Wheat–rye chromosome translocations involving small terminal and intercalary rye chromosome segments in the Portuguese wheat landrace Barbela

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The old Portuguese wheat landrace aggregate, known as Barbela, shows good productivity under low fertility conditions often associated with acid soils. Using genomic *in situ* hybridization with rye DNA, we were able to show that Barbela wheat lines contain small, spontaneously occurring, rye chromosome segments representing up to 5 per cent of a chromosome. Two independent accessions were studied which included terminal rye segments, and a further accession had one chromosome pair with a terminal segment and a second pair with an intercalary chromosome segment (3.5 per cent) of rye origin. Small alien chromosome segments are valuable in cereal breeding for transfer of useful characteristics into wheat without deleterious characteristics from the alien. These results show that such translocations may occur spontaneously and be of such high agronomic value that they are selected by farmers as landraces.

Keywords: biodiversity, *in situ* hybridization, landraces, plant breeding, rye, wheat.

Introduction

‘Barbela’ wheat (*Triticum aestivum* L. em Thell.) is an aggregate of old Portuguese landraces that has been discussed in the literature for more than a century (Lapa, 1865; Coutinho, 1884). The Barbela wheat population is variable and several morphological variants (erect, semi-erect and prostrate) are recognized, as well as a subrace with a characteristic large kernel, Barbela ‘Grosso’ or Tick Barbela (Vasconcelos, 1933; Monteiro, 1935). Barbela landraces show impressively wide adaptation to different environments. The landrace produced a worthwhile crop in low fertility conditions, often associated with acid soils—where it was only exceeded in yield by the regional rye populations—as well as in good fertile situations, although it often lodged (Guedes-Pinto *et al.*, 1982; Bernard & Guedes-Pinto, 1983). In the 1930s, Barbela was the most cultivated wheat in

Portugal (Monteiro, 1935; Castilho, 1954) showing winter hardiness in the northern, inner region and drought tolerance in the semi-arid and hot regions of the south. Now Barbela is out of the national catalogue of varieties but a programme to recover and characterize the subraces is being undertaken at the University of Trás-os-Montes and Alto Douro (UTAD).

Analysis of the genotype × environment interaction for agronomic data (Guedes-Pinto *et al.*, 1982; Bernard & Guedes-Pinto, 1983) shows that Barbela has a pattern like the regional rye populations. The similarity could be a response of two different species submitted to the same edaphoclimatic selection pressure genetically evolving similar adaptations (Marchenay & Lagarde, 1987), or indicate that there is spontaneous introgression of rye germplasm into the wheat. In particular, its agronomic behaviour and aluminium tolerance studies in nutritive solution suggested that Barbela lines could carry a rye chromosomal translocation (Silva *et al.*, 1991), and the fact that Barbela is frequently culti-

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vated with regional ryes, gives the opportunity for introgression.

Genomic *in situ* hybridization is a sensitive and accurate method that has been used to identify various alien chromosomes and chromosome segments in wheat (Schwarzacher *et al.*, 1992). In particular the method is effective in identifying rye chromosome arms in wheat (Heslop-Harrison *et al.*, 1991). Because of the unknown number, size and genetic composition of rye segments potentially present in the landrace, *in situ* hybridization was considered to be an efficient method to search for rye chromatin introgression, in preference to methods using molecular markers that would need many tests per line. Results from *in situ* hybridization can be informative about the size and chromosome location of any rye chromosomes or chromosome segments detected. In this study we searched for rye segments in the Barbela chromosomes using genomic *in situ* hybridization with rye DNA as a probe and 18S-25S rDNA as a second probe to assist chromosome identification.

Materials and methods

Plant material

Lines for screening by *in situ* hybridization were selected by their agronomic behaviour from one hundred lines each coming from a single spike collected from separate fields or localities in the northern, inner region of Portugal under 'Project Barbela' (PDRITM no. 2; Fig. 1). Seeds used in the investigation here were from the second generation

after collection. Code numbers of lines are given in the results (in the format: individual-ear-code/collection-locality-code; collection locations are shown in Fig. 1 and detailed locality codes are available from Project Barbela, Department of Genetics and Biotechnology, UTAD). In each experiment, a triticale (cv. Lasko), rye (cv. Halo) or wheat variety carrying a 1B-1R translocation (cv. Beaver) was used as a control for the *in situ* hybridization. *Secale cereale* L. (rye) cv. Petkus was used for probe preparation.

