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Reduction of the lenght of 1 RS.1BL translocation in the bread wheat variety "Yugoslavia"

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ABSTRACT

This study was conducted to estimate the survival of 1RS.1BL wheat-rye translocations in a bread wheat (*Triticum aestivum* L. em Thell.) variety "Yugoslavia" including "Aurora" as a donor of translocation in its pedigree. The cultivar was cytologically checked by N-banding for the presence of 1RS.1BL translocation. Fluorescence *in situ* hybridization using total genomic biotin labelled rye DNA as a probe was used for identification and localisation of the translocation breakpoints and their size. At metaphase and interphase the introgressed rye segment was observed and photographed. Digital image analysing system with cooled CCD camera was used for overlapping the pictures of the probe signals and counterstained wheat chromosomes and helped to determine the position and extent breakpoint of this translocation. The translocation in this variety is probably restricted to the NOR region only, unlike in other 1RS.BL translocation lines ("Kavkaz") where the translocation carries at least the major part of the 1RS arm.

Key words: bread wheat, *Triticum aestivum* L. em Thell., N-banding, fluorescence *in situ* hybridization, 1RS.1BL translocation.

IZVLEČEK

ZMANJŠANJE DOLŽINE 1RS.1BL TRANSLOKACIJE PRI KULTIVARJU NAVADNE PŠENICE "YUGOSLAVIA"

Raziskava je skušala ugotoviti preživetje 1RS.1BL translokacije pri kultivarju navadne pšenice "Yugoslavia". Kultivar je imel v rodovniku "Avroro" kot donorja translokacije. Sorta je bila citološko pregledan s pomočjo N-proganja na prisotnost translokacije. Uporabili smo tudi fluorescenčno *in situ* hibridizacijo s celotno genomsko probo rži za natančno opredelitev mesta translokacije in dolžine. V interfazi in metafazi je bil rženi segment na kromosomu opazovan in fotografiran. Slike smo posneli z digitalno CCD kamero in sliki tarče in protibarvanih kromosomov združili z digitalno analizo slike. 1RS.1BL translokacija je bila pri tem kultivarju pšenice omejena na NOR področje kromosoma 1B, z razliko od drugih translokacij („Kavkaz"), kjer je translokacija obsegala vsaj večji del rženega kromosomskega kraka 1RS.

Ključne beside: navadna pšenica, *Triticum aestivum* L. em Thell., N-proganje, flurescenčna *in situ* hibridizacija, 1RS.1BL translokacija

1 INTRODUCTION

Rye chromatin has been successfully incorporated in many wheat varieties all over the world, especially in eastern Europe and Mexico. Even recent surveys show that sometimes more than 45 % of breeding material may contain those translocations (Zhou et al., 2007) or 55 % of CIMMYT bread wheat germplasms. In Hungary 53 % of wheat cultivars registered during the last twenty years carry the 1RS translocation (Hoffmann, 2008). This translocation has been deemed that it has been incorporated into more than 60 wheat varieties, including the prominent Veery spring wheat lines that occupy more than 50 % of all developing country wheat area, almost 40 million hectares. Their most important phenotypic deviation from common wheat cultivars is the so called wheat-rye desease resistance to races of powdery mildew and rusts (Bartos and Banes, 1971; Zeller, 1972). The desease resistance is linked with decreased breadmaking quality (Zeller et al. 1982), good ecological adaptability and yield performance (Rajaram et al., 1983; Schlegel & Meinel,

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1994; Singh et al., 1990). Indirect sources for 1RS.1BL wheat-rye chromosome translocations in Yugoslav breeding programs were the substitution lines Weique and Neuzhücht, which carried chromatin from Petkus rye. The Soviet cultivars Aurora, Kavkaz and Skorospelka 35 were donors of 1RS.1BL translocations in Yugoslav wheat varieties. During the past 20 years (1967-1986) Yugoslav breeding programs released 148 cultivars of bread wheat (Jošt & Cox, 1990).

Chromosome 1R of rye is a usefull source of genes for desease resistance and enhanced agronomic performance in wheat. The genes encoded on the 1RS chromosome arm of rye carry disease resistance genes (Zeller and Hsam, 1983), including powdery mildew (Pm8) (Zeller, 1973; Zeller and Fuchs, 1983; Heun and Fishbeck, 1987), leaf rust (Lr26), stem rust (Sr31) and tolerance to greenbug (Gb) (Sebesta and Wood, 1978).

