http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 5(8): 869-870, 2002
© Asian Network for Scientific Information 2002

An Improved Genomic *In situ* Hybridization (GISH) Method to Differentiate Chromosomes from Closely Related Genomes

[1]N. Iqbal, [2]S.M. Reader, [3]P.D.S. Caligari and [2]T.E. Miller
Nuclear Institute for Agriculture and Biology (NIAB),
P. O. Box 128, Jhang Road Faisalabad, Pakistan
[2]John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK
[3]Department of Agricultural Botany, School of Plant Sciences, University of Reading, Whiteknights, Reading RG6 6AS, UK

Abstract: Previously reported genomic *in situ* hybridization protocols failed to differentiate *Aegilops uniaristata* chromosomes in a polyploid wheat background. Different protocols and hybridization temperatures were tested to optimize the differentiation of N genome *A. uniaristata* chromosomes from those of the A, B and D genomes of wheat. A combination of pre-hybridization of cytological preparations, pre-annealing of the genomic probe with blocking DNA and hybridization at elevated temperatures proved successful. A genomic *in situ* hybridization protocol for the differentiation of chromosomes from very closely related genomes in a polyploid background is reported.

Key words: Genomic *in situ* hybridization, GISH, *A. uniaristata*, wheat, addition lines

Introduction

Identification of alien chromosomes and chromosome segments in a particular genetic background is very important in plant cytogenetic studies. The genomic *in situ* hybridization (GISH) technique has become a popular cytogenetic technique in recent years. This technique uses total genomic DNA, from the donor species, as a probe on cytological preparations to identify the alien chromatin material. Total genomic DNA has been used as a probe to differentiate chromosomes of alien species in wheat (Le *et al.*, 1989), to identify alien chromosome segments (Schwarzacher *et al.*, 1992) and to study meiotic pairing in hybrids (Miller *et al.*, 1994).

The use of high concentrations of fragmented unlabeled genomic DNA from recipient species, blocking DNA, with the labeled DNA probe at hybridization was reported to improve the discrimination in GISH analysis (Anamthawat-Jonsson *et al.*, 1990). Pre-annealing of two differentially labeled total genomic DNA probes was also reported by Anamthawat-Jonsson and Reader (1995) to remove the common sequences and enhance chromosome differentiation. Here we report a GISH protocol that combines the pre-blocking of common sequences on chromosome preparations with pre-annealing of probe and blocking DNA, at elevated temperatures, to successfully discriminate the chromosomes of closely related genomes.

Materials and Methods

The work was conducted at the John Innes Centre, Norwich, UK. under the Islamic Development Bank Scholarship Program. Material used in this study was generated at John Innes Center.

Six addition lines of the chromosomes of *Aegilops uniaristata* (2n = 2x = 14, NN) in bread wheat *Triticum aestivum* cv. Chinese Spring (2n = 6x = 42, AABBDD) and one *T. aestivum/A. uniaristata* translocation line 3BL/3NL were used in this study along with two parents.

Total genomic DNA was isolated according to Davis *et al.* (1986). Mitotic slides were prepared following the method of Schwarzacher *et al.* (1989) and probes were labeled by the Nick translation method (Leitch *et al.*, 1994). Fluorescent-labeled nucleotide from New Life Sciences were used for the labeling of DNA. The rapid *in situ* hybridization method of Reader *et al.* (1994) was modified as described below.

Slide preparation: Mitotic chromosome slides were treated with 0.05 M HCl for two minutes and incubated with pepsin (5 μg ml^{-1}) for 15 minutes at 37°C. The slides were then washed with water and 2 x SSC (0.3 M NaCl, 30 mM trisodium citrate) for 2 minutes and 10 minutes respectively. Slides were treated with RNase and fixed with paraformaldehyde as described by Reader *et al.* (1994). Chromosomes on slides were denatured by formamide solution (70% formamide in 2 X SSC) at 70°C for two minutes following the dehydration through an ice-cold ethanol series.

