Volume 56

2013

An NRC Research Press Journal

Une revue de NRC Research Press

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# Genome

# Génome

In cooperation with the Canadian Society for Molecular BioSciences

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NRC Research Press

ARTICLE

# Differentiation of triticale cultivars through FISH karyotyping of their rye chromosomes

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**Abstract:** The aim of this work was to cytogenetically characterize triticale cultivars through fluorescence in situ hybridization (FISH) analysis of their rye chromosomes. In the present work, we studied six cultivars of triticale ('Cayú-UNRC', 'Cumé-UNRC', 'Genú-UNRC', 'Ñinca-UNRC', 'Quiñé-UNRC', and 'Tizné-UNRC'), released by the Universidad Nacional de Río Cuarto (UNRC), Córdoba, Argentina. The cultivars were obtained from the International Center for the Improvement of Maize and Wheat (CIMMYT) and improved for fresh forage, haymaking, and feed grain at UNRC. The distribution and organization of highly repetitive DNA sequences of *Secale cereale* (pSc74, pSc200, pSc250, and pSc119.2) using FISH analyses revealed a specific localization of the signals for several rye chromosomes, which allowed us to distinguish the cultivars. Cluster analysis showed a great cytogenetic similarity among the rye cultivars used to originate these hybrids. The knowledge of the variability among triticale cultivars is necessary to propose future crosses in breeding programs. This study will also be valuable to identify commercial seeds and to analyze the possible association between agronomic characters and the presence of certain rye chromosomes or specific regions in these chromosomes.

*Key words:* cytogenetic similarity, FISH, pSc74, pSc119.2, pSc200, pSc250.

**Résumé :** Le but de ce travail était de réaliser une caractérisation cytogénétique de cultivars du triticale au moyen d'une analyse de leurs chromosomes provenant du seigle par hybridation in situ en fluorescence (FISH). Dans ce travail, les auteurs ont étudié six cultivars de triticale ('Cayú-UNRC', 'Cumé-UNRC', 'Genú-UNRC', 'Ñinca-UNRC', 'Quiña-UNRC', 'Quiñé-UNRC' et 'Tizné-UNRC') développés à l'Universidad Nacional de Rio Cuarto (UNRC), à Córdoba, en Argentine. Tous ont été obtenus de CIMMYT et améliorés pour leur production de fourrage frais, de foin et de grains d'alimentation animale à l'UNRC. Au moyen d'analyses FISH, la répartition et l'organisation de séquences répétitives d'ADN du *Secale cereale* (pSc74, pSc200, pSc250 et pSc119.2) ont été étudiées et ce travail a révélé une localisation spécifique des signaux chez plusieurs chromosomes du seigle, ce qui a permis de distinguer les cultivars. Une analyse de groupement a montré une grande similarité cytogénétique parmi les cultivars de seigle employés pour produire ces hybrides. Une connaissance de la variabilité parmi les cultivars du triticale est également utile pour distinguer les lots de semences commerciales ou pour analyser de possibles associations entre les caractères agronomiques et la présence de certains chromosomes ou de certaines régions spécifiques des chromosomes du seigle. [Traduit par la Rédaction]

*Mots-clés :* similarité cytogénétique, FISH, pSc74, pSc119.2, pSc200, pSc250.

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## Introduction

Triticale can serve as a donor of genes governing tolerance to abiotic and biotic stresses to wheat (Gill et al. 2008); chromosome arms 1RS and 2RL are the most notable in this regard (Graybosch 2001).

The variations in the amount and distribution of heterochromatin have facilitated the identification of rye chromosomes in different triticales (Seal and Bennett 1981). The heterochromatin in the telomeric regions of rye chromosomes could be directly involved in meiotic instability, reduced fertility, seed shrivelling, and somaclonal variation (Soler et al. 1990; Kaltsikes and Bebeli 1992; Bebeli et al. 1993).

