

TELOMERIC HETEROCHROMATIN, SEEDS AND MEIOTIC CHARACTERISTICS IN TWO TRICEPIRO LINES

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Abstract. Fradkin, M.; E. Greizerstein, L. Poggio, H. Paccapelo & M. R. Ferrari. 2011. Telomeric heterochromatin, seeds and meiotic characteristics in two tricepiro lines. *Darwiniana* 49(1): 25-31.

A hybrid, named tricepiro, was obtained in 1972, by crossing a hexaploid triticale ($2n=6x=42$) and an octoploid trigopiro ($2n=8x=56$). The lines achieved include the tricepiro Don René INTA, which has shriveled kernels, and FA-L2, which has smooth ones. The relationship between the heterochromatin content of the rye chromosomes, the seed weight, the presence of meiotic abnormalities and the kernel shriveling has been documented previously in other intergeneric hybrids. The purpose of this study was to determine the average percentage of heterochromatin content in the rye chromosomes of the two tricepiro lines mentioned above and to relate this feature to some characteristics of the meiosis and seeds. We confirmed that the two lines have the same total chromosome number ($2n=42$) and the same number of rye chromosomes (14). We found that both lines have a complete chromosome mating until late diakinesis, but differ in the percentage of cells with univalents outside the equatorial plate in Metaphase I (Don René 42.85% and FA-L2 14.00%). In addition, the two lines differed in their meiotic index (Don René 66.47% and FA-L2 87.90%) and seed weight (Don René 0.029 ± 0.000 g and FA-L2 0.038 ± 0.001 g). The C-banding in rye chromosomes in mitotic metaphase indicated that the average percentage of heterochromatin content did not differ significantly between the two lines. In contrast to our expectations, the meiotic behavior and seed characteristics were not related to the heterochromatin percentage of the rye chromosomes of the tricepiro lines studied.

Keywords. Heterochromatin, micronuclei, shriveled kernels, tricepiro, univalents.

Resumen. Fradkin, M.; E. Greizerstein, L. Poggio, H. Paccapelo & M. R. Ferrari. 2011. Heterocromatina telomérica, semillas y características meióticas en dos líneas de tricepiro. *Darwiniana* 49(1): 25-31.

Al cruzar un triticale hexaploide ($2n=6x=42$) y un trigopiro octoploide ($2n=8x=56$) se obtuvo, en 1972, un híbrido denominado tricepiro. Entre las líneas que se lograron están tricepiro Don René INTA que posee semillas arrugadas y FA-L2 con semillas lisas. El contenido de heterocromatina de los cromosomas de centeno en otros híbridos intergenéricos ha sido relacionado con la rugosidad y peso de las semillas, así como con la presencia de anomalías meióticas. El objetivo del presente trabajo fue determinar, en ambas líneas mencionadas anteriormente, el porcentaje promedio de heterocromatina presente en los cromosomas de centeno y relacionar este valor con características de la meiosis y de las semillas. Confirmamos que las dos líneas tienen el mismo número cromosómico ($2n=42$) y el mismo número de cromosomas de centeno (14). Encontramos que ambas presentan un apareamiento completo de los cromosomas hasta diacinesis tardía, pero difieren en el porcentaje de células con univalentes fuera de la placa ecuatorial en Metafase I (Don René 42,85 % y FA-L2 14,00 %). Además, ambas líneas difie-

ren en el Índice Meiótico (Don René 66,47 % y FA-L2 87,90 %) y en el peso de las semillas (Don René $0,029 \pm 0,000$ g y FA-L2 $0,038 \pm 0,001$ g). El análisis de las bandas C, hecho en metafases mitóticas, indicó que el porcentaje promedio del contenido de heterocromatina presente en los cromosomas de centeno, no difiere significativamente entre las dos líneas. Contrariamente a nuestras expectativas, el comportamiento meiótico y las características de las semillas no mostraron relación con el porcentaje de heterocromatina presente en los cromosomas de centeno de las líneas híbridas estudiadas.

Palabras clave. Heterocromatina, micronúcleos, semillas arrugadas, tricepiro, univalentes.