Preparation of metaphases for *in situ* hybridization

The seeds were germinated in distilled water on filter paper in Petri dishes at 25°C. When the root-tips were one or 2 cm long, they were excised and transferred to ice water at 0°C for 24 h and finally fixed in 3:1 (v/v) 100 per cent ethanol:acetic acid. Root-tip spread preparations were made as described by Schwarzacher *et al.* (1994) and slides pretreated with RNase (100 µg/mL) for one hour at 37°C. The method was optimized to give extended, prometaphase-like chromosomes, rather than highly condensed metaphase chromosomes so that the size and location of any rye chromosome segments detected in the Barbela lines could be measured with high resolution.

Probe and blocking DNA preparation

Total genomic DNA from rye was isolated using standard methods (Sambrook *et al.*, 1990), sonicated

Fig. 1 A map of the north-east of Portugal showing the origin of the Barbela wheat lines used for analysis. 1, Line 34/66, Pinedo, Vimioso–Bragança; 2, 56/92, Vale da Carvalho, Silva–Miranda do Douro; 3, 16/95, Agual Vivas, Miranda do Douro; 4, 39/114, Rio Frio–Bragança; 5, 38/119, Izeda–Bragança.

to produce 10–12 kb fragments and labelled with digoxigenin-11-dUTP (Boehringer) by nick translation for use as a probe for *in situ* hybridization. Total genomic DNA from *T. aestivum* cv. Chinese Spring was fragmented to pieces 50–100 bp long by autoclaving and used as blocking DNA (Heslop-Harrison *et al.*, 1988, 1990; Anamthawat-Jónsson *et al.*, 1990).

The ribosomal rDNA sequence pTa71 contains a 9 kb *EcoRI* fragment of rDNA isolated from wheat *T. aestivum* L. em. Thell. (Gerlach & Bedbrook, 1979) recloned into pUC19; it contains coding sequences for the 18S, 5.8S and 25S rRNA genes and the intergenic spacer sequences (the rDNA, located at the nucleolar organizing regions, NORs, on the chromosomes). pTa71 was labelled with tetramethyl rhodamine isothiocyanate (TRITC)-coupled dUTP (Amersham, Fluorored) by nick translation.

In situ hybridization and probe detection protocols followed Schwarzacher *et al.* (1994) with minor modifications. The probe concentrations (ng/slide) used in the hybridization mixture were: 75 ng of total genomic DNA from rye, 75 ng of pTa71 and blocking DNA at 35 times the amount of genomic probe DNA. The hybridization mixture also contained 50 per cent formamide, 1 × SSC (20 × stock: 3 M NaCl, 0.3 M Na citrate), 20 per cent (w/v) dextran sulphate, 3.75 per cent salmon sperm DNA and 0.25 per cent (w/v) sodium dodecyl sulphate (SDS). The hybridization mixture was denatured at 70°C for 10 min, chilled on ice for 5 min and 40 µL was applied to the chromosome preparation. After covering with a plastic coverslip, slides were denatured at 70°C for 5 min and hybridization was carried out overnight at 37°C in a humid chamber (Heslop-Harrison *et al.*, 1991), followed by stringent washes in 20 per cent (v/v) formamide in 0.1 × SSC at 42°C for 10 min. Final stringency was typically 80–85 per cent, based on the formula of Meinkoth & Wahl (1984) for DNA hybrids of more than 50 nucleotides in solution, where $T_m = (0.41 (\% \text{ GC}) - 500/n - 0.61 (\% \text{ formamide} + 16.6 \log I + 81.5))^\circ\text{C}$ (T_m = dissociation temperature; I = ionic strength, mol/L; GC = base composition, taken as 55 per cent for cereals; and n = number of nucleotides in the probe). Experimental modulation of stringent wash conditions following *in situ* hybridization to chromosome preparations indicates that calculated hybridization stringency is correct (Schmidt & Heslop-Harrison, 1996).