In different genera of the tribe Triticeae, repetitive DNA families are essential to identify species. The probe designated pSc119.2, which contains a 120-bp highly repeated sequence (Bedbrook et al. 1980), has proved to be extremely useful to characterize rye heterochromatin (McIntyre et al. 1990). This highly repeated sequence has been reported to predominantly occupy telomeres, as well as some major interstitial sites, in rye chromosomes and to show cross-hybridization with wheat and other Triticeae (Jones and Flavell 1982; Rayburn and Gill 1985). Other highly repetitive sequences, such as pSc74 and pSc200, have been isolated from *Secale cereale*. These sequences are organized as long tandem arrays of 380–382 bp, and they account for 2.5% of the *S. cereale* genome (Bedbrook et al. 1980; Appels et al. 1986; Vershinin et al. 1995). Another sequence, pSc250 (~550 bp), accounts for 1% of the genome of *S. cereale* (Vershinin et al. 1995). Using double-target fluorescence in situ hybridization (FISH), Vershinin et al. (1995, 1996) found that both pSc200 and pSc250 are located together as prominent bands, with a relatively distal location as compared with pSc119.2.

Received 3 August 2012. Accepted 6 May 2013.

Corresponding Editor: J.P. Gustafson.

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 Published at www.nrcresearchpress.com/gen on 8 May 2013.

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From the point of view of improvement, the low genetic variability in rye and triticale crosses is a problem. This situation could lead to future difficulties due to lack of adaptation and stability in the new varieties, especially when the crop is cultivated more extensively (Mellado et al. 2008).

Different cultivars were improved in Argentina, and six of them, which are suitable for grazing, haymaking, and feed grain, are analyzed in the present paper. Some of the characteristics of the cultivars are as follows: 'Quiñé' and 'Tizné' have a short-intermediate life cycle, whereas the other cultivars have an intermediate cycle. 'Cayú' and 'Tizné' have good diseases resistance as well as cold hardiness and drought stress tolerance. 'Ñinca' has moderate salinity tolerance at germination and emergence. The average dry matter yield varies between 4 and 5 tons per hectare for all cultivars. 'Cumé' provides more than 50% of their total biomass during the first grazing. 'Genú' and 'Tizné' have continuous production during the growing season, while 'Cayú' remains green until the heading. Regarding grain yield, all cultivars have above 2 tons per hectare; 'Quiñé' and 'Cayú' frequently exceed this average.

The aim of this work was to cytogenetically characterize these triticale cultivars through FISH analysis of their rye chromosomes. The knowledge of the variability among triticale cultivars is necessary to propose futures crosses with the lowest possible number of chromosome irregularities so as to be successful in breeding programs.

## Materials and methods

### Plant material

We used six cultivars of triticale released by the Universidad Nacional de Río Cuarto (UNRC), Córdoba, Argentina. The cultivars were obtained from the International Center for the Improvement of Maize and Wheat (CIMMYT) and improved for fresh forage, haymaking, and feed grain at UNRC. According to the information obtained in CIMMYT records, different rye lines could have been used to get the triticale cultivars, but we do not know the certain origin of each one. Their current fancy names and original denomination (in parentheses) are as follows: 'Cayú-UNRC' ('UM Tcl'), 'Cumé-UNRC' ('Kiss-IGA'), 'Genú-UNRC' ('Alabama A&M University'), 'Ñinca-UNRC' ('XII TC'), 'Quiñé-UNRC' ('Bgl-IRA'), and 'Tizné-UNRC' ('Cachirulo-M2A').

### DNA probes and labeling

Total genomic DNA was extracted from young leaves using the Wizard genomic DNA purification kit (Promega).

The probes containing highly repetitive DNA sequences from *S. cereale* were the clones pSc74, pSc119.2, pSc200, and pSc250. The DNA probes pSc74 and pSc119.2 were kindly supplied by A. Cuadrado, Department of Cell Biology and Genetics, University of Alcalá de Henares (Spain).

Polymerase chain reaction (PCR) was used to obtain the pSc200 and pSc250 probes. Two pairs of primers were designed to amplify these regions from their published sequences: (*i*) pSc200, primer 1: 5′-GAGTCTCGATCAATTTCGG-3′ and primer 2: 5′-GCAAGTGAG GAGACAAGC-3′; (*ii*) pSc250, primer 1: 5′-GTTCGAAAATAATGG GCC-3′ and primer 2: 5′-CCAACCACTAAATCATTCG-3′.

PCR was performed with total genomic DNA under the following conditions: 5 min at 94 °C; followed by 30 cycles of 30 s at 94 °C, 30 s at 56 °C, and 90 s at 72 °C; with a final extension of 5 min at 72 °C (Vershinin et al. 1996).

