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Detection of Interspecies Hybrids in Plants by Fluorescence in situ Hybridization, Using Total Genomic DNA as a Probe

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Summary

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Total genomic DNA probe preparation and the non-isotopic biotin probe labelling technique are described. The use of an excess of unlabelled competitor DNA from a related plant species gives more constant results in determing whole plant genomes with in situ experiments on plant hybrids. Results of fluorescence in situ hybridization for detecting alien rye chromatin and localisation on metaphase chromosomes and interphase nuclei in the bread wheat cultivar "Yugoslavia" which carries a 1BL.1RS translocation are shown.

Introduction

In situ hybridization has become a powerful diagnostic tool, it is possible to detect single and low copy DNA sequences, perform physical mapping of genes, detect chromosomal aberrations like deletions, translocations, substitutions etc. Repetitive sequences, whole chromosome and whole genome detections (Nakahara & Yamamoto 1993) are also possible with specially prepared and labelled probes. The results of in situ hybridization contributed a new understanding of plant genome organisation to phylogenetic plant studies (LEITCH & al. 1991). The plant

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cell wall can effect the accessibility of a labelled probe to a chromosome and the plant protoplasm can cause a relatively high background in the commonly used squash preparations. AMBROS & al. 1986 attempted to overcome the problem by the use of the protoplasm drop technique and enzymatic cell wall digestion.

Materials and Methods

The in situ hybridization technique was applied to alopoliploid bread wheat *Triticum aestivum* L. em Thell. in search for rye (*Secale cereale* L.) 1BL.1RS translocations in the cultivar (Cv.) Yugoslavia. We used the total genomic rye DNA extracted from young leaves. The technique for fluorescence in situ hybridization (FISH) was after BUSCH & al. 1994.

Plant material

Seeds of bread wheat (*Triticum aestivum* L. em. Thell.) Cv. "Yugoslavia were germinated on moist paper at 22°C in the dark. Roots of seedlings were cut of and stored overnight in ice water. The material was then fixed in ethanol:acetic acid and stored at –20°C until use. Roots were rinsed in tap water and the root tips (2 mm) cut off for digestion in 250 µl of Pectolyase Y-23, Cellulase R 10 in 75 mM KCl and 7,5 mM EDTA (pH 4.0) at 25°C for 55 minutes after PAN & al. 1993. The lysate of 15 root tips was filtered through a 80 µm mesh net. The protoplasts were resuspended in 75 mM KCL and spun down at 80x g for 5 minutes. The pellet was resuspended four times in fixative and spun down again. The protoplasts were finally resuspended in 120 µl of 3:1 fixative and dropped on ice-cold, cleaned slides.

Chromosome preparations

The slides were immersed in 3:1 fixative for 30 min. at room temperature, slides were incubated 5 min. in 2xSSC at room temperature (RT), dried and incubated with 100 µl/ml RNAse A for 60 min. at 37°C in a moist chamber. After removal of coverslips, they were washed twice in 2xSSC at room temperature (5 min each) in 1xPBS at 37°C pH 7.2 for 5 min. and digested for 10 min. in 0.005% Pepsin. They were washed twice in 2x SSC, pH 7.2, for 5 min and then dehydrated in a graded ethanol series (70%, 80%, 90%, 99%) 3 min. each and air dried.

Probe preparation and labelling

For each slide preparation two probes were used: the biotin labelled genomic probe DNA and unlabelled competitor DNA from a related species, that was used at the same time as a carrier DNA. Total rye DNA was extracted from the first leafs of young plants of Cv. "Danko". Competitor wheat DNA was extracted from young leaves of Cv. "Chinese Spring. Total genomic probe and competitor DNA were enzymatical shared using DNase I, the fragment lengths (100-500 bp) were checked by electrophoresis on 1% agarose gel (PHW802 Gibco) with ethidium bromide staining. The shared and Sephadex G–50 column purified genomic probe DNAs were labelled by nick translation (nick translation kit, BLR) with Biotin-14-dATP. The probe was purified on a Sephadex G-50 spin column to remove unincorporated nucleotides and by ethanol precipitation.

Probe and chromosome denaturation

Total genomic probe DNA from different species and competitor DNA were denatured for 15 minutes at 80°C and cooled on ice. The chromosome preparations were denatured in 50% formamide-2xSSC mixture (pH 7.0) at 70°C for 2 minutes. The slides were transferred to graded ice cold 3 min. each and air dried.

