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Evidence of Crossing-over Inhibition in Rye Anthers Cultured with Colchicine

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Abstract. In order to investigate wheter colchicine affects crossing-over, rye anthers of an inbred line of rye forming bridges and fragments at anaphase I produced by erroneous chiasmata, and anthers of plants heterozygous for a conspicuous heterochromatin band, were cultured in a medium with colchicine. Anthers planted at zygotene did not show bridges at AI in the inbred line. In the heterozygotes no difference between associated chromatids in respect to the heterochromatin band, resulting from crossingover, were observed. In anthers planted at pachytene both bridges and chromosomes showing difference between associated chromatids were observed at a stage equivalent to AI with the same frequency as in anaphase I cells of untreated anthers. This demonstrates that crossing-over or a prerequisite to crossing-over is established at zygotene, and also that absence of chiasmate association at later stages is not due to precocious slipping off of chiasmata.

Introduction

In a previous paper (Peña and Puertas, 1978) colchicine induction of C-meiosis and asynapsis in cultured anthers of rye was reported. Two anthers of a floret were cultured and given the treatment indicated, the third was used to determine the stage at time of planting. Anthers planted at mid zygotene showed asynapsis in PMCs with 14 X-shaped (lax) chromosomes (X-l cells) coexisting with unaffected PMCs at diplotene or, depending on the duration of the treatment, in PMCs with short chromosomes (X-s cells) apparently corresponding with diakinesis. Anthers planted at later stages showed normal synapsis till metaphase I; chiasma release was possible in the absence of a functional spindle and chromosomes were associated pairwise (X-p cells). However, this earlier experiment did not permit the distinction between absence of chiasmata (asynapsis or desynapsis) and precocious terminalization followed by slipping off of chiasmata. In order to distinguish between these possibilities an inbred line of rye forming bridges and fragments at anaphase I produced by erroneous chiasmata (Giraldez

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and Lacadena, 1978) and plants heterozygous for a heterochromatin band which is apparent with C-banding techniques, have been used.

Material and Methods

Inbred lines P, M and A earlier studied by Giraldez and Lacadena (1976) were employed.

Using C-banding techniques, the M line shows chromosome pair 3 with a conspicuous telomeric band (Fig. 1 a) which is absent in line A (Fig. 1 b). The F_1 of M and A was made and heterozygous F_2 plants were raised (Fig. 1 c).

Anthers from line P and from MA heterozygotes were cultured with and without colchicine, starting at mid zygotene, according to the technique described by Peña and Puertas (1978). After the period of culture all anthers were fixed in acetic alcohol 1:3. P line anthers were stained using the Feulgen method. Preparations were made permanent with Sandeural.

MA anthers were C-banded according to the technique of Giraldez et al. (1979). Briefly: air dryed slides were immersed in 0.2 N HCl at 60° C for 3 min, washed in tap water and placed in a saturated solution of Ba $(OH)_2$ at room temperature for 10 min, washed again in tap water and immersed in 2 × SSC at 60° C for 1 h. Slides were then stained with a Giemsa solution prepared with 3 ml of Giemsa Gurr's R 66 and 100 ml phosphate buffer pH 7. Staining was checked for appropriate contrast and subsequently the slides were washed in tap water and rapidly air dryed, immersed in xylene for 5 min and mounted in DPX.

