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Visualization of *Thinopyrum bessarabicum* chromosomes in a *Triticum aestivum/Th. bessarabicum* amphiploid by genomic *in situ* hybridization

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ABSTRACT

Identification of alien chromosomes in wheat/alien species hybrids facilitates subsequent detection of cryptic alien chromatin introgressions into wheat. In this study, genomic *in situ* hybridization was used to detect alien chromatin in mitotic metaphase chromosome spreads of *Triticum aestivum* L./*Thinopyrum bessarabicum* amphiploid. *Th. bessarabicum* DNA was isolated and labeled with biotin-11-dUTP. DNA was extracted from the donor wheat, Chinese Spring, and sheared. The labeled alien and unlabeled wheat DNA's were then mixed in a hybridization solution. After hybridization and detection with a streptavidin — horseradish peroxidase protocol, all 14 of the *Th. bessarabicum* chromosomes appeared totally brown. The 42 wheat chromosomes appeared blue due to counterstaining with Giemsa. The results demonstrate the usefulness of genomic *in situ* hybridization in detecting *Th. bessarabicum* DNA introduced into wheat.

Key words: Genomic blocking, Intergeneric hybridization.

INTRODUCTION

Wide hybridization between species of *Triticum* and related genera provides an important mechanism to improve the genetic diversity of wheat. Use of related genera for the improvement of wheat typically requires selective sexual transfer of the desirable gene into wheat. Transfer of extraneous alien DNA must be minimized so as to exclude genes conferring undesirable traits. In addition, the transferred alien DNA segment should be readily identifiable in the wheat background. New molecular cytogenetic techniques allow for the rapid recognition of alien DNA in wheat backgrounds. Now that these techniques are available, it is imperative that they be incorporated into wheat-alien breeding programs.

One of the most potentially useful molecular cytogenetic techniques for wheat alien introgression is genomic *in situ* hybridization in plants (Le *et al.*, 1989; Schwarzacher *et al.*, 1989). Several studies have demonstrated the potential significance of genomic *in situ* hybridization in plant breeding (Leitch *et al.*, 1990; HeslopHarrison *et al.*, 1990; Anamthawat-Jonsson *et al.*, 1990; Mukai and Gill, 1991). We therefore have attempted to explore the usefulness of genomic *in situ* hybridization in developing wheat with improved salt tolerance transferred from *Thinopyrum bessarabicum* (Savul and Rayss) Löve.

Diploid *Th. bessarabicum* ($2n = 2x = 14$; JJ) is a self-fertile maritime species noted for its high level of salinity tolerance. Incorporation of the salinity tolerance into wheat encompasses intergeneric hybridization and F_1 hybrid advance by backcrossing or topcrossing, leading ultimately to cryptic introgressions (Mujeeb-Kazi *et al.*, 1987). Genetic mechanisms such as the *Ph* mutant or irradiation techniques may also be employed to promote recombination. This progression necessitates diagnosis of alien chromosomal segments in a wheat background. While techniques such as N-banding, C-banding, isozyme analysis, and RFLP analysis may be employed for identification of alien DNA in wheat, large portions of alien genomes are not observed by these methods. Genomic *in situ* hybridization has a decided advantage in the confirmation of cryptic alien introgression.

The purpose of this study was to determine the usefulness of genomic *in situ* hybridization in detecting the presence of *Th. bessarabicum* chromosomes in a wheat background.

MATERIALS AND METHODS

Seeds of the wheat cultivar Chinese Spring ($2n$ = 6x = 42) were obtained from the late Dr. E.R. SEARS (University of Missouri, Columbia, MO). Seeds of *Th. bessarabicum* were obtained from the late Dr. D.R. DEWEY (USDA/ARS Logan, UT). In a joint project supported by British Overseas Development Agency (ODA), the then Plant Breeding Institute (PBI) at Cambridge, UK, the University of Bangor, Wales, UK, and CIMMYT, Mexico, hybrids and fertile amphiploids between those two species were produced (FORSTER *et al.*, 1987; MUJEEB-KAZI *et al.*, 1987). Seeds from the amphiploid ($2n$ = 8x = 56; AABBDDJJ), produced at PBI, were used in this study.

For chromosome preparation, seeds of the amphiploid were germinated and treated as described by RAYBURN and GILL (1985). Briefly, seeds were placed on wet blotting paper and allowed to imbibe for 24 h. The germinating seeds were placed at 4°C for 24-48 h, and the seedlings were then placed at room temperature for 24-48 h. Root tips were harvested and pretreated for 24 h at 0°C. After fixation in a 3:1 (Ethanol:Acetic acid) solution for 2-4 d, the root tips were stained in 1% acetocarmine and squashes made. Slides were stored at −70°C until ready for hybridization.