INTRODUCTION

Tricepiro is a synthetic forage crop obtained in Argentina in 1972 by crossing a hexaploid triticale (AABBRR $2n=6x=42$) and an octoploid trigopiro (AABBDDJJ, $2n=8x=56$) (Covas, 1976, 1989, 1995). Different lines, all with $2n=42$ chromosomes, were obtained after several years of breeding (Tosso et al., 2000). Two of these lines, named Don René INTA and FA-L2, were studied in the present work. The genomic composition of tricepiro in different lines is quite similar to triticales, but tricepiros have *Thinopyrum* introgression (Ferrari et al., 2005; Fradkin et al., 2009a). Both lines of tricepiro analyzed here differ in their kernel characteristics: Don René has shriveled kernels while FA-L2 has smooth ones.

Kernel shriveling and meiotic instability have been considered important problems of synthetic amphiploid triticales. These characteristics have been frequently related to the presence of large terminal heterochromatin blocks on the rye chromosomes (Thomas & Kaltsikes, 1974; Merker, 1976; Bennett, 1977; Roupakias & Kaltsikes, 1977; Gustafson & Bennet, 1982; Naranjo & Lacadena, 1982; Soler et al., 1990; Jouve & Soler, 1996). However, in some cases, the size of the terminal heterochromatin blocks seems not to be associated with kernel shriveling and meiotic instability (Varghese & Lelley, 1983; Papa et al., 1990).

Triticale meiotic instability can be originated by causes that are not related to the large rye terminal heterochromatin bands, such as mutations, that could disturb the correct rye meiotic mechanisms (Jenkins et al., 2005, 2008; Sosnikhina et al., 2005, 2007).

The aim of the present work is to analyze the relationship between the percentage of the rye terminal heterochromatin bands and the presence and number of univalents in Metaphase I, the Meiotic

Index, and the kernel characteristics (shriveled or smooth kernels and weight) in the tricepiro lines Don René and FA-L2.

MATERIALS AND METHODS

Seeds of the tricepiro Don René INTA, tricepiro FA-L2 and *Secale cereale* L. were provided by Ing. G. Covas. Plant samples from which seeds were obtained were deposited at Facultad de Agronomía de la Universidad Nacional de La Pampa, Argentina. Plants were grown in irrigated beds in the greenhouse of the Instituto Fitotécnico de Santa Catalina, Llavallol, Buenos Aires Province, Argentina.

Meiotic studies

Young spikes were fixed in absolute alcohol: acetic acid (3:1). The anthers from immature flowers were squashed in 2% acetic hematoxylin as stain and 1% ferric citrate as mordant. Slides were made permanent by freezing with CO₂, removing the cover slip, dehydrating in absolute alcohol and mounting in Euparal.

Meiotic Index

The number of tetrads with and without micronuclei (normal) of each line was determined from the observation of at least 100 tetrads. The Meiotic Index (MI) was obtained according to the following formula:

$$MI = (\text{number of normal tetrads} / \text{number of total tetrads analyzed}) \times 100 \text{ (Love, 1949).}$$

C-banding

C-banding was performed according to Tito et

al. (1991) with minor modifications. Roots (1 cm long) were pre-treated in ice-cold water for 36 hours and fixed in absolute alcohol: acetic acid (3:1) for 24 hours at room temperature and stored at -20°C. Fixed roots were washed in 0.01 M citric acid-sodium citrate buffer (pH 4.6) to remove fixative, and transferred to an enzyme solution containing 2% cellulase and 20% liquid pectinase. The softened material was washed again in buffer solution. Finally, slides were prepared using the squash technique in a drop of 45% acetic acid and chromosomes were stained with 4'-6-Diamidino-2-phenylindole (DAPI).

Chromosome measurements

The lengths of rye chromosomes and their terminal heterochromatin bands were measured with the computer application MicroMeasure version 3.01, available on the Internet at <http://www.colostate.edu/Depts/Biology/MicroMeasure>.

The lengths of the rye terminal heterochromatin bands were normalized dividing the length of each band by the total length of the corresponding chromosome.

The percentage of normalized heterochromatin present in the rye chromosomes of each line was calculated using the following formula: $(\sum NLH / n) \times 100$ (NLH=normalized length of heterochromatin terminal bands, n=total number of rye chromosomes in each line). Twenty cells in each line were analysed.

Kernel weight

One hundred seeds of each line were individually weighed in a digital scale and the seed average weight of each line was obtained.