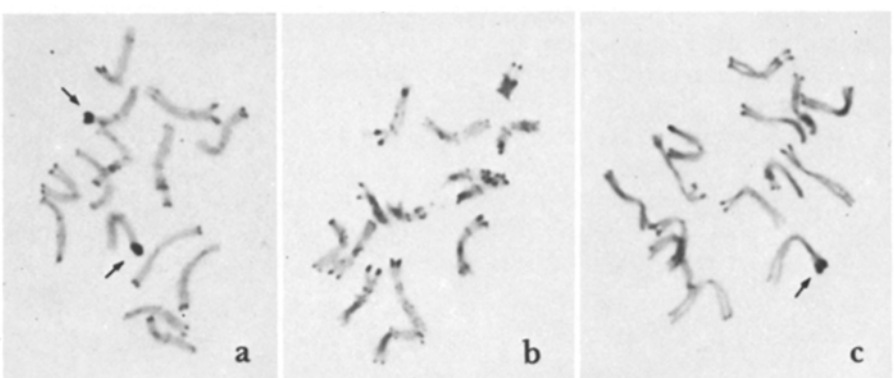

Fig. 1. a C-banded somatic metaphase of M line showing chromosome pair 3 with a conspicuous heterochromatin band (arrowed). **b** C-banded somatic metaphase of A line, the big band is absent. **c** Heterozygous MA plant, arrow marks chromosome 3

Results

Anthers of the P line were cultured for periods varying from 5 to 30 h, while in MA heterozygotes this varied from 8 to 24 h. Colchicine did not alter either the duration nor the synchrony of meiosis, as could be deduced by comparing cultures with and without colchicine.

PMCs with C-mitotic morphology induced by colchicine in P and MA plants showed the same appearance and distribution pattern as in the cultivar Ailés (Peña and Puertas, 1978). In line P two novel types of PMCs were found:

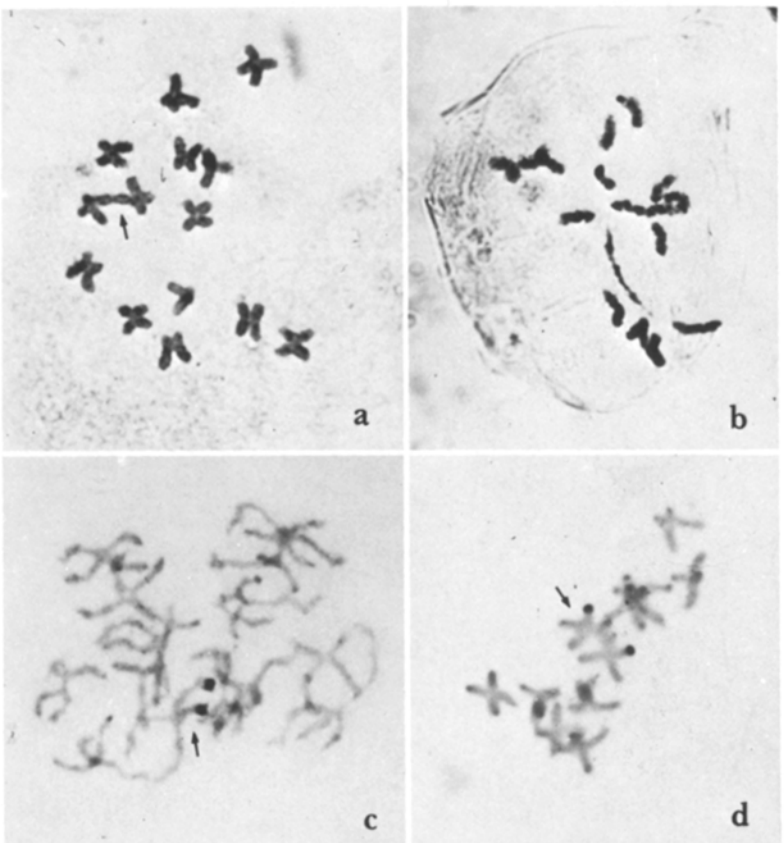

Fig. 2. a X-p cell of P line showing one bridge (arrowed), pairs of chromosomes have been separated due to strong squash to make the bridge apparent. **b** C-anaphase cell of P line showing one bridge. **c** X-l cell of aMA heterozygote showing the parental pattern for the heterochromatin band (arrowed). **d** X-p cell of a MA heterozygote showing the recombinant pattern (arrowed)

X-p and C-anaphase cells with bridges and fragments (Fig. 2a, b). The number of bridges in X-p cells varied from 1 to 4 (in 31–36% of the cells) while no more than 1 bridge was found in C-anaphases. It is remarkable that neither X-1 nor X-s cells appeared with bridges. The frequencies of these cell types are shown in Table 1.