Pre-annealing of probe: For pre-annealing 200 ng of labeled probe DNA was mixed with the same amount of blocking DNA in the hybridization mixture (10% dextran sulphate, 2 X SSC, 0.25 % SDS) in a total volume of 60 μl. The mixture was heated at 100°C in a hot block for 3 minutes, centrifuged briefly and incubated at 76°C for 2 hours.

Pre-hybridization of slides: Pre-hybridization mixture was prepared which contained only blocking DNA (0.5 μg), i.e. omitting the probe DNA. The mixture was denatured by heating at 100°C for 3 minutes and chilled on ice for a further 3 minutes after brief centrifugation. It was then put on to the previously denatured chromosome preparations, covered with a plastic cover slip, and pre-hybridized for 2 hours at 76°C.

Hybridization and washing: Slides, which have been subjected to the Pre-hybridization protocol above, were then hybridized with pre-annealed probe mixture at 74°C for 4 hours. After hybridization, slides were washed twice (5 minutes each) with 2 X SSC at 74°C, transferred to 37°C and then to room temperature. Slides were then stained with DAPI (0.125 μg ml^{-1}), mounted with Vectashield (Vector laboratories) and examined with a Nikon fluorescent microscope.

Results and Discussion

Pre-annealing of probes and the use of blocking DNA in the hybridization mixture has been described before (Anamthawat-Jonsson *et al.*, 1990, Anamthawat-Jonsson and Reader, 1995) to increase the efficiency of GISH. These procedures separately and at temperatures described in published protocols failed to differentiate *A. uniaristata* chromosomes from wheat chromosomes, especially those of its D genome. After trying several temperature regimes and methods the above-described protocol proved successful in distinguishing the N genome chromosomes of *A. uniaristata* from those of the A, B and D genomes of wheat. The *A. uniaristata* chromosomes in wheat background (Fig. 1a and b) and 3BL/3NL translocation line where both arms, originating from different genomes, can clearly be identified on the basis of differential hybridization intensities (Fig. 1c). Previously described methods have been employed mostly on diploid species or for the discrimination of chromosomes from different genera in wheat and all use lower pre-annealing and hybridization temperatures. The genus *Aegilops* is closely related to wheat with two of the genomes (the B and D genomes) being

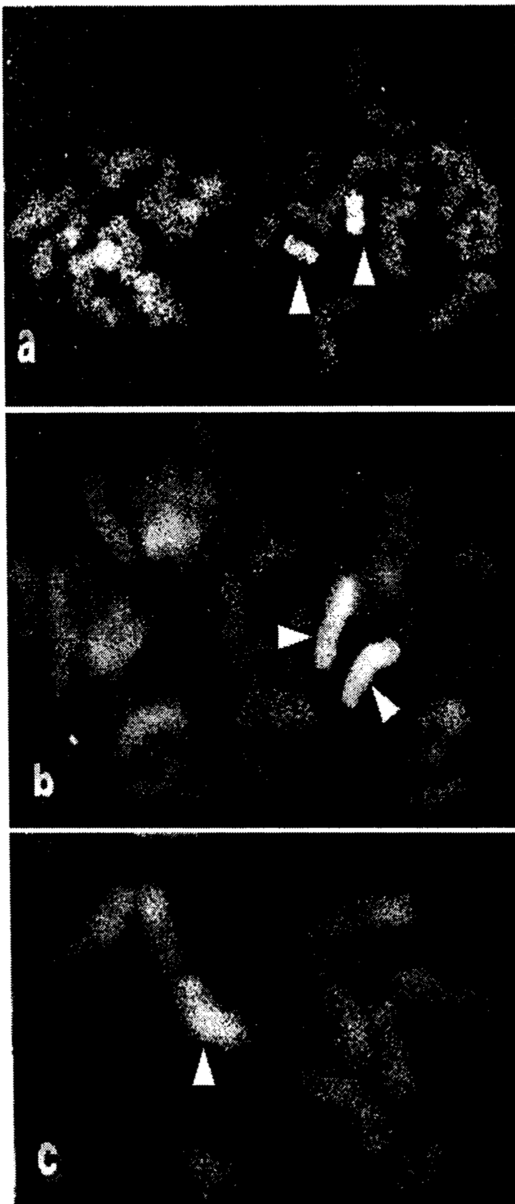

Fig. 1: GISH technique performed on the somatic metaphase chromosomes of *Aegilops uniaristata* addition and translocation lines using *A. uniaristata* DNA as labeled probe. Arrows indicate the added chromosomes 1N (a) and 4N (b) in Chinese spring background and long arm of chromosome 3N in 3BL/3NL translocation line (c).