All the probes were labeled with digoxigenin DIG High Prime (ROCHE) or biotin nick-translation kit (Invitrogen). To detect digoxigenin-labelled probes, slides were treated with sheep antidigoxigenin FITC (fluorescein isothyocyanate) (green); for biotin-labelled probes, they were treated with conjugate streptavidine–Cy3 (red).

### In situ hybridization

FISH was performed in mitotic cells according to Ferrari et al. (2005) with minor modifications. Briefly, 1 cm long roots were pretreated in ice-cold water for 48 h and fixed in 3:1 absolute alcohol – acetic acid for 24 h at room temperature. Fixed roots were washed in 0.01 mol/L citric acid – sodium citrate, pH 4.6 buffer (pH 4.6) to remove the fixative, and transferred to an enzyme solution containing 2 mL of 2% (*w*/*v*) cellulase Onozuka R10 (Merck) and 20% (*v*/*v*) liquid pectinase (Sigma). The incubation was performed at 37 °C during 3 h, and then the sample was squashed in a drop of 45% acetic acid. Slides were selected by phase-contrast light microscopy. After removal of coverslips by freezing, the slides were air dried. Slide preparations were incubated in 100 mg/mL DNase-free RNase in 2× SSC (100 μg /mL) for 1 h at 37 °C in a humid chamber and then washed three times in 2× SSC for 5 min at room temperature. The slides were post-fixed in freshly prepared 4% (*w*/*v*) paraformaldehyde in water for 10 min, washed three times in 2× SSC for 5 min, dehydrated in a graded ethanol series, and air dried. The hybridization mixture consisted of 50% *w*/*w* deionized formamide, 10% *w*/*v* dextran sulphate, 0.1% *w*/*v* SDS, and salmon sperm DNA 0.3 ng /mL in 2× SSC. A volume containing 50 ng of the labeled probe was then added to 30 μL of this hybridization mixture for each slide. The hybridization mixture was denatured for 15 min at 75 °C, loaded onto the slide preparation, and covered with a plastic coverslip. The slides were placed on a thermocycler for 7 min at 75 °C (denaturation), 10 min at 45 °C, and 10 min at 38 °C, and subsequently incubated overnight at 37 °C to allow hybridization. Following this hybridization step, coverslips were carefully floated off by placing the slides in 2× SSC for 3 min at 42 °C. The slides were then given a stringent wash in 20% formamide (*v*/*v*) in 0.1× SSC for 10 min at 42 °C. The slides were washed in 0.1× SSC for 5 min at 42 °C, followed by 2× SSC for 5 min at 42 °C, transferred to detection buffer (4× SSC/0.2% *v*/*v* Tween 20) for 5 min at 42 °C, and finally treated in detection buffer for 1 h at room temperature. To detect digoxigenin-labeled probes, slides were treated with sheep antidigoxigenin FITC (fluorescein isothyocyanate) (green), whereas to detect biotin-labeled probes, slides were treated with streptavidin–Cy3 conjugate (red). Slides were treated with 2.5% *w*/*v* bovine serum albumin (BSA) in detection buffer, incubated in a solution of 1/20 of the corresponding antibody in detection buffer containing 2.5% *w*/*v* BSA for 1 h at 37 °C, and washed three times in detection buffer for 10 min at room temperature. Slides were counterstained with 1 mg/mL 4′, 6-diamidino-2-phenylindole (DAPI) in distilled water for 15 min at room temperature and then mounted in antifade solution (Vector Labs). A minimum of 85 cells were analyzed for each cultivar of triticale. Slides were examined with a Leica DMLB epifluorescence microscope. Photographs were taken using the IM50 (version 4.0) software (Leica Microsystem, Cambridge, UK). Images were analyzed using Adobe Photoshop 7.0 software.

### Numerical taxonomy

The NTSyS PC (version 2.2f) software (Rohlf 2002) was used to establish relations of similarity between the six triticale cultivars. We built a matrix with 46 characters that corresponded to all the signals observed on the rye chromosomes from the six cultivars with the probes pSc74, pSc200, and pSc250. Each character had two alternatives: presence of the signal (1) and absence of the signal (0). The previous matrix was reduced to 30 signals to implement the NTSyS program (signals without variation between cultivars were not considered in the NTSyS analysis). We applied the Jaccard coefficient of association and the cultivars were grouped with the UPGMA clustering method (Sneath and Sokal 1973; Crisci and Lopez Armengol 1983).