Total genomic DNA was isolated from both Chinese Spring and *Th. bessarabicum* chromosomes by the procedure of HOISINGTON (1992). The Chinese Spring DNA subsequently used as blocking DNA was sheared to 400-800 bp by passing it 10 times through a 1 ml tuberculin syringe with a 24 G needle attached. The *Th. bessarabicum* DNA was nick-translated with biotin 11 dUTP using a kit supplied by Enzo Diagnostics, Inc. (New York, NY).

For *in situ* hybridization, the slides were removed from the −70°C freezer and the coverslip removed. The chromosomes were denatured and rapidly dehydrated according to RAYBURN and GILL (1985). The procedures for the genomic *in situ* hybridization were those of MUKAI and GILL (1991). The detection of the biotinylated probe was performed with the detek1-hrp kit from Enzo Diagnostics. The substrate for the horseradish peroxidase enzyme was changed to diaminobenzidine tetrahydrachloride (DAB) (Sigma). The detection conditions were as described by MUKAI and GILL (1991).

RESULTS AND DISCUSSION

Upon genomic *in situ* hybridization of the Chinese Spring/*Th. bessarabicum* amphiploid, 14 of the 56 chromosomes were brown in color. The remaining 42 chromosomes were all light blue in appearance (Fig. 1). A 1:1 ratio of *Th. bessarabicum* to Chinese Spring DNA was used. The unlabeled Chinese Spring DNA effectively kept the labeled *Th. bessarabicum* DNA from hybridizing to the 42 wheat chromosomes. When Chinese Spring DNA was not used as a blocking DNA, all 56 of the amphiploid chromosomes appeared brown (data not shown).

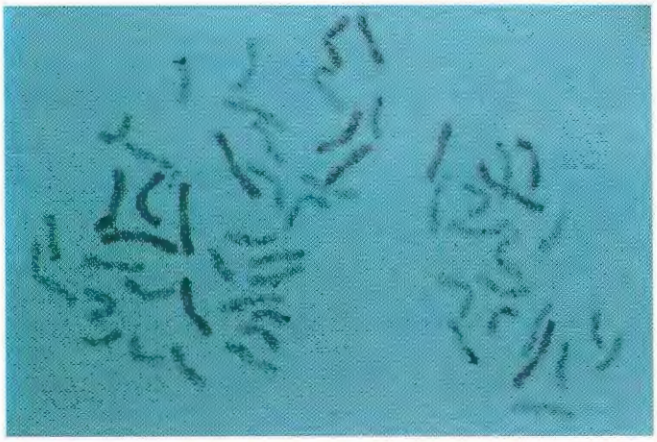

FIGURE 1 - Detection of brown-colored *Thinopyrum bessarabicum* chromosomes in its amphiploid with *Triticum aestivum* cv. Chinese Spring (2n = 8x = 56) using biotin labelled genomic DNA of *Th. bessarabicum* as a probe.

These results indicate that enough homoeology exists between *Th. bessarabicum* and wheat that unless wheat DNA is used for blocking *Th. bessarabicum* and wheat DNA, duplexes are formed during DNA-DNA hybridization conditions. Evidence of residual homoeology was also obtained by varying the stringency of the post hybridization washes. When the 37°C temperature used by MUKAI and GILL (1991) was used in the present study, the 42 wheat chromosomes were slightly brown rather than blue. Differentiation between the light brown wheat and dark brown *Th. bessarabicum* chromosomes was still possible but not optimal. Upon raising the washing temperature to 45°C, the 42 wheat chromosomes appeared blue with very little in any brown clolor. These results indicated that there was enough homoeology between chromosomes of wheat and *Th. bessarabicum* that more stringent washing conditions were necessary. The conditions used in this study were more stringent than those used by HESLOP-HARRISON *et al.* (1990), i.e., 42°C in 50% formamide. However, HESLOP-HARRISON *et al.* (1990) used 20 to 30 times more blocking DNA than used in our study. This difference in blocking DNA concentration could be a factor in obtaining cross hybridization between alien and wheat DNA's. Obtaining optimum differentiation between wheat and alien chromosomes with genomic *in situ* hybridization may be a function of a proper balance between amount of blocking DNA and stringency of post hybridization washing conditions.

Another factor which was determined to be critical in this study was slide preparation. Excess heating of the root tip or slide during slide prepar-

ation resulted in either nonspecific binding of the labeled DNA to the chromosomes or in nonspecific binding of the horseradish peroxidase — streptavidin complex to the chromosome. In either case, the chromosomes and interphase nuclei all appeared brown with little if any differentiation between alien and wheat chromosomes. Optimum differentiation occurred when slight heat was applied just before squashing the root tips on the slide. Any other heating caused disruption of the technique.

In conclusion, genomic *in situ* hybridization was found to be a rapid method by which *Th. bessarabicum* chromosomes could be detected in a wheat background. The resolution of the technique is such that the stage is now set to apply the genomic *in situ* hybridization to analysis of advanced derivatives of this cross. The significance of the genomic *in situ* hybridization technique will be in detecting subtle alien DNA introgression in a wheat background that may otherwise go undetected by more conventional methods.

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