For detection of sites of probe hybridization, we used sheep anti-digoxigenin conjugated to fluorescein (anti-digoxigenin-FITC; Boehringer) for digox-

igenin-labelled probes and streptavidin-Cy3 (Sigma) for the biotin-labelled probes. Slides were transferred into detection buffer (4 × SSC, 0.2 per cent (v/v) Tween-20) for 5 min, treated with 5 per cent (w/v) bovine serum albumin (BSA) in detection buffer for 5 min, and then incubated in 5 mg mL⁻¹ CY3-labelled streptavidin and 20 mg mL⁻¹ anti-digoxigenin-fluorescein in detection buffer containing 5 per cent (w/v) BSA for 1 h at 37°C. After incubation the slides were washed in detection buffer three times, 8 min each, at 37°C. Chromosomes were counterstained with 4'-6' diamidino-2-phenylindole (DAPI, 4 mg mL⁻¹). Slides were mounted in Citifluor-Glycerol and analysed on an epifluorescence Leica Aristoplan microscope with appropriate filters.

Photographs were taken with appropriate epifluorescence filters on Fuji Colour 400 Super G colour negative film and digitized using the Kodak Photo CD system. Prints were made using Adobe Photoshop with contrast optimization applied equally to the whole image, and cropping or over-laying as appropriate.

Results

Genomic *in situ* hybridization with rye DNA as a probe was able to detect rye chromosome segments in a wheat background (Figs 2–6).

Controls: triticales, 1B-1R wheats, rye

In situ hybridization to triticales, using the same labelled batches of genomic rye DNA probe as for the Barbela lines, revealed the 14 chromosomes of rye origin by their bright fluorescence, whereas other chromosome arms, of wheat origin, showed only a very weak signal (Fig. 2). DAPI staining of chromosomes from rye also revealed bright terminal bands of heterochromatin, whereas *in situ* hybridization with the same genomic rye probe labelled chromosomes uniformly brightly, with a similar hybridization strength to the terminal regions and the NOR. Careful inspection of prints where the rye chromosomes were greatly overexposed during photography showed some wheat-origin chromosome arms where the labelling level was variable but slightly heavier, and gaps where no label at all was detected. With some batches of probes, the rRNA genes at the major nucleolar organizer regions of wheat origin were labelled and visible as bands; this also occurred on the Barbela metaphases (cf. Figs 3 and 4). Apart from these rDNA sites, no labelling patterns that could be described as bands or punctate sites were

detected under any circumstances on wheat-origin chromosomes. Depending on the batch of probe used, the terminal heterochromatin on rye-origin chromosomes was labelled equally, slightly more weakly or slightly more brightly than the rest of the rye chromosomes.

In situ hybridization to wheats carrying a centromeric 1BL-1RS translocation, revealed the 1R chromosome arm by its bright fluorescence, whereas other chromosome arms showed only very weak signal (see Heslop-Harrison *et al.*, 1991). DAPI staining revealed bright terminal bands on both of the 1R origin chromosome arms, and there were smaller DAPI-bright segments visible in wheat-origin chromosome arms.

Barbela lines 56/92 and 38/119

These two lines showed a small terminal segment on one pair of chromosomes with bright green fluorescence following *in situ* hybridization with rye total genomic as probe, indicating the presence of a wheat-rye translocation (Figs 3 and 4). In both lines, the segment was located on the short arm of a submetacentric chromosome, and occupied about 5 per cent of the chromosome. No clear differences were detected between the lines, although the data presented here are not conclusive. Two major NORs were detected by *in situ* hybridization with the rDNA probe on chromosomes that were identified as wheat chromosomes 1B and 6B. Minor sites, presumably originating from wheat chromosomes 5D and 1A were usually revealed, but under the hybridization and detection (including photographic) conditions used, we would not expect to see smaller NORs as they would be below the threshold for

visualization. No NOR was detected on translocated chromosomes.