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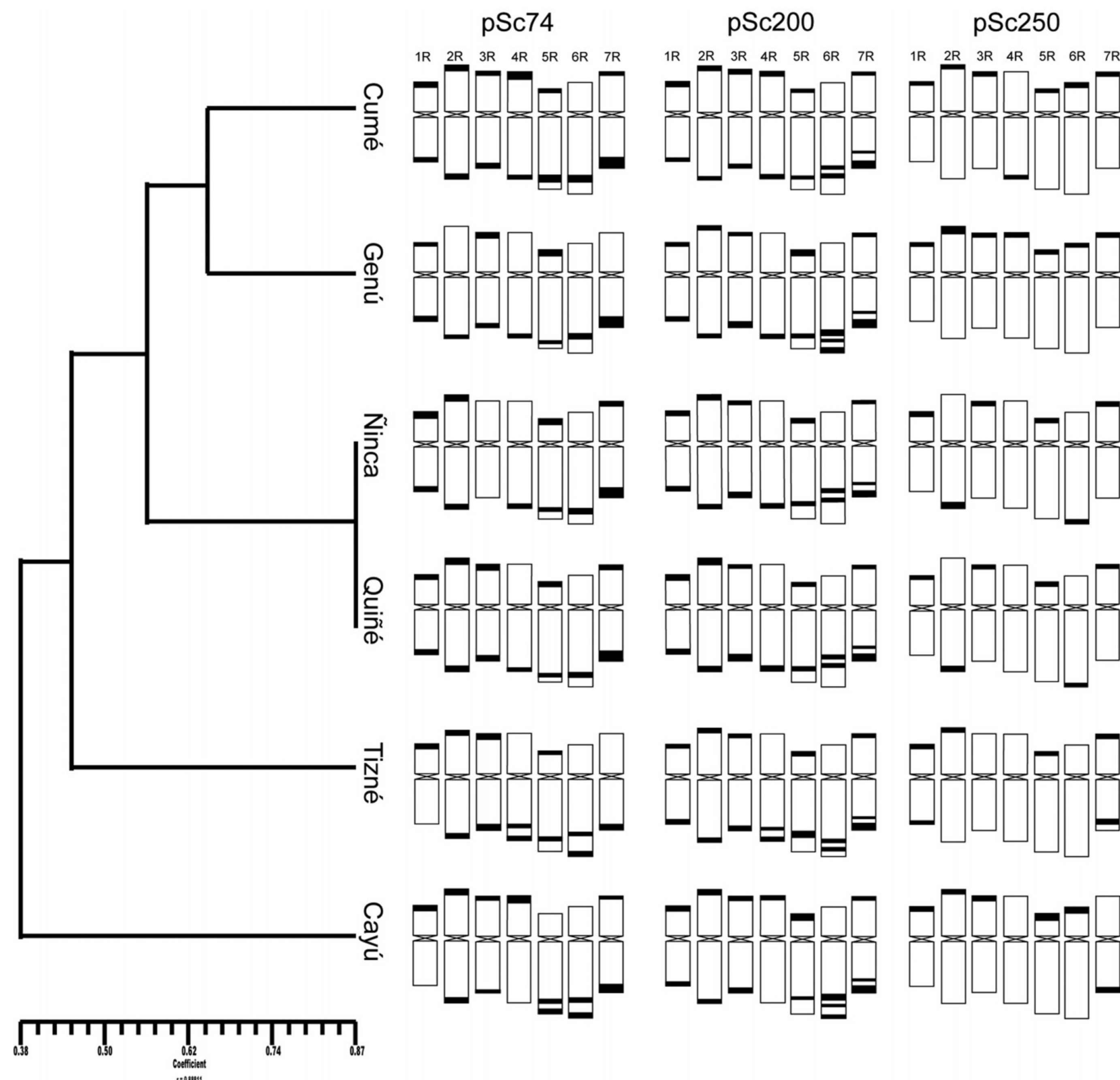
**Fig. 1.** Phenogram of six triticale cultivars based on UPGMA cluster analysis of association coefficients on 30 hybridization signals obtained with the pSc74, pSc200, and pSc250 probes. The cophenetic correlation coefficient was $r = 0.88811$. The idiogram based on the pSc74, pSc200, and pSc250 probes provided the informative patterns of FISH signals to compare the six triticale cultivars.