Novel tertiary wheat-rye recombinant lines were produced carrying different lengths of of rye chromosome arm 1 RS. The larger root biomass of the 1RS translocation lines contributes to an increased drought resistance, combined with less yield decrease.

The bread wheat variety "Yugoslavia" was introduced by the Institute for Crops, Agricultural Faculty, University in Novi Sad in 1980. The crosses of NS646/Bezostaja1/Aurora are involved in the pedigree of "Yugoslavia". The cultivar has good winter cold resistance, good resistance to leaf rust (*Puccinia graminis*), to stem rust (*Puccinia graminis* tritici) and to powdery mildew (*Erysiphe graminis).* The potential yield of the cultivar was more than 10t/ha. In 1988 on 280.000 ha the winter wheat cultivar "Yugoslavia" was grown (18% of wheat grown areas in Yugoslavia). The cultivar is still nowadays in Slovenia.

Fluorescence *in situ* hybridization (FISH) using total genomic rye DNA, together with an access of unlabelled wheat competitor DNA was used for identification, breakpoint and size of the introgresed rye segment in the bread wheat cultivar "Yugoslavia".

FISH has become an increasingly powerfull diagnostic tool, the plant cell wall can effect the accessibility of the probe to a chromosome preparation and plant cellular debris can cause a relative high background in the commonly used squash preparations. Ambros et al. (1986) have attempted to bypass this problem by the use of protoplast drop technique and modified *in situ* hybridization technique (Bush et. Al., 1994; Lichter & Cremer, 1992). The cultivar "Yugoslavia" was also cytological checked with the differential Giemsa N-banding staining technique (Javornik et al. 1991), for the presence of 1RS.1BL translocations and chromosome identification. B-genome chromosomes of common wheat provided significant N-bands, while the alien 1RS arm none. Our thesis on the basis of N-bands and FISH experiments is that in some Yugoslav varieties, these translocations carry less than the whole chromosome arm.

2 MATERIAL AND METHODS

Seeds of bread wheat (*Triticum aestivum* L. em Thell.) cv. "Yugoslavia", were germinated on moist paper at 22°C in the dark. For fluorescence *in situ* hybridization, roots of young seedlings were cut of and stored overnight in ice water, as described in Doležel et al. (1992) & Pan et al. (1993). The material was then fixed in 3:1 (v/v) ethanol:acetic acid and stored at -20ºC untill use. Roots were rinsed in tap water and the root tips (2 mm) cut off for digestion in 250 µl of Pectolyase Y-23 (Kikkoman), Cellulase R 10 (Onozuka) in 75 mM KCl and 7.5 mM EDTA (pH 4.0) at 25°C for 55 minutes after Pan et 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 80 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 fixative and dropped on ice-cold, cleaned slides.

2. 1 Probe preparation:

Total rye DNA was extracted from first leafs of young plants of cv. "Danko". Competitor wheat DNA was extracted from young leaves of cv. "Chinese Spring". Total genomic competitor DNA from "Chinese Spring" and total genomic rye DNA were enzymaticaly shared using DNAse I, the fragment lengths (100-500 bp) were chequed with electrophoresis on 1% agarose gel (PHW802 Gibco) with ethidium bromide staining. The shared and Sephadex G-50 column purified genomic rye DNA, was labelled by nick translation (nick translation kit, BLR) with Biotin-14-dATP. The rye probe was purified by Sephadex G-50 spin column to remove unincorporated nucleotides and by the ethanol precipitation step.

2. 2 *In situ* hybridization and signal detection:

For hybridization 12 ng/µl rye Bio-14 dATP was used together with unlabelled 360ng/µl competitor wheat DNA was dissolved in 50% formamide, 10% dextran sulphate and 2xSSC. Total genomic rye DNA probe and competitor wheat DNA were denatured for 15 minutes at 80°C and cooled on ice.

Slide preparation:
Slides were incubated 5 min. in 2XSSC at room temperature (RT), digested with RNAse (1h at 37°C), washed twice for 5 min in 2XSSC at RT, 5 min in 1xPBS at 37°C, digested for

10 min in 0.005% Pepsin according to Wiegant et al. (1991). They were washed twice in 2 x SSC, pH 7.2 for 5 min and then dehydrated in a graded ethanol series (70%, 80%, 90%, 99%), 2 min each. The preparations were denatured in 50% formamide-2xSSC mixture (pH 7.0) at 70°C for 2 minutes. The slides were transferred to ice cold ethanol (70%, 80%, 90%, 99%) 3 minutes each and air dried.

