ISSN 0373-580 X
Bol. Soc. Argent. Bot. 49 (4): 541-546. 2014

Genome characterization of a synthetic *Triticum* x *Thinopyrum* (Poaceae) amphiploid using in situ hybridization

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Summary: "Trigopiros" derive from crosses between different species of *Triticum* L. and *Thinopyrum* Löve. These synthetic amphiploids are designed with the aim to obtain cereals similar to wheat but which are perennial, resistant to diseases and to the salinity of the soils. Moreover, they allow the transfer of the agronomic attributes of *Thinopyrum* to wheat. "Trigopiro" Don Noé INTA, which is currently grown in Argentina, presents valuable agronomic traits as well as a high content of seed proteins. In the present work, the use of classical cytogenetic techniques allowed us to confirm that the chromosome number of "Trigopiro" Don Noé is 2n=56. The application of *in situ* hybridization (FISH-GISH) allowed us to postulate its genomic composition for the first time. This artificial hybrid has 14 chromosomes from genome J of *Thinopyrum* and 2 chromosomes pairs with putative translocations between *Triticum* and *Thinopyrum*. The rest of chromosomes belong to A, B and D genomes of *Triticum*.

Key words: "Trigopiro", FISH, GISH.

Resumen: Caracterización del genoma de un *Triticum* x *Thinopyrum* (Poaceae) sintético amfiploide utilizando hibridación *in situ.* Los "Trigopiros" derivan de cruzamientos entre diferentes especies de *Triticum* y *Thinopyrum*. El objetivo es obtener cereales con características similares al trigo, perennes, resistentes a enfermedades y a la salinidad de los suelos. Además estos híbridos sintéticos son útiles para transferir al trigo atributos agronómicos de *Thinopyrum*. "Trigopiro" Don Noé INTA, que se cultiva actualmente en Argentina, presenta rasgos agronómicos valiosos, así como un alto contenido de proteínas seminales. En el presente trabajo se confirmó que el número de cromosomas de "Trigopiro" Don Noé es 2n = 56. Técnicas de hibridación *in situ* (FISH-GISH) permitieron postular su composición genómica desconocida hasta el momento. Este híbrido artificial posee 14 cromosomas del genoma J de *Thinopyrum* y 2 pares de cromosomas con posibles translocaciones entre *Triticum* y *Thinopyrum*. El resto de los cromosomas pertenecen a los genomas A, B y D de *Triticum*.

Palabras clave: "Trigopiro", FISH, GISH.

Introduction

The name "Trigopiro" refers to cereals derived from crosses between *Triticum* L. and *Thinopyrum* Löve. These artificial amphiploids are also designated with the Latin names *Agroticum*, *Agrotriticum*, or *Tritipyron* (Covas *et al.,* 1980). They were obtained with the aim to have a cereal similar to wheat but which were, resistant to diseases and to the salinity of soils. Besides, they are currently subject of cytogenetic studies because they may have structural rearrangements which cause variations in valuable agronomic traits (Han *et al.*, 2004; Brasileiro–Vidal *et al*., 2005; Qi *et al*., 2010; Chen *et al*., 2012).

Thinopyrum is a relatively young genus within the tribe Triticeae that includes species with different ploidy levels. According to Dewey (1984), the species *Th. elongatum* (Host) D.R. Dewey, (2n=2x=14) would carry genome E whereas

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Th. bessarabicum (Savul. et Rayss) Á. Löve, (2n=2x=14) would carry genome J. Genomic *in situ* hybridization (GISH) has shown that genomes E and J are closely similar in their repetitive DNA (Kosina & Heslop-Harrison, 1996). Based on molecular cytogenetic studies, Chen *et al*., (1998) suggested that *Th. ponticum* (Podp.) Barkworth et D.R. Dewey, (2n = 10x = 70) would have a basic JJJ^SJ^S genomic constitution and that genome J^S is characterized by having specific sequences of the genome S of *Pseudoroegneria strigosa* (M. Bieb.) Á. Löve, in regions close to the centromere.

In Argentina, SH16 INTA and Don Noé INTA are the "trigopiros" currently used in improvement assays. In previous studies, we have found that SH16 INTA is hexaploid, with 2n=42 chromosomes, 14 of which belong to the J genome of *Thinopyrum* and 28 to wheat genomes. We also found that 14 of the latter belong to genome B, 4 to genome D (chromosome pairs 2D and 4D) and the remaining ones probably to genome A (Fradkin *et al*., 2011).