## Results

### In situ hybridization analysis

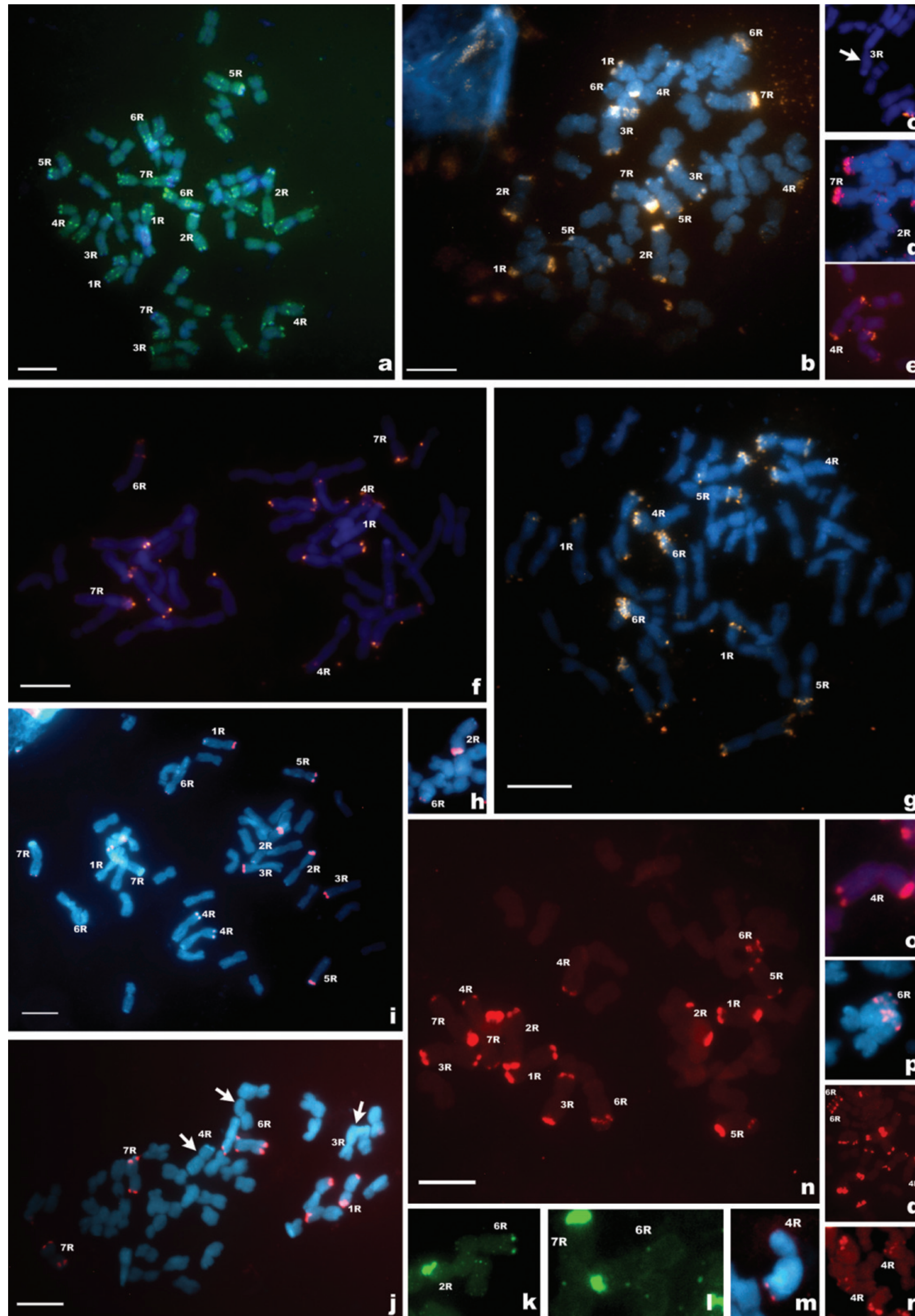
In situ hybridization carried out with the repetitive sequence families of pSc119.2, pSc74, pSc200, and pSc250 allowed the unambiguous identification of the individual rye chromosomes present in the six Argentinean triticale cultivars and indubitable discrimination between these cultivars. The idiograms presented in Fig. 1 show the hybridization patterns of the three last probes.