Fifteen µl of DNA probe were loaded per slide, sealed with a 24x36 mm cover slip and hybridized in a moist chamber at 37° C overnight. 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. On 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.

Signal detection:
Signal detection of biotinylated genomic rye probe was performed according to Pinkel et al. (1988) with Avidin-Cy3 (1µg/ml). After the final washing in 0.05% Tween-20, 4XSSC, pH 7.2, slides were rinsed twice in phosphate-buffered saline at room temperature and dehydrated in graded ethanol series. Chromosomes were counterstained with 0.5 µ L/ml DAPI and mounted in Vector-Shield antifade solution. The Zeiss filter set 487909 (Avidin Cy-3), the Zeiss filter set 487901 (DAPI) and Zeiss-Axioplan epiflurescence microscope, equipped with cooled CCD camera and digital image analysing system was used for the interpretation of results and generation of counterstained chromosome and probe signals.

2. 3 N-banding

For N-banding the root tips were incubated in bromonaftalene for 4h at room temperature, fixed in 3:1 (v/v) ethanol:acetic acid and stored in deep-freeze.

N-banding of mitotic metaphase chromosomes was carried out as described by Gerlach (1977) and improved by the technique of Endo and Gill (1984), which allows the recognition of 16 wheat chromosome pairs out of 21, including the whole B-genome of wheat. Approximately nine root tips from three plants were cytologicaly checked and photographed with Zeiss-photomicroscope at 1000x magnification. Results are based on three spread 1B or 1RS.1BL wheat chromosomes. From the spread chromosomes a typical B-genome karyotype of "Yugoslavia" bread wheat cultivar was prepared, with bands compared to the cultivar "Chinese Spring" (Endo and Gill, 1984). Because of heterochromatin polymorphism among different cultivars, best results were obtained in combining bands with arm ratios. We used the non-occurrence of the telomere band on the short arm of the 1B chromosome as N-band marker for the 1RS.1BL translocation.

3 RESULTS AND DISCUSSION

The recognition of normal and translocated common wheat chromosomes by N-banding is based on the distribution of bands and arm ratios. Normal 1B chromosomes posses strong centromeric bands, telomeric bands on the short, and long arms. They have some interstitial bands on the long arm and are submetacentric (Fig. 1.B).

Translocated 1RS.1BL chromosomes from the cultivar "Yugoslavia" were identified by similar bands on the 1BL arm and similar arm ratio, but no bands on the telomeric part of the 1RS arm (Figure 1. A).

Figure 1. Normal 1B (A) from the cultivar “Dukat” and translocated 1RS.1BL bread wheat chromosome (B) from the cultivar “Yugoslavia”, determined by N-banding (magnification ca. 3000x)

Figure 1. Normal bread wheat B-genome karyotype from the cultivar "Dukat" (Figure 1.A) and translocated 1RS chromosome (Figure 1.B) from the cultivar "Yugoslavia", determined by N-banding (magnification ca. 2000x)

Typical B-genome karyotype from the bread wheat cultivar "Dukat" was prepared from B-genome chromosomes spreads (Figure 2) and the chromosomes were measured.

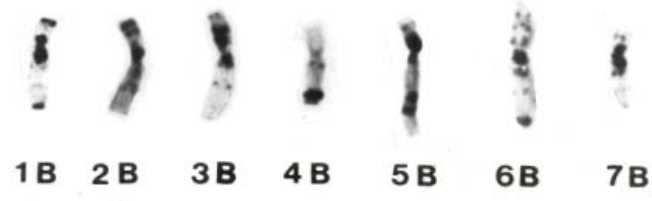

Figure 2. Normal haploid bread wheat B-genome karyotype from the cultivar "Dukat", determined by N-banding (magnification ca. 2000x)

The B-genome karyotype is based on measurements of six B-genome chromosome spreads from different cells. Our measurements are based on 4 hours of monobromonaftalene pretreatment.

The translocated 1RS satellite was identified by the unbanded short arm on the 1B chromosome, and characteristic N-bands on the long arm of this wheat chromosome. We observed an extra pericentromeric band on the 1RS arm as evidence for that this translocation carries less than the whole 1RS arm. The translocation was in a homozygous state.

However the exact location of translocation breakpoint is difficult to pinpoint with banding techniques alone (Cai and Liu, 1989). The coupling with the use of fluorescence *in-situ* DNA hybridization using biotin labelled total genomic rye DNA was needed to confirm categorically the translocation breakpoint, localization and size. The reduced length of the 1RS1BL translocation is probably the reason of relative good bread making quality of the cultivar "Yugoslavia"

Genomic *in situ* DNA hybridization:
Probe hybridization sites were detected by Avidin-Cy3 conjugate with red fluorescence under yellow light excitation (Zeiss filter-set 487915) and allowed visualisation of the introgressed rye segment. Chromosomes were counterstained with DAPI (UV excitation with Zeiss filter-set 487901). A Zeiss-Axioplan epifluorescence microscope and digital image analysis system was used for generating signals of probe and counterstained chromosomes. The biotin labelled rye segment (red signal with Avidin Cy-3 conjugate) was restricted to the 1B NOR region only (Figure 3.A) and was in a homozygous state. Figure 3.B shows the typical distribution of rye signals in interphase nuclei of wheat cultivar "Yugoslavia" after the FISH technique.

Figure 3. Hybridization sites of biotin labelled total rye genomic probe in the bread wheat cultivar "Yugoslavia", on metaphase chromosomes (A) and on interphase nucleus (B), after FISH.

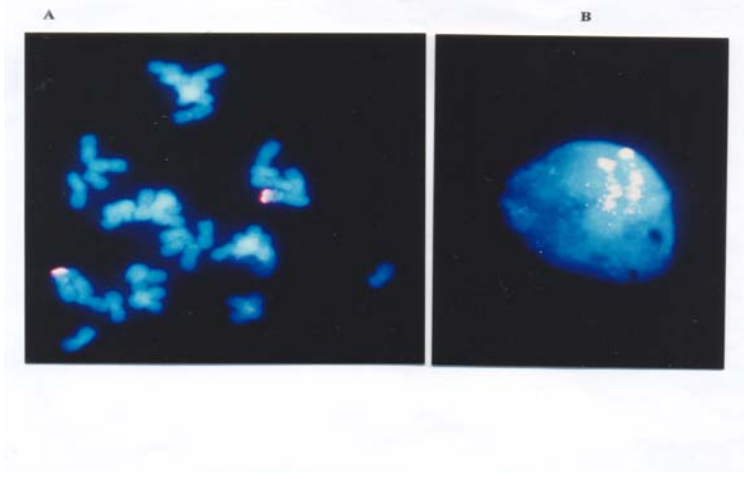

Figure 3. Hybridization sites of biotin labelled total rye genomic probe in the bread wheat cultivar "Yugoslavia", on metaphase chromosomes (A) and on interphase nucleus (B) after FISH.

Translocated 1RS arms have probably recombined with 1BS arms of normal wheat during breeding to form short arms composed of centromeric regions of the 1BS near the centromere and satellited 1RS region. Further analyses of storage proteins (HMW and LMW glutenin subunits), using SDS PAGE electrophoresis would be reasonable for this wheat cultivar.

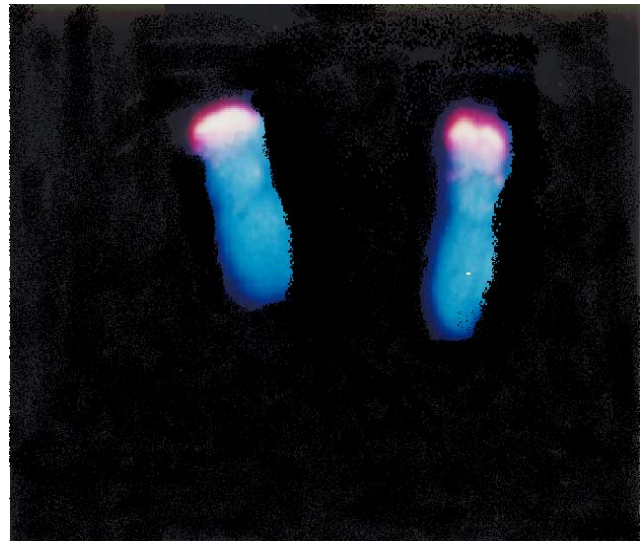

Figure 4. The 1B/1R translocations are restricted to the 1B chromosome SAT region. The two translocated chromosomes are from one metaphase cell of the bread wheat cultivar "Yugoslavia". Red dots represent biotin labelled rye signals (Avidin – Cy3, magnification ca. 3000x).

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