derived from *Aegilops* species. There are high DNA sequence homoeologies between the genomes and especially so between the N and D genomes. Fluorescent *in situ* hybridization studies with repetitive DNA sequences (Iqbal *et al.*, 2000a) and recombination of *A. uniaristata* chromosome with those of wheat also revealed close homoeologies between these two species (Iqbal *et al.*, 2000b). In this situation the normal pre-annealing of the two genomic DNAs does not efficiently counter their close homoeology. This together with a hybridization temperature of only 65°C is not high enough to reduce the cross hybridization of the probe DNA fragments to different genomes of the chromosome preparation.

The protocol described here uses higher pre-annealing and pre-hybridization temperatures to eliminate the repetitive sequences, and DNA fragments with very high homoeology, so that they are thus not available at the hybridization step. The hybridization temperature is also higher (74°C) than that usually used for GISH studies (65°C), allowing association of sequences with very high homoeologies, and thus reducing the chances of cross hybridization. Concentration of probe DNA was also increased so that enough genome specific sequences are available for hybridization after pre-annealing and pre-blocking steps.

The method has proved successful in this case and may be valuable for the discrimination of closely related genomes in similar polyploid situations.

Acknowledgments

N. Iqbal Thanks the Islamic Development Bank for the Ph.D. scholarship, which enabled this work to take place. This study was partly funded by the UK Department of International Development.

References

Anamthawat-Jonsson, K., T. Schwarzacher, A. R. Leitch, M. D. Bennett and J. S. Heslop-Harrison, 1990. Discrimination between closely related Triticeae species using genomic DNA as a probe. Theor. Appl. Genet., 79: 721-728.

Anamthawat-Jonsson, K. and S. M. Reader, 1995. Pre-annealing of total genomic DNA probes for simultaneous genomic *in situ* hybridization. Genome, 38: 814-816.

Davis, L. G., M.D. Dibner and J.F. Battery, 1986. Basic Methods in Molecular Biology. Elsevier, New York.

Iqbal, N., S.M. Reader, P.D.S. Caligari and T.E. Miller, 2000a. Characterization of *Aegilops uniaristata* chromosomes by comparative DNA marker analysis and Repetitive DNA sequence *in situ* hybridization. Theo. Appl. Genet., 101: 1173-1179.

Iqbal, N., S.M. Reader, P.D.S. Caligari and T.E. Miller, 2000b. production and characterization of recombination between chromosome 3N of *Aegilops uniaristata* and chromosome 3A of wheat. Heredity, 84: 487-492.

Le, H.T., R.C. Armstrong and B. Miki, 1989. Detection of rye DNA in wheat-rye hybrids and wheat translocation stocks using total genomic DNA as a probe. Pl. Mol. Biol. Reptr., 7: 150-158.

Leitch, A.R., T. Schwarzacher, D. Jakson and J. Leitch, 1994. *In situ* hybridization- A practical guide. BIOS Scientific, Oxford.

Miller, T.E., S.M. Reader, A. Mahmood, K.A. Purdie, and I.P. King, 1994. Determination of the frequency of Wheat/rye chromosome pairing in wheat x rye hybrids with and without chromosome 5B. Theor. Appl. Genet., 89: 255-258.

Reader, S.M., S. Abbo, K.A. Purdie, I.P. King and T.E. Miller, 1994. Direct labeling of plant chromosomes by rapid *in situ* hybridization. Trend. Genet., 10: 265-266.

Schwarzacher, T., A.R. Leitch, M.D. Bennett and J.S. Heslop-Harrison, 1989. *In situ* localization of parental genomes in a wide hybrid. Ann. Bot., 64: 315-324.