The hybridization pattern of the pSc119.2 probe, which was the same in the six cultivars, identified the seven pairs of rye chromosomes (Fig. 2*a*). Hybridization with this probe was also observed in the chromosomes of the B genome of wheat and in some chromosomes of the A genome.

Since the pSc74, pSc250, and pSc200 probes presented no signal in the wheat chromosomes, all the signals detected with these probes correspond only to the rye chromosomes. Hybridization signals with the pSc74, pSc250, and pSc200 probes are shown in Figs. 2*b*, 2*i*, and 2*n*, respectively. The patterns of these cells are the most frequently observed in the cultivars analyzed, while Figs. 2*c*–2*h*, 2*j*–2*m*, and 2*o*–2*r* show some chromosomes or sets of chromosomes that differed from the most common patterns.

The cells were in all cases counterstained with DAPI, which allowed the observation of the morphology of the chromosomes

**Fig. 2.** Photomicrographs showing the distribution of several repetitive DNA sequences of *Secale cereale* on metaphase chromosomes of six triticale cultivars. (*a*) FISH using pSc119.2 probe (triticale 'Cumé') allowed the identification of the 14 rye chromosomes. (*b*, *i*, and *n*) More characteristic pattern of hybridization signal distribution observed in the six triticale cultivars using probes (*b*) pSc74 ('Quiñé'), (*i*) pSc250 ('Genú'), and (*n*) pSc200 ('Ñinca'). (*c*–*g*) pSc74 probe showing some chromosomes that are characteristic of each cultivar: (*c*) the arrow shows the 3R pair of the triticale 'Ñinca', which does not present hybridization signal with this probe; (*d*) 2R and 7R pairs of 'Genú'; (*e*) 4R pair of 'Cumé'; (*f*) 1R, 4R, and 7R pairs of 'Tizné'; and (*g*) 1R, 4R, 5R, and 6R pairs of 'Cayú'. (*h*–*m*) pSc250 probe showing some variations in the distribution of FISH signals that differ with the most typical distribution observed in most of the cultivars for this probe: (*h*) 2R and 6R pairs of 'Quiñé'; (*j*) The arrows show that the 3R, 4R, and 6R pairs do not have hybridization signal of 'Tizné'; (*k*) 2R and 6R pairs of 'Ñinca'; (*l*) 7R pair of 'Cayú'; and (*m*) 1R pair of 'Cumé'. (*o*–*r*) pSc200 probe: (*o*) 4R pair of 'Cumé'; (*p*) 6R pair of 'Genú'; (*q*) 4R, 5R, and 6R pairs of 'Cayú'; and (*r*) 4R pair of 'Tizné'. All the cells, except figures *k*, *l*, *n*, *q*, and *r*, were counterstained with DAPI. Figures (*a*) and (*b*) have 42 chromosomes, while (*i*) and (*n*) are incomplete and show 41 and 40 chromosomes, respectively. Scale bars = 10 μm.



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and contributed to their recognition. The simultaneous use of the probes allowed the unequivocal identification of all the rye chromosomes.

While the pSc119.2 probe did not allow the distinguishing of the six cultivars analyzed, the simultaneous use of this probe with the remaining three allowed the identification of all the rye chromosomes, obtaining patterns characteristic of each cultivar. Even 'Ñinca' and 'Quiñé' were distinguished with the pSc74 probe, even though they had the lowest taxonomic distance in the phenogram shown in Fig. 1 and hybridization signals identical with the pSc119.2, pSc250, and pSc200 probes. This is because the 3R chromosome in the 'Ñinca' cultivar presented no bands, while 'Quiñé' presented telomeric bands in both arms, similar to the other cultivars.

The six triticale cultivars showed different hybridization pattern in the chromosomes of rye, showing between two and four different patterns for each chromosome (Fig. 1).

### pSc74 probe

The 1R pair of 'Cumé','Genú','Ñincá', and 'Quiñé' showed hybridization signals in the telomeric region of both chromosome arms (Figs. 1 and 2*b*), whereas that of 'Tizné' and 'Cayú' showed only one signal in the short arm (Figs. 1, 2*f*, and 2*g*).

The 2R pair of 'Genú' presented signal only in the telomeric region of the long arm (Figs. 1 and 2*d*), whereas that of the rest of the cultivars presented hybridization signal in both arms.

