybrids.

Key words: *Brassica* species - *Orychophragmus violaceus* - intergeneric hybrids - chromosome complement - genomic *in situ* hybridization

INTRODUCTION

Sexual hybridizations between the six cultivated *Brassica* (*Cruciferae*) species (three diploids: *B. rapa* L., *B. nigra* (L.) Koch and *B. oleracea* L.; and three tetraploids: *B. juncea* (L.) Czern. & Coss, *B. napus* L. and *B. carinata* A. Braun, originating from pair crosses of the three diploids; U 1935) and *Orychophragmus violaceus* (L.) O.E. Schulz produced various progenies with different chromosome constitutions which gave new insights about chromosome behaviour in wide crosses of plants (Li et al. 1995, 1996, 1998; Li and Heneen 1999). Except the hybrid with *B. oleracea*, which was karyotypically stable and sterile, the other five ones were karyotypically unstable and partially fertile mixoploids. Complete or partial separation of parental genomes was suggested to occur during mitosis in these mixoploids and to produce cells with parental complements, or parental complements plus additional chromosomes from the other parent, or partially parental complements, or substituted complements. The cells with *Brassica* complements or with chromosomes mainly from *Brassica* parents seemed to be more competitive during plant growth and hence gamete formation. The possible occurrence of the above phenomena, which may produce *Brassica* aneuploids and homozygous plants, demonstrates the need to study the chromosome constitutions of these hybrids and their progenies by applying genome *in situ* hybridization (GISH). The results obtained in this study are reported here.

MATERIALS AND METHODS

Plant material. With *O. violaceus* as pollen parent, crosses with *B. juncea* (accessions no. GJ19), *B. carinata* (GO 11) were made by hand emasculation and pollination. The seeds obtained from these crosses, selfed seeds of a plant (2n=17-26) from the cross with *B. nigra* cv. Giebra (Li and Heneen 1999) were germinated in an incubator at 22°C and then potted in the greenhouse.

Cytological preparations. Roots from seedlings and styles from young flower buds were used to study mitotic chromosomes. Flower buds were fixed for meiotic analysis. Materials to be used in preparing squashes for GISH were treated with an aqueous solution of pectinase and cellulase.

In situ hybridization. Genomic *O. violaceus* DNA and ribosomal DNA from *Triticum aestivum* (pTa71) were labelled with digoxigenin-11-dUTP (Roche) by nick-translation. GISH was carried out according to Leitch et al. (1994). The hybridization mixture contained 3 μg ml^{-1} of labelled *O. violaceus* DNA and 20 μg ml^{-1} of *Brassica* blocking DNA. For detecting the rDNA loci on *O. violaceus* chromosomes, the hybridization mixture contained 1 μl of labelled rDNA and 2 μl of salmon DNA. The digoxigenin at the hybridization sites was detected using fluorescein-conjugated anti-digoxigenin IgG (Roche).

RESULTS AND DISCUSSIONS

B. juncea (2n=36) x *O. violaceus* (2n=24) hybrids

The mixoploid plants from the cross were divided into three groups according to their mitotic and meiotic chromosome constitutions. In the first group, comprising two partially fertile plants, most cells in styles from young flower buds had a chromosome number higher than 36 (up to 42). In these cells, all the chromosomes of *B. juncea* and additional chromosomes of *O. violaceus* were included. Cells with 36 chromosomes showed 0-4 *O. violaceus* chromosomes. A few cells contained less than 36 chromosomes with or without *O. violaceus* chromosomes. Different number of labelled univalents or bivalents were observed in PMCs at diakinesis and metaphase I (MI). Most PMCs at anaphase I (AI) had chromosome number higher than 36 and contained the *B. juncea* chromosomes and additional *O. violaceus* chromosomes. In most cases, the *O. violaceus* chromosomes were equal in number in the two polar groups. In a few PMCs (2n=36), the chromosomes were unequally segregated and 0-2 *O. violaceus* chromosomes were included in each polar group. When *O. violaceus* chromosomes were lacking, these PMCs showed 18:18 segregations. When selfed, the two plants showed low fertility and produced some short pods which contained brown coated seeds, similar to that of *O. violaceus*. *B. juncea* plants showed nearly normal fertility when pollinated by the hybrid plants, and produced yellow seeds with brown sectors. In the root cells of seedlings belonging to the selfed and backcrossed progeny seedlings, 36 *B. juncea* chromosomes and some *O. violaceus* chromosomes were observed. The plants (mainly, 2n=30-36 in the styles) in the following two groups were morphologically quite similar to mother plants *B. juncea* and showed nearly normal fertility. A group of 5 plants produced seeds whose coats were not entirely yellow, as in *B. juncea*, but showed brown sectors. The chromosome constitutions of their PMCs can be seen from Table 1. In 3 plants, all PMCs had 18 bivalents at diakinesis and MI and showed 18:18 segregation at AI. Among them, some contained 1-4 *O. violaceus* bivalents or 1-4 *O.violaceus* chromosomes in each polar groups at AI. Only one plant (No.5 in Table 1) had *O. violaceus* chromosomes in all PMCs, the other plants had PMCs both with or without *O. violaceus* chromosomes. In 2 plants, some PMCs having fewer than 36 chromosomes were found. The majority of these PMCs showed 32 or 34chromosomes and contained 0-2 pairs of *O .violaceus* chromosomes. In the 5 plants studied, 88.8% of PMCs having 36 chromosomes showed some *O. violaceus* chromosomes. Two-four *O.violaceus* chromosomes were included in the root cells (2n=36) of several seedlings obtained from the seeds of plant No.5. The third group was made of 11 plants which had PMCs with 18 bivalents at diakinesis and MI and 18:18 segregation at AI, and did not show *O. violaceus* chromosomes, though *O.violaceus* chromatin was detected in some nuclei of the anther-wall cells.

