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Development of DNA markers based on Randomly Amplified Polymorphic sequences in a *Triticum aestivum* L. × *Thinopyrum bessarabicum* amphiploid

M.D.H.M. William [1], L. Gray [2], A.L. Rayburn [3] and A. Mujeeb-Kazi [1]

[1] *International Maize and Wheat Improvement Center (CIMMYT), Lisboa 27, Apartado Postal 6-641, 06600 México, D.F., Mexico*

[2] *USDA/ARS, University of Illinois, Urbana, Illinois, USA*

[3] *Department of Agronomy, University of Illinois, Urbana, Illinois, USA*

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ABSTRACT

Randomly Amplified Polymorphic DNA (RAPD) markers were developed using the polymerase chain reaction (PCR) for the amphiploid of *Triticum aestivum* L. cv. Chinese Spring and *Thinopyrum bessarabicum* (Savul. and Rayss) Löve. Polymorphisms observed with seven arbitrary oligonucleotide decamer primers are reported. There was no polymorphism among different DNA extracts from different individual seedlings of Chinese Spring. The amplification patterns generated with primers for the two plant species were repeatable. The marker bands specific to *Th. bessarabicum* may be helpful in tracking its chromatin in the wheat background when chromosome additions, substitutions, translocations or subtle chromosomal interchanges are produced.

Key words: Disomic chromosome additions, Polymerase Chain Reaction, Randomply Amplified Polymorphic DNA, Salt tolerance.

INTRODUCTION

Thinopyrum bessarabicum (Savul. and Rayss) Löve ($2n = 2x = 14$) is a self-fertile maritime grass with a high level of tolerance to drought and salinity, and is genomically designated as JJ. The amphiploid of *Th. bessarabicum* with *Triticun aestivum* L. ($2n = 6x = 42$) also expressed a high level of salt tolerance associated with disomic chromosome 5J (Forster *et al.*, 1988), a finding that varied from subsequent observations by Mujeeb-Kazi (1992). Despite this inconsistency, *Th. bessarabicum* is a potentially valuable germplasm source for improving salt tolerance in cultivated wheat. Incorporation of salinity tolerance into cultivated wheat necessitates further development of the remaining alien disomic chromosome additions, production of their substitution lines and effecting cryptic transfers of salt tolerance from lines identified as crucial into wheat.

Genetic markers serve an important diagnostic role in the development of alien addition or substitution lines. Markers traditionally used are morphological, cytological or seed proteins/isozymes. Restriction Fragment Length Polymorphisms (RFLPs) have also been successfully utilized as have markers based on polymerase chain reaction (PCR) (Weining and Landridge, 1991; D'Ovidio *et al.*, 1990). The RFLP markers have been extensively used in the construction of genetic linkage maps for a wide range of crops. However, in wheat and its relatives, possibly due to polyploidy and genomic complexity, RFLP markers have so far not been very informative (Weining and Landridge, 1991).

Based on PCR, another potential set of markers involves the use of Randomly Amplified Polymorphic DNA sequences (RAPDs) (Mullis and Faloona, 1987; Saiki *et al.*, 1985). In the use of RAPDs, oligonucleotide primers whose sequences are arbitrarily derived are used to generate amplified DNA sequences. The polymorphisms generated using RAPDs are based upon the distance between a sequence complimentary to the oligonucleotide primer and the same sequence in reverse orientation in the genomic DNA.

Here we report the development of PCR-based RAPD markers in the amphiploid of *T. aestivum* ×

Th. bessarabicum ($2n = 8x = 56$; AABBDDJJ). These markers will eventually be assigned to specific *Th. bessarabicum* chromosomes using disomic additions of *Th. bessarabicum* in a wheat background. These additions are currently being characterized by cytological and protein/isozyme procedures.

MATERIALS AND METHODS

Seed material. Seeds of *Triticun aestivum* cv. Chinese Spring ($2n = 6x = 42$; AABBDD) were obtained from the late Dr. E.R. SEARS (University of Missouri, Columbia, MO) and those pf *Thinopyrum bessarabicum* ($2n = 2x = 14$; JJ) from the late Dr. D.R. DEWEY (USDA/ARS; Logan, UT). The hybrid and the colchicine doubled fertile amphiploid between the two species were produced in a joint project sponsored by the British Overseas Development Agency between the Plant Breeding Institute (PBI), United Kingdom, and CIMMYT, Mexico. Seeds of the amphiploid ($2n = 8x = 56$; AABBDDJJ) produced at PBI were utilized in the study.