"Trigopiro" Don Noé INTA comes from an original crossing probably made at the University of California (USA) and then introduced in Argentina by the agronomist Roberto Leiboff about 45 years ago. This material, which was markedly heterogeneous, was selected at the Anguil Experimental Station of the National Institute of Agricultural Technology of Argentina (INTA) and used to obtain the variety Don Alfredo (Covas *et al*. 1978, 1980).

The use of genealogical selection in plants of this variety allowed obtaining a new improved variety with higher forage and grain productivity, characterized by its increased content of seminal proteins.

In this work we discuss the chromosome number and genome composition of this valuable artificial amphiploid with the aim to achieve a more efficient genetic improvement.

Materials and Methods

Plant material: Seeds of the "trigopiro" Don Noé INTA, *Triticum aestivum* L., var. Chinese Spring and *Th. ponticum* were kindly donated by Ing. Agr. G. Covas, Ing. Agr. H. Paccapelo and Prof. Ing. V. Ferreira.

Preparation of cells: Roots (1 cm long) were pretreated at 0°C in water in equilibrium with ice for 36 h, fixed in 3:1 absolute alcohol:glacial acetic acid for 24 h at room temperature, and subsequently kept at -20°C. The processing of the roots to obtain metaphase cells was carried out following Fradkin *et al.* (2011).

Feulgen reaction: The Feulgen staining technique was performed according to Greizerstein *et al.* (1997).

DNA and probes: DNA from *T. aestivum* and *Th. ponticum* and the probes of satellite DNA pSc119.2 and pAs1 were used. Genomic DNA was isolated from adult leaves of *T. aestivum* var. Chinese Spring and from *Th. ponticum* using the Wizard® Genomic DNA purification Kit (Promega), following the manufacturer's instructions with minor modifications. GISH was performed using genomic DNA from *Thinopyrum* labeled with biotin and unlabeled DNA from wheat as blocking DNA (1:60). FISH was performed with the probes pSc119.2 and pAs1. The probes were labeled using the BioNick Labeling System (Invitrogen) following the manufacturer's instructions. All the probes used were revealed with streptavidin Cy3. *In situ* hybridization was performed following Ferrari *et al.* (2005), with minor modifications.

Results

Observations done on mitotic cells stained with the Feulgen Reaction (n=25), revealed a chromosome number 2n = 56 (Fig. 1a).

The use of pSc119.2 allowed us to observe until 28 chromosomes with hybridization signals (n=25). We recognized 18 chromosomes of wheat: 14 of the B genome and the pairs 4A and 5A. The chromosomes 2B, 4B, 5B y 6B showed interstitial signals and the remaining labeled chromosomes presented only terminal signals (Fig. 1 b and c).

FISH experiments, using pAs1, revealed chromosomes of the D genome of wheat and other ones, that based on their small size and the distribution of their hybridization signals, would belong to *Thinopyrum* genome (n=10) (Fig. 1 d).

In the current work, genomic DNA of *Th. ponticum* as a probe and DNA of wheat as a block were applied to mitotic cells (n=10), strong hybridization signals were observed in 14 chromosomes. Two chromosome pairs had an arm

