VISUALIZATION OF SECALE CEREALE DNA IN WHEAT GERM PLASM BY GENOMIC IN SITU HYBRIDIZATION

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ABSTRACT
Total genomic DNA from rye was labelled with biotin and used as a probe for in situ hybridization to show the sizes and translocation points of the rye chromosome segments in wheat mutant forms K1 and K2, which carry a translocation between wheat chromosome 1B, and the short arm of rye chromosome 1R (1B/1R). The translocation breakpoints were at or very near to, the centromere. Total genomic rye DNA used as a probe was also able to distinguish 12 rye chromosomes in 6x triticale-MT47 by genomic in situ hybridization. The techniques using genomic probes are useful for detecting, characterizing and following alien chromosomes or chromosome segments by genomic hybridization- GISH.

Introduction
Hexaploid (2n=6x=42) wheats which contain a segment of the rye chromosome 1R translocated onto long arm of the 1B chromosome of wheat (1B/1R) are widely used in agriculture. Alien genetic transfer programs in wheat and cereals have been associated with exploiting the genetic variability of different Criticize relatives (intergeneric hybridization) as well as the variation prevalent amongst the closer wheat relatives (interspecific hybridization) (12). These hybridization methods allow for the incorporation of novel genetic variation into wheat for biotic/abiotic stresses as a consequence of alien DNA exchange mechanisms. For intergeneric introgression the choice of an alien species has tremendous significance and the use of an alien diploid species is a priority of which Secale cereale L., is one option. However, the presence of the translocation is associated with poor bread making quality (17). Because of the possible correlation between the presence of the translocation and increased disease resistance or yield, it is desirable to detect its presence in early breeding lines, when a yield advantage of a few per cent may be statistically undetectable (7).

A wide range of cytogenetically methods has been applied to identify the 1B/1R translocation. The 1R chromosome arm can be distinguished from all other chromosome arms in Giemsa stained root-tip metaphase cells of hexaploid wheats carrying the translocation by the presence of a nucleolar organizing region (NOR) and a telomeric band. However the number of C-bands in cereales are limited. Appels et al., (1) suggested the use of a cloned rye rDNA spacer 2.4 kb long to show the presence of the rye NOR, but this probe gives no information about the size of the translocation. A further method which has been used to identify the presence of the translocation or alien chromosomes in wheats is in situ hybridization. Rayburn and Gill (14) used a cloned, rye specific probe to look at rye chromosomes in triticale, while Islam-Faridi and Mujeeb-Kazi (9) used genomic in situ hybridization for visualization the
presence of rye chromosomes in wheats. Hybridization with total genomic DNA can be used to distinguish chromosomes and chromosome segments originating from different genomes in barley x rye species hybrids (6; 15) and in triticale (wheat x rye) and 1B/1R wheats (10). The genomic probing in situ hybridization potentially allows accurate determination of chromosome break points.

In our study we apply the genomic in situ hybridization technique to detect the rye segments in bread wheat (Triticum aestivum L.) and rye chromosomes in sphaero-coccum mutant form 6x Triticale-MT47. Nonradioactive in situ hybridization using labelled total genomic DNA of the alien species was successfully utilized with the unlabelled blocking DNA of T. aestivum or T. durum (2n=6x=42, AABBDD or 2n=4x=28, AABB). These results are presented together with the details of the procedures that give high resolution of alien genetic materials.

Materials and Methods

Plant materials
The structural mutant forms K1, K2 and MT47sph were produced by EMS treatment (0,5% EMS) of the dry seeds of T. aestivum cv. Kavkaz, which contains reciprocal translocation between 1BL/1RS and 6x MT triticale. K1 was selected in M1 progeny as a heterozygous semi-sterile plant. Homozygotes were found in self-pollinated progeny of this plant (3, 4).

Chromosome preparation
Root tips collected from germinating seeds in petri dishes were pretreated 0,05% colchicine plus α-bromonaphthalene for 3-3,5h and incubated in 3 ethanol: 1 acetic acid. The preparation was made by the ordinary squash method.

Plant DNA preparation and probe labelling
DNA of T. aestivum, K1, MT47 and S. cereale was extracted following standard protocol (8). Prior to use, the DNA was mechanically sheared to 10-20 kb by passing the DNA about 200 times through a 26-gauge, 100μl syringe. DNA of the species to be used for blocking was autoclaved to degrade it into 300- to 1000-bp fragments. Both sheared and autoclaved DNA was stored at 4 °C. Total genomic DNA was labeled by nick-translation using biotin-14-dATP (Gibco, BRL) as the in situ probe. Unincorporated nucleotides were removed by a Sephadex G50 column. The label incorporation was evaluated by means of dot blots using the streptavidin-alkaline phosphatase-detection system (Gibco, BRL).