Statistical Analysis

Differences in the seed weight and size of telomeric heterochromatin between the two lines were tested through the t-Test. The number of cells with and without univalents at Metaphase I and the number of tetrads with and without micronuclei were tested using the Chi-square test. All the statistical analyses mentioned above were carried out with the program INFOSAT (Di Rienzo et al., 2008).

Table 1. Seed and meiotic characteristics of Don René INTA and FA-L2 tricepiro lines. Abbreviations: n1, number of cells; n2, number of tetrads; n3, number of seeds; SD, standard deviation.

	Don René INTA	FA-L2
Kernel	Shriveled	Smooth
Rye chromosome heterochromatin mean (%)±SD	12.55±0.17 ^a	12.79±0.23 ^a
n1	20	20
Cells with I (%) in MI	42.85 ^a	14.00 ^b
Range of I/cell	1-11	1-4
n1	77	40
Meiotic index	66.47	87.90
Range of micronuclei/tetrad	1-9	1-6
n2	173	211
Seed weight average (g)±SD	0.029±0.000 ^a	0.038±0.001 ^b
n3	100	100

Different superscript letters in each column denote significant differences ($p < 0.05$)

RESULTS

We confirmed that individuals belonging to the two tricepiro lines studied had the same chromosome number ($2n=42$) (Fig. 1A, B) and 14 rye chromosomes with conspicuous DAPI (+) terminal heterochromatic bands (Fig. 1A-C). No differences were found in the heterochromatin percentage of the two lines ($p < 0.05$) (Table 1).

A complete pairing of chromosomes, forming 21 bivalents, up to late diakinesis was observed in both lines (Fig. 2A, B). However, differences in the number of univalents outside the equatorial plate in Metaphase I were found between Don René and FA-L2 (Fig. 2C, D). FA-L2 had a lower percentage of cells with univalents outside the equatorial plate in Metaphase I and a narrower univalent range (Table 1).