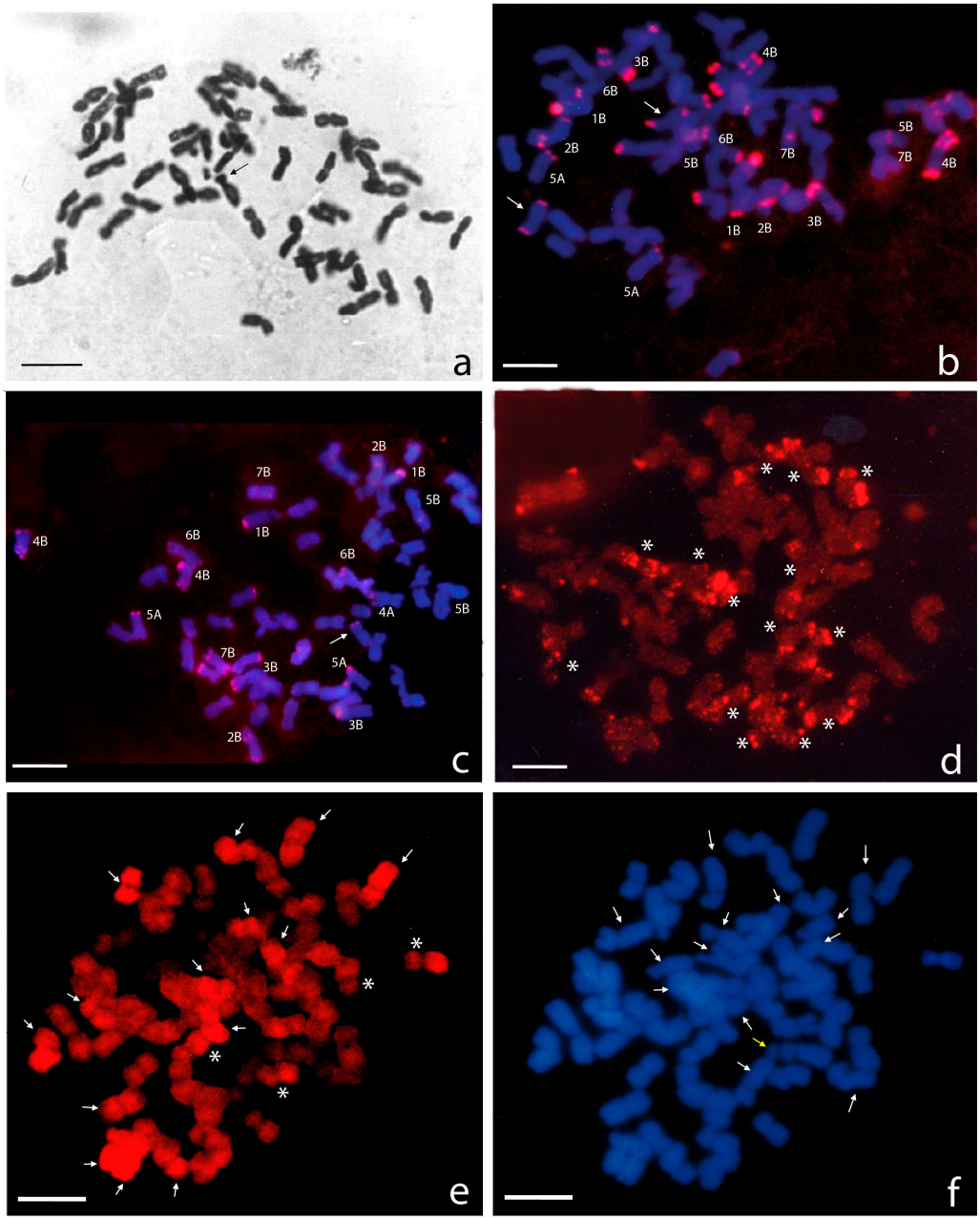

Fig. 1. Mitotic metaphase of "trigopiro" Don Noé INTA. **a:** Feulgen reaction. A total of 56 chromosomes are observed. The arrow indicates the 6B chromosome. **b, c:** FISH with pSc119.2 from *Secale cereale* (red). The photomicrograph shows 14 chromosomes of the B genome of wheat and the pairs 4A and 5A of wheat. The arrows correspond to chromosomes of J genome. **d:** FISH with pAs1 of *Aegilops squarrosa* (red). The asterisks indicate the presence of 14 chromosomes of the wheat D genome. **e:** GISH: the arrows show 14 chromosomes with intense hybridization signal with total genomic DNA from *Thinopyrum ponticum* (red) and the asterisk indicate putative translocation *Triticum- Thinopyrum*. **f:** Same cell as (e), counterstained with DAPI. Arrows show chromosomes with null or almost null hybridization signal with total genomic DNA from *Thinopyrum ponticum*. Yellow arrow points 6B chromosome. All the probes were labeled with biotin and revealed with Cy3. Bars 10 µm.

intensely colored and the other lightly colored. The remaining chromosomes showed light or null coloration (Fig. 1 e). DAPI counterstaining allowed us to observe clearly all the chromosomes in each cell (Fig.1 f).

Discussion

"Trigopiro" Don Noé, a cultivar with a high content of seminal proteins and which is used to obtain improved lines of wheat and synthetic hybrids within the tribe Triticeae (Covas *et al.*, 1980; Ferreira *et al.,* 2007; Ruiz *et al.,* 2007; Castro *et al*., 2011). Thus, it is important to know its genome composition to establish strategies for its use in breeding programs.