The 3R pair of 'Ñinca' did not present any hybridization signal (Figs. 1 and 2*c*), whereas all the other cultivars showed one signal in the telomeric region of both arms.

The 4R pair was the chromosome that showed the highest variability between cultivars. 'Cayú' showed one hybridization signal in the telomeric region of the short arm (Figs. 1 and 2*g*), whereas 'Tizné' showed two signals (a telomeric and a subtelomeric one), both in the long arm (Figs. 1 and 2*f*). 'Cumé' presented one telomeric hybridization signal in each arm (Figs. 1 and 2*e*) and the other three cultivars only one signal in the telomeric region of the long arm (Figs. 1 and 2*b*).

The 5R pair showed a telomeric signal in the short arm and a subtelomeric signal in the long arm in all the cultivars (Figs. 1 and 2*b*), except in 'Cayú', which exhibited a telomeric and a subtelomeric signal, both in the long arm (Figs. 1 and 2*g*).

The 6R pair of 'Tizné' and 'Cayú' showed hybridization signal in the telomeric and subtelomeric region in the long arm (Figs. 1, 2*f*, and 2*g*), whereas that of the other four cultivars showed only one signal in the subtelomeric region of the long arm.

The 7R pair showed hybridization signal in the telomeric region in both arms in Cayú', 'Cumé', 'Ñinca', and 'Quiñé' (Figs. 1 and 2*b*). However, 'Genú' and 'Tizné' have hybridization signals only in the telomeric region of the long arm (Figs. 1 and 2*f*).

### pSc200 probe

The 1R, 2R, 3R, 5R, and 7R pairs showed identical patterns of hybridization in all the cultivars (Figs. 1 and 2*n*).

The 4R pair showed differences in the number and distribution of the signals between the cultivars studied. 'Genú', 'Ñinca', and 'Quiñé' presented only one signal in the telomeric region of the long arm (Fig. 2*n*), whereas 'Tizné' presented two signals in the long arm (a telomeric and a subtelomeric one) (Fig. 2*r*) and 'Cumé presented signals in the telomeric region of both arms (Figs. 1 and 2*o*).

The 6R pair showed differences between cultivars in number and distribution of the signals. 'Cayú' and 'Genú' showed three signals in the long arm: one telomeric and two subtelomeric ones (Figs. 1, 2*p*, and 2*q*), while the other four cultivars had two subtelomeric signals in the same arm (Figs. 1 and 2*n*).

### pSc250 probe

The 1R pair of all the cultivars presented only one signal in the telomeric region of the short arm (Figs. 1 and 2*i*). 'Tizné' also had a second signal in the telomeric region of the long arm (Figs. 1 and 2*j*).

The 2R pair of all the cultivars had only one signal. 'Ñinca' and 'Quiñé' had the signal in the telomeric region of the long arm (Figs. 1, 2*h*, and 2*k*), while the rest of the cultivars had the signal in the telomeric region of the short arm (Figs. 1 and 2*i*),

The 3R pair of 'Tizné' did not present any hybridization signal (Figs. 1 and 2*j*), while all the other cultivars showed one signal in the telomeric region of the short arm (Figs. 1 and 2*i*).

The 4R pair did not show hybridization signal in most of the cultivars, except in 'Cumé', which presented signal in the telomeric region of the long arm (Figs. 1 and 2*m*), and in 'Genú', which presented signal in the telomeric region of the short arm (Figs. 1 and 2*i*).

The 5R pair of all the cultivars showed one hybridization signal in the telomeric region in the short arm (Figs. 1 and 2*i*).

The 6R pair of 'Ñinca' and 'Quiñé' showed signal in the telomeric region of the long arm (Figs. 1, 2*h*, and 2*k*), whereas that of 'Tizné' did not show any signal along the entire chromosome (Figs. 1 and 2*j*) and that of the remaining three cultivars had only one signal in the telomeric region of the short arm (Figs. 1, 2*i*, and 2*l*).

The 7R pair of most of the cultivars presented only one hybridization signal in the telomeric region of the short arm (Figs. 1 and 2*i*). 'Tizné' had an additional signal in the subtelomeric region of the long arm (Figs. 1 and 2*j*), and 'Cayú' had only one signal in the telomeric region of the long arm (Figs. 1 and 2*l*).