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Hybridization of probe and chromosome DNA sequences

For hybridization Biotin-14 dATP labelled probe DNA at a final concentration of 12 ng/µl was used together with unlabelled 360 ng/µl competitor DNA, both dissolved in a hybridization mixture of 50% formamide, 10% dextran sulphate and 2xSSC. Fifteen µl of DNA probe in the hybridization mixture were loaded per slide, sealed and hybridized in a moist chamber at 37°C overnight.

Slide preparation and washing

After hybridization the coverslips were removed in 50% formamide 2xSSC (pH 7.2), at 42°C for 15 minutes in a shaking water bath. The samples were then washed in 50% formamide-2xSSC at 45°C three times for 5 min and 5 min in 2xSSC at 45°C. To the drained slides 180 µl of blocking solution 4xSSC and 3% BSA (fraction V) was added. The slides were covered with a 24x36mm cover slip and incubated for 30 min. at 37°C. The cover slips were taken off, excess fluid drained and 80 µl detection solution was added.

Detection of probe hybridization sites

Signal detection of biotinylated genomic rye probe was performed with the fluorochrome Avidin-Cy3 in the detection mixture at a concentration of 4µg/ml in 80 µl detection solution per slide. After the final washing in 0.05% Tween-20, 4xSSC, pH 7.2, slides were rinsed twice in phosphate-buffered saline (PBS) at room temperature and dehydrated in a graded ethanol series. Chromosomes were counter stained with 0.5 µl/ml DAPI and mounted in an antifade solution.

Visualisation and image analysis

The Zeiss filter set 487909 (Avidin Cy-3), a Zeiss filter set 487901 (DAPI) and a Zeiss-Axioplan epiflurescence microscope, equipped with a cooled CCD camera and digital image analysing system was used for the interpretation of results.

Results and Discussion

The results from fluorescence in situ hybridization in the bread wheat cultivar "Yugoslavia" are presented. Rye probe hybridization sites were detected by the Avidin-Cy3 conjugate which fluoresced red under green light excitation (Zeiss filter-set 487909)) and allowed visualisation of the introgressed rye segment in the bread wheat cultivar "Yugoslavia". Chromosomes were counter stained with DAPI, which fluoresced blue under UV excitation with the Zeiss filter-set 487901. A Zeiss-Axioplan epifluorescence microscope and image analysis system were used for generating signals of probe and counter stained chromosomes. The biotin labelled rye probe segment (red signal with Avidin Cy-3) was restricted to the 1B NOR region only (Fig. 1.A) and was in a homozygous state showing the 1BL.1RS wheat-rye translocation. Fig. 1.B shows a typical distribution of rye signals in the interphase nuclei of the wheat cultivar "Yugoslavia" after FISH technique.

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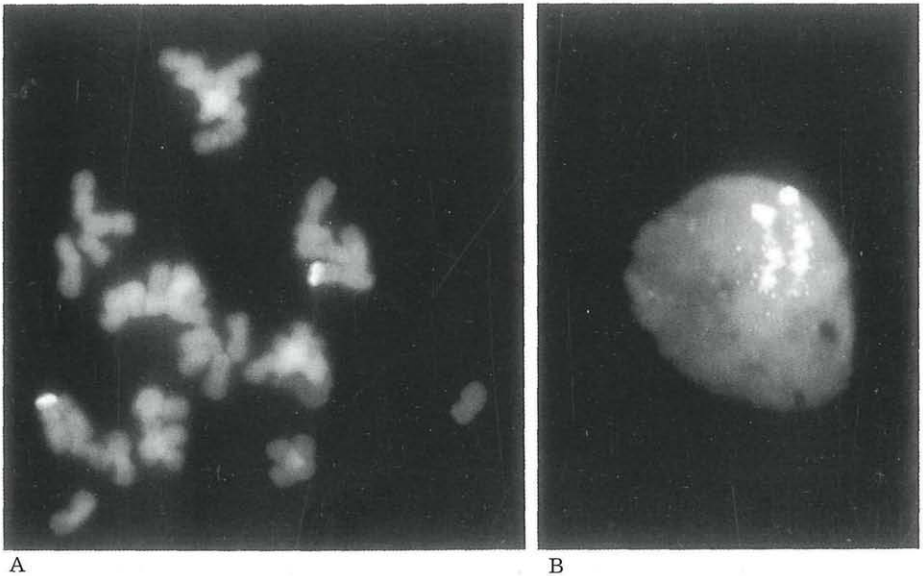

A B

Fig. 1. Hybridization sites of biotin labelled total rye genomic probe in the bread wheat cultivar "Yugoslavia", on metaphase chromosomes (Fig.1.A) and in interphase nuclei (Fig. 1.B), after in situ DNA hybridization.

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