The chromosome number determined in the present work for "trigopiro" Don Noé INTA was 2n=56, which confirms the observations made by other authors (Ruiz *et al.,* 2000; Tosso *et al.,* 2000).

FISH experiments using different probes enable to know the genome composition and to recognize chromosomes in diverse species and in natural and artificial hybrids. The probe designated pSc119.2, which contains a 120-bp highly repeated sequence (Bedbrook *et al*., 1980), was isolated from *Secale cereale* and thus allows identifying the rye chromosomes. Besides, it is characterized by the ability to hybridize with various species of the tribe Triticeae (Heslop-Harrison, 2000). This probe identifies the complete B genome, the pairs 4A and 5A and some chromosomes of D genome of *Triticum* (Mukai *et al.,* 1993). Also gives signals in *Thinopyrum* and discriminates between E and J genomes. Hybridization sites are distributed throughout genome E chromosomes and at the terminal regions of the genome J chromosomes (Lapitan *et al.,* 1987; Kosina & Heslop-Harrison 1996; Brasileiro-Vidal *et al.*; 2003, Sepsi *et al.,* 2008).

In the present work, using the probe pSc119.2 in mitotic cells of "trigopiro" Don Noé, we recognized the seven chromosomes pairs of the wheat B genome and the pairs 4A and 5A. The remaining labeled chromosomes showed only terminal signals, hence they would belong to the genomes J of *Thinopyrum* and D of wheat.

For a more complete analysis of the genome composition of "trigopiro" Don Noé INTA, FISH experiments using pAs1 as a probe were made. This probe was isolated from the D genome of *Aegilops squarrosa* auct. non L. = *Ae. tauschii* Coss, (Rayburn & Gill, 1986). In the variety Chinese Spring, *in situ* hybridization with this probe allows discriminating the seven chromosomes of the D genome (Mukai *et al.,* 1993). FISH experiments using this probe reveals chromosomes of the D genome of "trigopiro" Don Noé INTA and other chromosomes that based on their small size and their hibridization signals distribution would belong to *Thinopyrum* genome.

In situ hybridization with genomic DNA (GISH) has been previously used to determine the genome composition of many interspecific hybrids, as well as to detect the presence of introgressions and translocations (Ferrari *et al.*, 2005; Wang *et al.,* 2005; Zheng *et al.,* 2006; Chen *et al.,* 1998; Qi *et al.,* 2010).

In the present work, GISH using *Th. ponticum* as a probe and *T. aestivum* as blocking allowed us to recognize 14 *Thinopyrum* chromosomes with a bright hybridization and two pairs of *Triticum-Thinopyrum* translocated chromosomes. The rest of wheat chromosomes had different intensity of hybridization indicative of different levels of cross hybridization. According to Liu *et al.* (2007) genetic relationships among J *Thinopyrum* genome and the wheat A, B, and D genomes present differences, the J genome is closer to the D genome than to either the A or B genomes.

The presence of two pairs of chromosomes with hybridization signal in only one arm suggest that translocations *Triticum-Thinopyrum* would occurred during the breeding process of "trigopiro" Don Noé. Several authors have described the presence of translocations between wheat and *Thinopyrum* chromosomes (Zhang *et al.,* 1996; Brasileiro-Vidal *et al*., 2005; Oliver *et al*., 2006).

The cytogenetical results obtained in "trigopiro" Don Noé INTA showed important differences from those obtained in "trigopiro" SH16 INTA (Fradkin *et al*., 2011). Both hybrids currently used in breeding programs, present different levels of ploidy and different chromosome and genome composition.

Summarizing, "trigopiro" Don Noé INTA is a synthetic amphiploid with 2n=56 chromosomes that has 14 chromosomes from of genome J of

Thinopyrum, 2 chromosomes pairs have putative translocations between *Triticum* and *Thinopyrum* and the rest of chromosomes belong to A, B and D genomes of *Triticum*.

Acknowledgements

The authors thank Prof. Ing. Agr. H Paccapelo and Prof. Ing V. Ferreira for kindly providing "trigopiro" Don Noé INTA seeds. This research was carried out in Argentina and supported by grants from the "Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)" (PIP 0342), Agencia Nacional de Promoción Científica y Tecnológica (PICT bicentenario 2010-1665) and Universidad de Buenos Aires (UBACyT: 20020100100859).

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Recibido el 21 de marzo de 2014, aceptado el 20 de mayo de 2014.