### Numerical taxonomic analysis

The numerical analysis of the signals obtained with the in situ hybridization techniques using the pSc74, pSc200, and pSc250 probes allowed the construction of a phenogram for the six Argentinean cultivars (Fig. 1). The phenogram clearly showed the formation of a group between 'Ñinca' and 'Quiñé' cultivars, with the highest value of association. A second group was composed of 'Genú' and 'Cumé', whereas 'Tizné' and 'Cayú' showed a much lower association coefficient.

## Discussion

The cultivars analyzed in this work were improved for fresh forage, haymaking, and feed grain. They are characterized by their quick growth after grazing, good resistance to diseases, tolerance to frost and drought, and ability to maintain the forage quality until advanced stages of the crop cycle. These cultivars have differences in patterns of electrophoretic seminal gliadines, agronomic characters, and morpho-phenological traits (Gertie et al. 2004), and the in situ hybridization techniques also revealed cytogenetic differences between them.

In some wheat chromosomes, hybridization signals were observed with pSc119.2. Since these signals showed differences for the B genome of wheat, these data (not analyzed in the present work) will be useful in further characterization of these cultivars. Besides, the wheat chromosomes showed no hybridization signal with pSc74, pSc200, and pSc250 probes.

The pSc119.2 probe allowed the identification of the seven rye chromosome pairs, although no differences were found in the hybridization pattern of the triticale cultivars. Cuadrado et al. (1995) analyzed several lines of rye with this probe and determined that the pattern was relatively stable.

On the other hand, the rye chromosomes showed variations in the distribution of the repetitive sequences present in the six triticale cultivars when analyzed with pSc74, pSc200, and pSc250. The hybridization signals obtained with pSc74 were localized in the telomeric and subtelomeric regions, and they showed variability between cultivars.

As indicated in the results section, pSc200 showed differences between some cultivars for the 4R and 6R pairs. The signal distribution pattern of pSc200 allowed easy recognition of the 5R, 6R, and 7R chromosomes. However, signals presented in 5R and 6R pairs differ from those obtained in other lines (Alkhimova et al. 1999; Schwarzacher 2003).

The pSc250 also showed variability between cultivars, except for the 5R chromosome. Some of the cultivars studied in the present work did not show hybridization signals in the 3R, 4R, and 6R chromosome pairs to pSc250 probe (Fig. 1). Previous studies in other cultivars have shown at least one telomeric signal in all rye chromosomes (Vershinin et al. 1996; Alkhimova et al. 1999; Schwarzacher 2003).

The cluster analysis originated a phenogram that showed that 'Ñinca' and 'Quiñé' cultivars are grouped with the highest value of association, while Cayú have the lowest value of association. Gertie et al. (2004) reported that the phenogram obtained by morpho-phenological traits revealed that "Cayú" has the lowest association level in respect to the rest of the cultivars.

The in situ hybridization with pSc119.2 together with pSc74, pSc200, and pSc250 revealed a specific localization signal for several rye chromosomes. This allowed the cytogenetical distinction of all the triticale cultivars, and also the identification of all the rye chromosomes unambiguously. Besides, in situ hybridization indicated that none of the six cultivars carries translocations.

The variability in the hybridization pattern observed in the different Argentinean triticale cultivars could have originated either from differences in the rye lines that gave rise to triticales or during the diploidization and stabilization process of the hybrids. Ma and Gustafson (2008) suggested that rapid and extensive sequence elimination is a major process involved in the evolution of triticales.

Information presented in this work could be used in future improvement plans involving triticale and other members of the tribe Triticeae. Hybrids between different cultivars would be useful to determine which chromosomes from each parent are either retained or lost, allowing the study of possible association between chromosomes and agronomic characteristics.

Triticales are used in the improvement of wheat, as they serve as donors of chromosomes or segments of chromosomes carrying genes that are valuable to wheat (Tyrka and Chelkowski 2004). The use of molecular cytogenetic techniques, which allowed the identification of translocations and chromosome loss and gain, would be a good technique for wheat improvement. Conventional triticale breeding is time-consuming and takes about 8–12 years. According to the results obtained in the present work, cytogenetic techniques would be important tools to speed up the release of new cultivars by improving selection strategies.

## Acknowledgements

We thank A. Wulff for his critical reading of the manuscript. This research was carried out in Argentina and supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) (PIP 112-200801-00342) and Universidad de Buenos Aires (X178).

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