Barbela line 39/114

Four chromosomes including labelled chromosome segments were detected. As in lines 38/119 and 56/92, one submetacentric chromosome pair had a small (5 per cent of the chromosome) terminal labelled segment on the short arm (Fig. 5). Notably, the other pair of labelled segments were interstitial, occupied 3.5 per cent of the chromosome and were located 75 per cent of the length of the arm from the centromere on the long arm of a chromosome with unequal arms (arm ratio 1.6; Figs 5 and 6). Results from the rDNA probe showed that no major NOR was present on either translocated chromosome pair.

Barbela lines 16/95 and 34/66

No segments of wheat chromosomes were seen to label with the rye DNA probe, indicating that rye chromosome segments were either absent or too small to detect conclusively with the technique described.

Discussion

The *in situ* hybridization results using rye genomic DNA as a probe show that two of the five Barbela wheat lines examined carefully include a pair of wheat chromosomes with small, terminal, rye-origin chromosome segments, and another line includes chromosomes with both a terminal and intercalary pair of segments.

Fig. 2 A triticales root-tip metaphase (chromosomes stained blue with the fluorochrome DAPI, panel (a) showing 14 chromosomes of rye origin hybridizing strongly with rye genomic DNA probe (detected yellow-green), whereas only weak and uniformly diffuse cross-hybridization is seen to the 28 wheat-origin chromosomes, with no bands. Chromosomes in (a) also show the major sites of rDNA hybridization (red). X indicates stain precipitate.

Fig. 3 A root-tip metaphase from Barbela accession 56/92, with chromosomes stained blue with DAPI (a), showing one pair of chromosomes with terminal rye-origin chromosome segments, labelled yellow-green with genomic rye DNA probe (b) on the short arm of a wheat chromosome (arrows).

Fig. 4 A root-tip metaphase from Barbela accession 38/119, with chromosomes stained blue with DAPI (a), showing one pair of chromosomes with terminal rye-origin chromosome segments in a partial metaphase hybridizing with labelled genomic rye DNA (b; green bands; arrows). The major rDNA sites (red in (a)) are also detected by the rye genomic DNA (green in (b)).

Fig. 5 A root-tip metaphase from Barbela accession 39/114, with chromosomes stained blue with DAPI (a), showing one pair of chromosomes with terminal rye-origin chromosome segments (arrows), and another pair with intercalary rye-origin segments (arrowheads) labelled green with genomic rye DNA probe (b). The major rDNA sites are also shown (red in (a)).

Fig. 6 A partial metaphase from a root-tip of Barbela accession 39/114, with chromosomes stained blue with DAPI (a), showing the two chromosomes with intercalary rye-origin segments (arrowheads) labelled green with genomic rye DNA (b, c). (c) Superimposition of the DAPI and genomic probe *in situ* hybridization results to show the location of the intercalary chromosome segment near the end of the long arm of a wheat-origin chromosome.

Control slides of triticale (Fig. 1) or a 1B-1R wheat variety revealed no small labelled chromosome segments, intercalary or terminal, of the type seen in the Barbela lines. Some repetitive sequences, including, for example, tandemly repeated sequences such as the rDNA and some tandem and dispersed repetitive sequences including retrotransposons, are common between wheat and rye, and could result in some hybridization signal from rye genomic DNA probe on wheat chromosomes. The controls show that only cross-hybridization of rDNA is detectable, and none of the other sequences present in the probe we used revealed any wheat chromosome segments. Furthermore, the lack of detectable hybridization in two of the Barbela lines examined confirms that the hybridization seen in the other three lines is not simply a property of a wheat variety that has not been previously investigated—caused, for example, by molecular amplification of a tandemly repeated DNA sequence that is homologous to a sequence in the rye probe.

No DAPI-positive heterochromatin is visible correlating with the rye chromosome segments in the Barbela lines, in contrast to the 1B-1R controls where terminal DAPI bands are visible on the 1R chromosome arm. Although the translocated terminal segments may arise from the long arms of rye chromosomes which do not carry terminal heterochromatin, it is known that some Portuguese rye varieties do not have detectable C-bands (L. Amado & W. Viegas, personal communication), and similar lines may have provided the source for introgressed chromatin in Barbela. The rye-origin probe would also hybridize to chromosome segments originating from other rye or closely related species, and we cannot rule out introgression of chromosome segments from such a species. Probing of rye genomic DNA to another rye species would be likely to label the chromosomes along their length, with gaps or regions of less hybridization (as with barley and wild *Hordeum* species; Anamthawat-Jónsson *et al.*, 1993) which could not be seen on small introgressed segments as here. However, no wild rye or closely related species occur in the same region as Barbela, whereas fields of rye and Barbela are often adjacent, so, along with the phenotypic indications, rye is likely to be the source of the alien chromosome segments.

