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

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Abstract. In order to investigate whether 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 crossing-over, 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 chiasmatic 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-I 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.
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 F₁ of M and A was made and heterozygous F₂ 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 dried 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 x 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 dried, immersed in xylene for 5 min and mounted in DPX.

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: