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Meiotic Irregularities in Argentinian Hexaploid Oats

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Structural rearrangements in oat hybrids have been reported by several authors (Rajhathy and Thomas 1974, Singh and Kolb 1991). However, intercultivar chromosome interchanges in domestic oats have not been extensively investigated.

Our objective in the present communication is to analyze the meiotic behavior of three hexaploid oat lines (*Avena sativa* L.) and three synthetic hybrids among them. This investigation was initiated to study the cytogenetic structure of argentinian oat experimental breeding lines and its likely implicances in further breeding programs.

Materials and methods

Lines and hybrids of hexaploid oats (*Avena sativa*, 2n=6x=42, AACCDD) were obtained from Ing. Agr. Hugo Chirichimo and Ing. Agr. Cecilia Fussé (Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Argentina). Both [111-4193/88] and [112-4282/88] progenitors are closed pedigree lines from Buck S.A. (Argentina). RIL is a recombinant inbred line.

Cross #1	Cross #2
Progenitors:	Progenitors:
P1=[111-4193/88]	P1=[111-4193/88]
RIL={PI 186270×[CI2931−Red Algerian (Hapar 30)]}	P2=[112-4282/88]
F1:	F1:
P1/RIL	P2/P1
RIL/P1	

Immature panicles were fixed in a 3:1 (absolute alcohol:acetic acid) solution. Anthers were squashed in 2% acetic haematoxylin. The pairing configurations were determined at diakinesis. Only cells with well-spread plates were scored. Mean chiasma frequencies were tested through ANOVA tests. Off-plate chromosome (OPC) and nonsegregating chromosomes (NSC) frequencies were compared through χ^2 tests.

Results and discussion

a. Meiosis I

Lines P1 and P2 formed 21 bivalents (II) in all cells studied. 100 cells were scored in each progenitor. P1/RIL and RIL/P1 exhibited 1 quadrivalent (IV)+19II at diakinesis in 60% of the polen mother cells analyzed (Fig. 1A). P2/P1 presented 1IV+19II in 50% and 2IV+17II in 17% of the sporocytes (Fig. 1B). 214 cells were scored in cross #1 and 97 in cross #2.

These results indicated that P1 and RIL differ for a single reciprocal translocation. On the other hand, P2 and P1 are differentiated by two independent interchanges. It should be noted that the frequency of sporocytes with the 1IV+19II configuration in the P2/P1 hybrid was

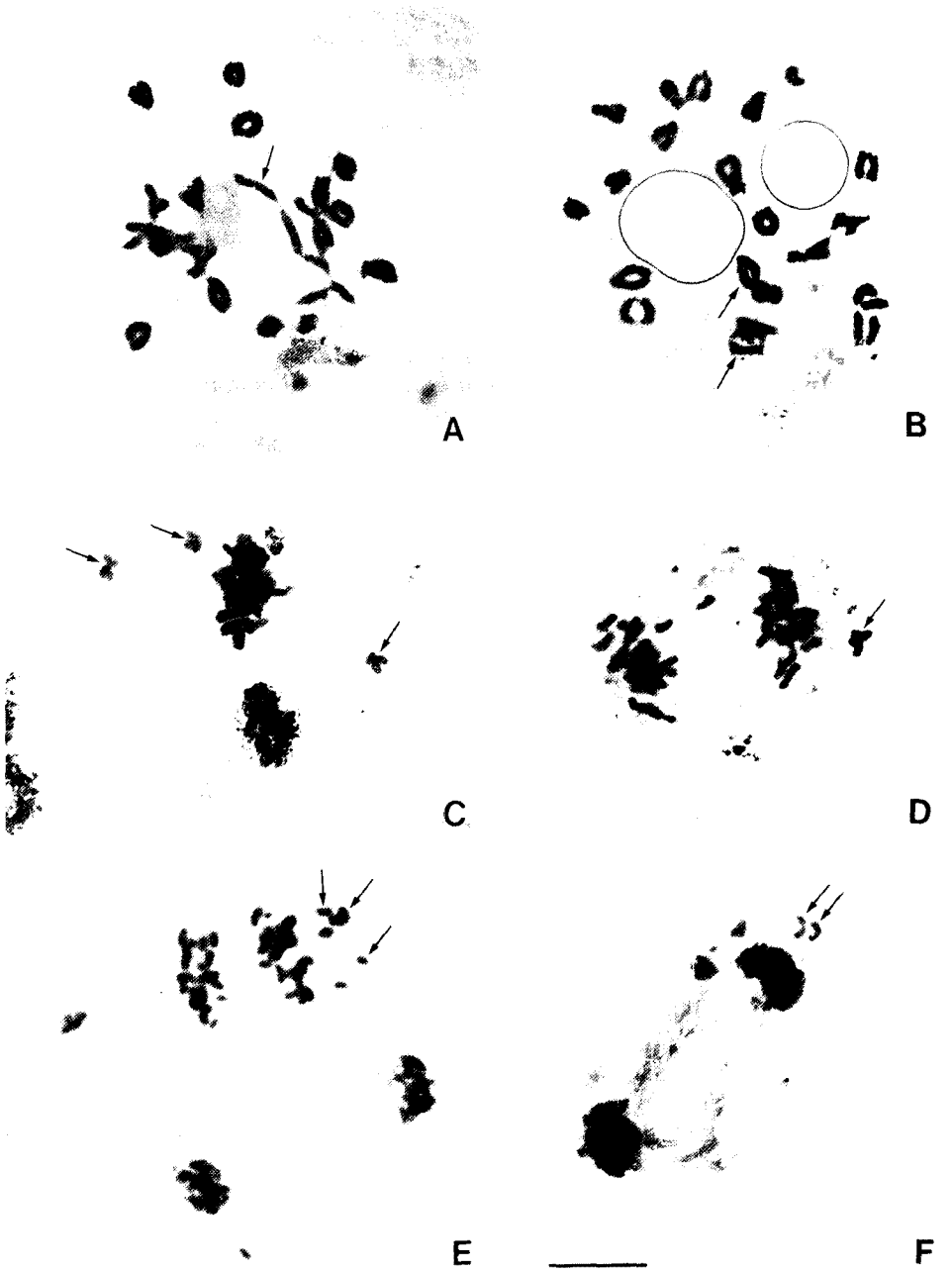

Fig. 1. Meiotic irregularities. A) Diakinesis in P1/RIL, 19II + 1IV (arrow). B) Diakinesis in P2/P1, 17II + 2IV (arrows). C) Metaphase II with three OPCs. D) Anaphase II with one NSC. E) Anaphase II with three NSCs. F) Telophase II showing two nonsegregating sister chromatids. Bar = 10 μm.

higher (50%) compared with the sporocytes with 2IV + 17II (17%). This suggests that the chromosome segments involved in one of the interchanges may be small.

Mean chiasma frequency per cell (Fig. 2) did not differ significantly among lines with 21II (P1 and P2), hybrids with one IV (P1/RIL, RIL/P1) and hybrids with up to 2IV per cell (P2/

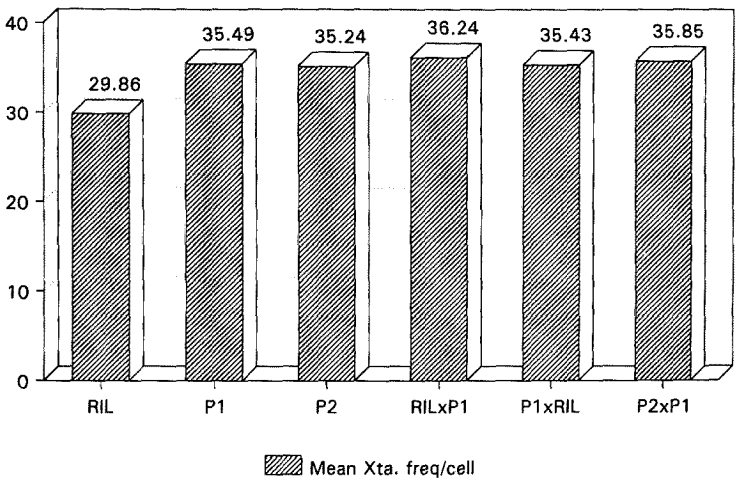

Fig. 2. Chiasma frequencies for lines and hybrids.

P1) ($F=1.36$; $CV=4.28$; $p\leq 0.05$). Therefore, heterocygosis for reciprocal translocations do not interfere with overall chiasma frequency in the present case.

RIL presented a significantly reduced chiasma frequency when compared with P1, P1/RIL and RIL/P1 ($F=10.64$; $CV=4.82$; $p\leq 0.01$), whose mean chiasma frequency was found to be similar ($F=2.98$; $CV=4.82$; $p\leq 0.01$). This could be indicating that recesive genes, defective for crossing-over events, might be present in RIL in homocigous condition.

b. Meiosis II

Table 1 shows the frequency of off-plate chromosomes (OPCs) in metaphase II (MII) and nonsegregating chromosomes (NSCs) in anaphase II and/or telophase II (AII-TII).