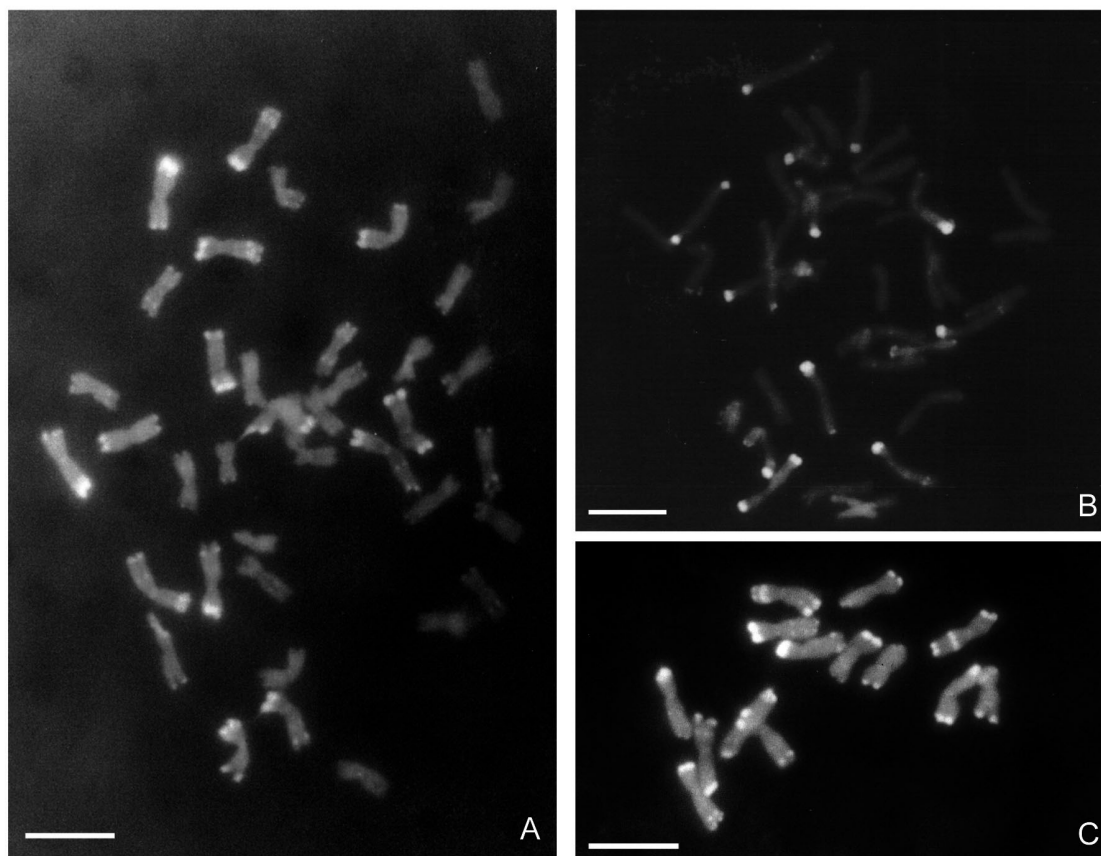


Fig. 1. C-bands in mitotic metaphase cells. **A**, tricepiro Don René INTA ($2n=42$). **B**, FA-L2 ($2n=42$). **C**, *Secale cereale* ($2n=14$). Bar=10 μm .

Although both lines had tetrads with micronuclei (Fig 2E, F), the number of tetrads with micronuclei was higher in Don René than in FA-L2. Besides, Don René had the highest number of micronuclei per tetrad (Table 1).

The meiotic index value was different in the two lines, being lower in Don René than in FA-L2. The seed weight also differed significantly between both lines, being lower in Don René than in FA-L2 (Table 1).

DISCUSSION AND CONCLUSIONS

The same chromosome number and similar rye chromosomes in both lines are a consequence of their common origin (Ferrari, 2004; Ferreira et al., 2007). The rye chromosomes were recognized by their typical terminal C-bands, in accordance with other authors (Papa et al., 1990; Cuadrado &

Jouve, 1994; Fradkin et al., 2009b). The common origin of the two lines can explain the similarity in heterochromatin percentage presented in the rye chromosomes. However, some of their morphological, agronomical and biochemical characteristics are different (Bertoni et al., 1995 a, b; Gros et al., 1995; Ferrari et al., 2001).

In the present work, these lines showed differences in the meiotic behavior: tricepiro Don René had a greater number of univalents in Metaphase I and micronuclei in the tetrads than FA-L2. Besides, Don René and FA-L2 differed in their kernel characteristics: the former had shriveled seeds whereas the latter had smooth ones with greater average weight.

The rye heterochromatic telomeric bands have been associated with abnormalities in the meiotic behavior, the shriveling and the weight of the seeds (Bennet, 1977; Soler et al., 1990). However, in the present study, the two tricepiro lines differed