X-p cells are cells in which apparently pairing had taken place, and probably chiasmata had formed, either due to "escape" from the treatment or because of having been at a post-pairing stage at the time of treatment. C-anaphases may be assumed to have resulted in low frequency from "escape" cells (X-p) and in the majority from "affected" (X-s) cells.

In anthers cultured in absence of colchicine 35% of anaphase I cells with 1–4 bridges were observed.

Results obtained in C-banded MA heterozygotes are shown in Table 2. X-l and X-s cells always appeared with the parental pattern (Fig. 2c), while in X-p

Table 1. Frequency of bridges in X-p and C-anaphase cells appearing in anthers of the P line in which treatment was initiated at zygotene and pachytene

Stage at initiation	Number of bridges per cell								
	X-p cells						C-anaphase cells		
	0	1	2	3	4	% of cells with bridges	0	1	% of cells with bridges
Zygotene	472	149	45	7	11	31	432	32	7.5
Pachytene	161	58	26	4	3	36.1	72	22	23.4

Table 2. Frequency of cells with or without recombination in MA heterozygotes. Treatment was initiated at mid-zygotene

	Cell types			Anaphase I of untreated anthers
	X-1	X-s	X-p	
PMCs without recombination	20	46	31	8
PMCs with recombination	0	0	105	29
% of recombination	0	0	77.22	78.38

cells recombination showed up (Fig. 2d) in 77.22% of the cases. The recombination frequency of anaphase I cells in untreated anthers was 78.38%.

An additional datum obtained from cultures without colchicine is an estimate of the meiotic duration in the P inbred line, according to the criteria of Bennett et al. (1971). It was found that both zygotene and pachytene took about 5 h. In the normal cultivar Ailés, the duration of both stages was double this time (10 h each). The duration of the remaining stages was similar both in P and Ailés (Peña, 1978).

Discussion

Bridges and fragments observed in inbred lines of rye are produced by erroneous chiasmata (Jones, 1968; Giraldez and Lacadena, 1978). Then, the presence of a bridge in a chromosome pair is evidence of the occurrence of the equivalent of at least one chiasma in this particular bivalent.

In line P studied by Giraldez and Lacadena (1978) the frequency of anaphase I cells showing bridges was 31.2%. A similar frequency was observed here in anthers cultivated without colchicine.

Therefore, under the assumption that colchicine produces asynapsis or desynapsis, it is expected that affected cells (X-1 and X-s) will not show bridges, while in X-p cells they will be formed with a frequency close to 31%. This was confirmed in our experiment (Table 1).

It has to be noted that X-p cells appeared in anthers planted both at zygotene and pachytene because colchicine does not affect pairing in all cells, while it blocks anaphase migration in almost every case. The frequency of X-p cells with bridges was very similar in both cases (Table 1).

In anthers planted at zygotene, C-anaphases may derive from both X-s and X-p cells, but mainly from X-s. In anthers planted at pachytene, C-anaphases will be formed only from X-p cells. This would explain that in anthers planted at zygotene only 7.5% of the C-anaphases showed bridges, while in anthers planted at pachytene the frequency came up to 23.4%. However, the expected frequency would be about 30% like in X-p cells. This difference may be due to the part of the cell population which was still at an earlier stage at time of planting. Anyway, the fact that in anthers planted at zygotene, bridge frequency at C-anaphase is very low, is a clear indication for absence of crossing-over in X-s cells.

The absence of recombination in X-l and X-s cells in MA heterozygotes also convincingly shows that colchicine applied at zytogene inhibits crossing-over. In X-p cells, however, crossing-over (77.22%) is not reduced compared to control (78.88%).

These results might be taken as a proof that crossing-over takes place at late zygotene or early pachytene. More probably, however, colchicine produces asynapsis, as a consequence of which crossing-over is inhibited.

This agrees with the time of action (zygotene) and with the results obtained in *Lilium* by Hotta and Sherpard (1973), and Shepard et al. (1974).

It is remarkable that the duration of zygotene and pachytene is much shorter in inbred line P than in normal cultivar Ailés. The shorter duration of these decisive meiotic stages may be the cause of failures and errors of crossing-over in inbred lines.

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