In situ hybridization, detection, and visualization
The wheat clone pTa71 (5) containing the 18S-5.8S-26S rRNA genes and the intergenic spacer sequence was labeled by nick translation using biotin14-dATP (Gibco BRL). The hybridization procedure was similar to that of Rayburn & Gill (14). Freshly prepared slides were treated with RNase (25 μg/ml) in 2 x SSC for 60 min at 37 °C. After washing with 2 x SSC, the slides were air dried for 30 min. The hybridization mixture contained 50% deionized formamide, 2 x SSC, 10% dextran sulfate and approximately 80 ng probe DNA. The blocking DNA was 15-20 times the amount of the probe DNA. The hybridization mixture (30-40 μl per slide) was denatured for 10 min at 80 °C and than kept on ice for 10 min at 78 °C and than overnight at 37 °C in a humid chamber.

After hybridization, the slides were washed in 2 x SSC twice (5 min each) at RT before detection of the hybridization sites by avidin-conjugated fluorochrome (avidin D, Vector Laboratory). The fluorochrome signal was amplified with biotinylated antiavidin D (Vector Laboratory) followed by another layer of avidin-conjugated fluorochromes (14). After amplification the slides were washed three times in BN detection buffer at 37 °C, drained and mounted in Vectashield Mounting Medium with PI (Vector Laboratory). The slides
were kept at 4 °C for 1-2 days to stabilize the fluorochromes before they were examined with a Nikon epifluorescence microscope.

**Results and Discussion**

The labelled total genomic probe hybridized strongly to the rye chromosome arms in wheat and rye chromosomes of 6x MT sph triticale metaphase cells. The 1RS translocation was visible as a discrete entity throughout the cell cycle (Fig. 1).

No other chromosome arms or chromosomes showed such strong hybridization in any of the mutant forms investigated. The specificity of probing was increased by blocking DNA because the wheat blocking DNA crosshybridized with sequences common to both the wheat and rye genome. In the present work, blocking DNA was used at 15-20 times the probe concentration.

Total genomic DNA of *S. cereale* as a probe enabled identification of complete (with the exception of 2R, data not showed after Giemsa staining) 12 rye chromosomes in MT47sph and wheat/rye chromosome translocation in metaphase cells of K1 (Figs. 1 and 2) or interfase cells (Fig. 3). As you can see from Fig. 2, the unlabelled wheat chromosomes are orange-red while the 12 rye chromosomes are labelled yellow.

In all our samples analyzed the quality of in situ hybridization was highly satisfactory for the diagnosis of alien chromosomes. The same was the results obtained by Islam-Faridi and Mujeeb-Kazi (9) in wheat/rye translocation lines. In our case the technique is enough sensitive and accurate for identifying the presence of alien chromosome segments which represent
tremendous potential for resolving cryptic wheat/alien exchanges both at metaphase and interphase. In our case we don’t use complete metaphases for identification the alien chromosomes in wheat backgrounds. Schwarzacher et al. (15) in their experiments also did not used complete metaphases for identification an array of alien chromosomes in wheat backgrounds. The interphase results are also of exceptional interest. According Schwarzacher et al. (15), Cremer et al. (2), Maluszynska and Heslop-Harrison (11) the cytogenetic analysis of interphase nuclei will be of increasing importance since high quality metaphase preparations require skilled methodology. The most important is the possibility of this technique to investigate the real disposition of different wheat and rye chromosomes (genomes) in interphase nuclei. As you can see from Fig. 3. the disposition of the two wheat and rye genomes are clearly in opposite area of the interphase nuclei. At interphase (Fig. 3) the rye genomes remained discrete with high fluorescence intensities. The present work shows that the 1RS and rye chromosomes tends to remain in an isolated domain within the cereal nucleus. This finding can be use as an accurate diagnostic procedure for screening S. cereale chromosomal sources toward practical agricultural goals. Genome in situ hybridization (GISH) could be use for testing the different genetic stocks that are in various developmental phases.

Conclusions

Genome in situ hybridization (GISH) by using genomic probes are of potentially wider application for detecting alien chromosomes or chromosome segments through breeding programmes. Genomic probing has a unique potential to detect changes in the physical size of an alien chromosome segment.

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REFERENCES