Rye chromosome introgression has been reported in numerous wheat cultivars (Zeller, 1973; Lukaszewski, 1990; Javornik *et al.*, 1991). However, the pedigrees of these cultivars indicate that they arise from relatively few original wheat-rye crosses, and include a translocation of the short arm of 1R and

long arm of 1B of wheat (Heslop-Harrison *et al.*, 1991). They have been selected by breeders because of their disease tolerance and other factors coming from the 1RS chromosome arm (Carver & Rayburn, 1994), although undesirable genes, such as those for proteins giving sticky dough, are also transferred. It has been widely suggested that use of smaller translocations would be useful to improve agronomic characters of wheat varieties, while maintaining the yield advantages (VillaReal *et al.*, 1991). Considerable efforts are being put into obtaining wheat-alien recombinant chromosomes with small alien chromosome segments carrying useful characteristics, and few or no linked deleterious genes (Koeberner & Shepherd, 1986). Methods widely used include the nulli-5B or *Ph* mutant which enables pairing and recombination between homologous chromosomes (Miller *et al.*, 1994) or X-irradiation (Mukai *et al.*, 1993), followed by extensive backcrossing. Another wheat variety, Viking, also has a small terminal rye-origin chromosome segment which has been identified by *in situ* hybridization (Schlegel *et al.*, 1993) and by marker loci on chromosome 5 of rye, translocated to chromosome 4 of wheat.

Pairing between alien and wheat chromosomes, even in the presence of the *Ph* gene, has been detected by *in situ* hybridization (Miller *et al.*, 1994). This type of pairing probably gave rise to the recombinants detected here, and it is notable that the segment transferred is small. Whether the intercalary recombinant occurred at a single meiosis or at two meioses is unknown. It is traditional to grow Barbela wheat in mixtures with rye in the northern, inner region of Portugal where hard winters, acid soils and poor fertility conditions are prevalent. It is also known that dry and hot spring and summer conditions are favourable for wheat outcross hybridization. Such conditions are not infrequent in a Mediterranean climate as in Portugal. Spontaneous wheat-rye hybrids are referred to in literature and thousands of hybrids appeared at the Agriculture Experiment Station of Saratov in 1918 (Meister in Müntzing, 1974). Natural introgression of rye germplasm into Barbela could, therefore, occur from spontaneous wheat-rye hybridization followed by backcrosses to Barbela wheat.

It is now well accepted that genes in wheat, rye and other Triticeae cereals are clustered (Heslop-Harrison, 1991; Lukaszewski, 1992; Gill *et al.*, 1993). A high proportion of the genetic length of each chromosome is represented by small physical regions, and it is clear that this reflects the physical distribution of recombination sites along chromosomes. Hence the small rye chromosome segments

in Barbela, if representing gene-rich regions, may include many genes. This could be confirmed by mapping of the genes on the segment, and the lines might provide a valuable resource for cloning rye-origin genes.

In situ hybridization is a labour-intensive screening method, but the results presented show that it is able to give definitive results about the presence and size of alien chromosome segments in unknown lines. The use of molecular markers for this type of study is more difficult as nothing is known about the molecular genetics of either the wheat or rye parent (and hence their RFLP patterns are unknown), nor are there suitable candidate markers for many of the agronomic performance characteristics present in the lines. The different positions and sizes of the rye segments in Barbela chromosomes seem to indicate that more than one wheat-rye hybridization event has happened. Nevertheless, the results show that landraces material may include lines of considerable interest to plant breeders, and emphasize the importance of the assessment and conservation of threatened genetic stocks. The landraces, primitive forms and wild species constitute a rich genetic resource (Tahir & Ketata, 1993) which deserves careful evaluation and preservation for further improvement of wheats.

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