OPCs lie outside the MII plate (Fig. 1C) and show a well defined centromeric region. Their sister chromatids are readily identifiable. NSCs lie beyond the array of segregating chromosomes and separate their sister centromeres after AII onset (Figs. 1D–E). Nevertheless, nonsegregating chromatids remain in close proximity, near the TII nuclei (Fig. 1F), and become micronuclei at the tetrad stage.

Table 2 shows a series of χ^2 tests performed in order to compare OPC and NSC frequencies in lines and hybrids. In all cases, those plants showing a high OPC frequency (P2 × P1, RIL × P1 and RIL) also presented a high NSC frequency. Furthermore, Table 1 show that OPC frequency is always higher or equal than NSC ones.

Several observations indicate that OPC/NSCs are actually unattached to the spindle by the moment of anaphase onset:

a. Statistical analysis, which show the posibility that NSCs derive from OPCs (Tables 1, 2).
b. OPC/NSC morphology and behavior, which strongly resemble c-metaphase and c-anaphase chromosomes respectively.
c. OPC/NSCs location, beyond the spindle zone (Figs. 1C–F.)

These data undoubtfully indicate that, contrarily to what is commonly agreed (e.g. Murray 1991) the presence of unattached chromosomes do not inhibit chromatid segregation, at least in hexaploid oats. Thus, complete metaphase II plate congression is not a prerrequisite for anaphase II onset in this case.

It is unclear whether the presence of OPC/NSCs affects the timing of AII onset in hexaploid oats. No definitive answer can be drawn from the present report. In an attempt to

Table 1. OPC and NSC frequencies

	MII			AII-TII			χ^2
	Normal	OPC	N° Cells	Normal	NSC	N° Cells	(MII/AII-TII)
Cross #1							
P1	86.36	13.64	154	100.00	0.00	191	—
RIL	66.29	33.71	89	74.03	25.97	77	1.17*
P1/RIL	81.37	18.63	102	99.20	0.80	125	21.13**
RIL/P1	48.39	51.61	93	76.47	25.96	85	14.83**
Cross #2							
P1	86.36	13.64	154	100.00	0.00	191	—
P2	91.23	8.77	57	98.48	1.52	66	3.47*
P2/P1	74.63	27.37	67	74.04	25.96	285	0.01*

* $p \leq 0.95$. ** $p \leq 0.99$.

Table 2. χ^2 comparissons between lines and hybrids with high and low OPC and NSC frequencies

	OPC (MII)	NSC (AII-TII)
Cross #1		
P1 vs RIL	13.70**	—
P1 vs RIL/P1	41.84**	—
RIL vs P1/RIL	5.63*	102.49**
P1/RIL vs RIL/P1	24.46**	29.04**
Cross #2		
P1 vs P2/P1	4.52*	—
P2 vs P2/P1	5.52*	19.66**

* $p \leq 0.95$. ** $p \leq 0.99$.

make a first approach to this question, a χ^2 test was performed in order to compare MII/AII-TII rates among plants with high (P2 × P1, RIL, RIL × P1) and low (P2, P1, P1 × RIL) OPC/NSC frequencies. At least 300 cells were scored for each plant. Nonsignificant differences were found ($\chi^2 = 1.33$; CV = 7.81; $p \leq 0.05$, cross #1; $\chi^2 = 4.42$; CV = 5.99; $p \leq 0.05$, cross #2). These results suggests that there is no delay for chromatid segregation onset in plants with high OPC/NSC frequency.

If this were the case, the inhibitory signal preventing sister chromatid segregation (Hartwell and Weinert 1989) would not result from unattached chromosomes, contrarily to McIntosh's proposal (1991). Earnshaw *et al.* (1991), on the other hand, argued that chromosomes unattached to the spindle seem to be excluded from the regulatory network and result invisible to the metaphase-anaphase checkpoint. The present results seem to support this latter hypothesis.

Summary

Three Argentinian oat lines (P1, P2 and RIL) and three synthetic hybrids among them were analized in the present communication. P1 and RIL differ for one reciprocal translocation. P1 and P2 presented two independent interchanges. For the first time meiotic irregularities during ecuational division in hexaploid oat hybrids are reported. This latter observation throw doubts on the dependence of anaphase onset on metaphase plate completion in oats.

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