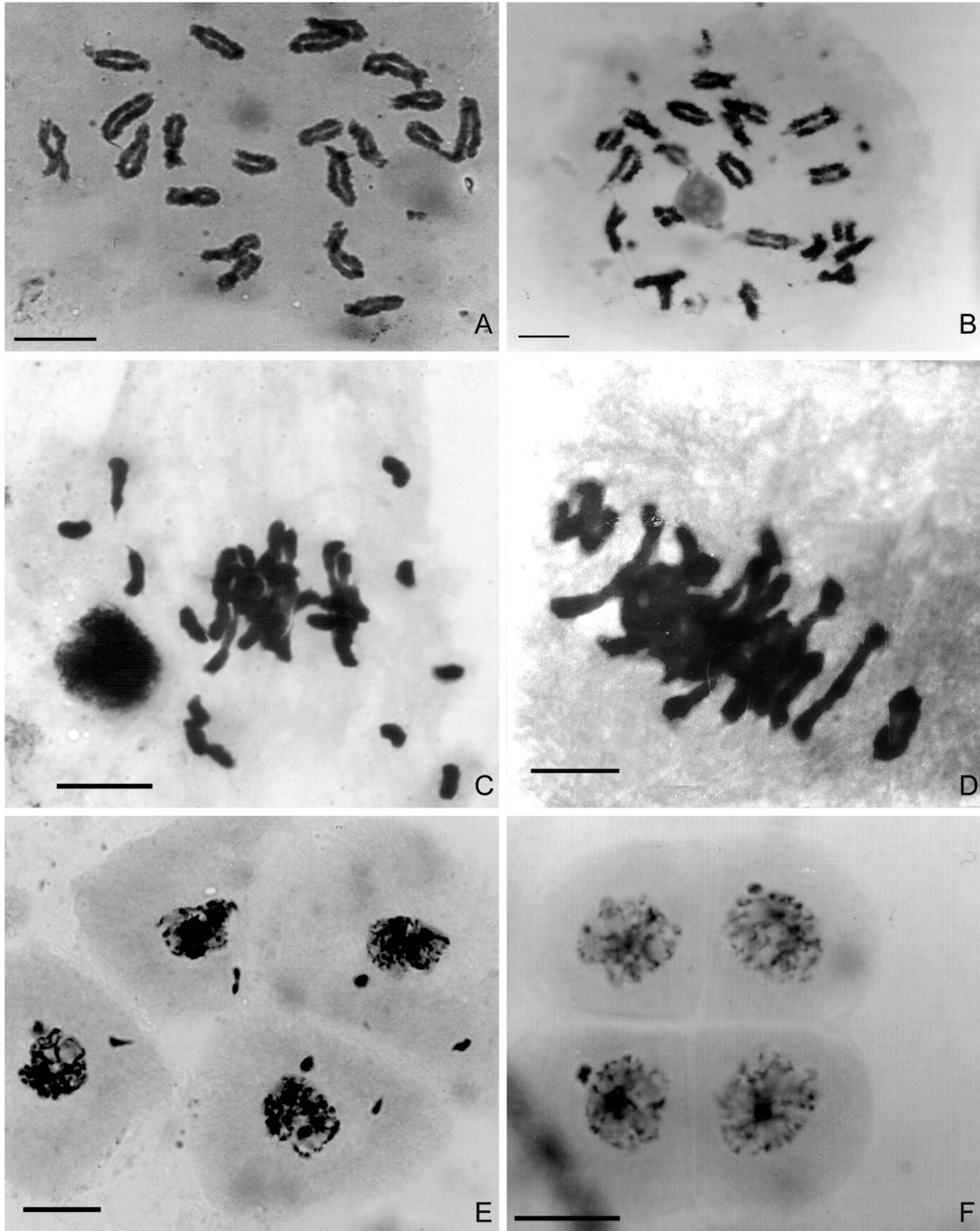


Fig. 2. Meiotic behaviour. **A, C, E,** tricepiro Don René. **B, D, F,** FA-L2. **A-B,** late diplotene 21 II. **C,** Metaphase I with 13 univalents. **D,** Metaphase I (ca 21 II). **E-F,** tetrads, with 6 and 2 micronuclei respectively. Bar=10 µm.

in their meiotic behavior and the weight and texture of their kernel, but had similar heterochromatin

content of the terminal areas of the rye chromosomes. This suggests that other mechanisms may be

involved in the origin of these differences. We thus hypothesize that one of the possible reasons is the presence of genes that modify the meiotic stability of the rye chromosomes. This idea has been supported by different authors who suggested that genes that induce asynapsis or desynapsis of homologous chromosomes could account for the origin of univalents in Metaphase I and of micronuclei in tetrads (Jenkins et al., 2005; Sosnikhina et al., 2005; Mikhailova et al., 2006).

The lower frequency of meiotic abnormalities in FA-L2 would enable this line to produce more balanced gametes than Don René. Therefore, the well-developed endosperm of FA-L2 would produce seeds with a smoother texture and a greater weight than those of Don René. We cannot rule out that physiological factors are involved in the shrivelling of the seed, as postulated by several authors (Klassen et al., 1971; Branlard et al., 1985; Heneen & Brismar, 1987; Bernardo et al., 1990).

As a consequence of the trigeneric origin of tricepiro (wheat, rye and wheatgrass), the genetic complexity could justify the variability found in the lines. Recent studies have shown that the allopolyploid formation in triticale and wheat is accompanied by diverse genetic and epigenetic events (Ma et al., 2004; Feldman & Levy 2005; Ma & Gustafson, 2006, 2008).

We postulate that the origin of the abnormalities in the meiotic behaviour and the shrivelling and weight of the kernels observed in the tricepiro lines studied are not related to the heterochromatin content of the rye chromosomes. The analysis carried out in the present work illustrates the importance of cytogenetic evaluation and multidisciplinary studies when analyzing different parameters in breeding programs.

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