B. carinata (2n=34) x *O. violaceus* hybrids

The mixoploid plants from this cross (mainly, 2n=29-34 in the styles) had the morphology of *B. carinata* mother plants and were nearly fertile. The chromosome constitutions of the PMCs of 9 of these plants can be seen from Table 1. Except for a few PMCs with fewer than 34 chromosomes (2n=24-32) which were observed in 2 plants, all PMCs had 17 bivalents at diakinesis and MI and 17:17 segregation at AI. However, in these PMCs, 2, 4, 6 chromosomes of *O. violaceus* might be present, which were paired at diakinesis and MI and equal in number in the two polar groups at AI. A plant (No. 6, in Table 1) had PMCs with only *B. carinata* chromosomes, in another (No. 14), *O. violaceus* chromosomes were present in all PMCs. In the 8 plants showing *O. violaceus* chromosomes, these were found in 42.5% of the PMCs.

Progenies of *B. nigra* (2n=16) x *O. violaceus* hybrids

In 4 selfed mixoploid plants, 8-10 chromosomes were included in each polar group of AI PMCs. Each polar group comprised 1-4 chromosomes of *O. violaceus*, which were present as uvivalents or bivalents at diakinesis and MI.

The chromosome set-up of plants observed in the *Brassica* species x *O. violaceus* crosses might have resulted from the complete or partial separation of the parental genomes, as proposed previously (Li et al. 1998; Li and Heneen 1999). In the mixoploid plant (2n=17-26) from the *B. nigra* x *O. violaceus* cross, reciprocal inclusion of chromosomes between the two parental genomes as well as chromosome addition was observed. The viability and high fertility of these plants having a partial *B. nigra* complement and additional *O. violaceus* chromosome indicated functional compensation between the two species and the polyploidy nature of *B. nigra*. This also backs the view that the three *Brassica* diploids were descended from hexaploid ancestors (Lagercrantz

1998). From the similar chromosome behaviors in the hybrids of *O. violaceus* with both *B. nigra* (genome B), *B. juncea* (AB) and *B. carinata* (BC), as well as from the karyotypical stability of the hybrids with *B. oleracea* (2n=18, CC, Li and Heneen 1999), we inferred that the chromosomes which were substituted in the *Brassica* complements belonged mainly to the B genome.

After *in situ* hybridization with the rDNA probe, up to 8 signals of different sizes were easily counted in interphase nuclei of *O. violaceous*. Four larger signals and 4 smaller ones were observed in most nuclei. Four chromosome pair bore rDNA cistrons at terminal locations, while only up to 6 satellites could be seen in metaphase plates. This result might support the view that *O. violaceus* is an autopolyploid species with a basic chromosome number of x=6 (2n=4x=24, Li et al. 1995, 1996).

Table 1 Chromosome constitution in PMCs of plants from crosses of *B. juncea* (1-5) or *B. carinata*(6-14) with *O. violaceus*

Plants	Number of PMCs with chromosome constitutions					
	a	*b*	*c*	*d*	*e*	*f*
1	7	44	2			13
2	14	5	6	1		
3	8	42	24	15	2	
4	6	42	17	7	4	16
5		14	31	17	5	
	g	*h*	*i*	*j*	*k*	
6	32					
7	20	10				
8	13	2				
9	13	2	3			
10	69	3	2			
11	45	11	8			
12	11	24	33	7	2	
13	24	7	5	1	10	
14		6	19	1		

a-e, PMCs (2n=36) with 18 bivalents at diakinesis, MI and 18:18 segregation at AI, but including 0 (*a*), 2 (*b*), 4 (*c*), 6 (*d*) or 8 (*e*) chromosomes of *O. violaceus*. The *O. violaceus* chromosomes were paired as bivalents or were equal in the two polar groups. *f*, PMCs with less than 36 chromosomes. *g-j* PMCs (2n=34) with 17 bivalents and 17:17 segregation, including 0 (*g*), 2 (*h*), 4 (*i*) or 6 (*j*) chromosomes of *O. violaceus*. The *O. violaceus* chromosomes were paired or segregated as above. k, PMCs with less than 34 chromosomes.

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