DNA isolation. Isolation of genomic DNA was in essence according to the protocols of HOISINGTON (1992). The DNA was extracted from approximately 300 mg of lyophilized, ground leaf tissue with 9.0 ml of CTAB extraction buffer for 90 min with continuous gentle shaking of the tubes in an oven at 65°C. A (24:1) chloroform/octanol mixture (4.5 ml) was added to each tube, mixed gently for 10 min at room temperature (RT) and then spun in a table top centrifuge for 10 min at 1500g at RT. The aqueous phase was transferred to fresh tubes and the chloroform/octanol extraction repeated. After spinning the samples again for 10 min at 1500g, the aqueous phase was pippetted to fresh tubes containing 50 µl of 10 mg/ml preboiled RNase A, mixed gently and incubated at RT for 30 min. The DNA was precipitated by adding 6 ml of isopropanol and the precipitated DNA was removed with a glass hook and resuspended in a 5ml tube containing 1 ml of TE (10mM Tris pH 8.0 and 1mM EDTA pH 8.0).

Polymerase chain reaction. A total of 26 oligonucleotide decamer primers (Operon Inc.) were evaluated and of the 13 primers that showed easily detectable polymorphisms seven are reported here. These seven primers had the following sequences:

1.	L	5	5′ ACGCAGGCAC 3′
2.	L	16	5′ AGGTTGCAGG 3′
3.	L	18	5′ ACCACCCACC 3′
4.	L	20	5′ TGGTGGACCA 3′
5.	N	1	5′ CTCACGTTGG 3′
6.	N	3	5′ GGTACTCCCC 3′
7.	N	5	5′ ACTGAACGCC 3′

The PCR reactions were carried out in a 25 µl reaction mixture consisting of 20 ng genomic DNA; 0.1 mM each dATP, dCTP, dGTP and dTTP (Perkin-Elmer Co., Norwalk, CT, USA); 0.001% gelatin (Sigma Chemical Co.); 2.0 mM M_gCl_2, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% triton X-100, 0.75 units of Taq polymerase (M_gCl_2, 10X dilution buffer; Promega Chemicals Co., Madison, WI) and 15 ng of primer. A hybaid thermocycler was used with one cycle of 2 min at 93°C, followed by 35 cycles at 92°C for 1 min, 36°C for 1 min and 71°C for 1 min. At the end of 35 cycles, a temperature of 72°C was maintained for 5 min. The reaction mixture were overlaid with 30 µl of mineral oil. Approximately 10 µl of the reactions were separated on a 1.1% agarose gel in a BRL Horizon 11.14 gel apparatus at 60V for 3.5 h, then stained with ethidium bromide.

RESULTS AND DISCUSSION

Fragments of genomic DNA can be amplified by single primers of arbitrary sequences using PCR (WILLIAMS *et al.*, 1990; WELSH and MCCLELLAND, 1990). Chromosome specific RAPD markers have also been developed in tomato (KLEIN-LANKHORST *et al.*, 1991). D'OVIDIO *et al.* (1990) reported amplification of wheat genomic DNA using primers specific for the gamma gliadin gene. Wheat genomic DNA has also been amplified by using random primers coupled with intron specific primers (WEINING and LANDRIDGE, 1991). The results of our study show that genomic DNA can also be amplified using random primers (DEVOS and GALE, 1992; VIERLING and NGUYEN, 1992).

An initial study using different concentrations of DNA and Taq polymerase indicated that under our conditions optimum results were obtained when 20 ng DNA and 0.75 units of Taq polymerase were used in the 25 µl reaction (data not shown). Because sequence information is limited in wheat and not available in *Th. bessarabicum* we propose that random primers could be useful in establishing more markers that may facilitate the detection of *Th. bessarabicum* chromatin in a wheat background.

A majority of the primers gave polymorphisms in the patterns of amplification products between Chinese Spring and *Th. bessarabicum*. Out of the 26 primers, 18 had amplification products in the amphiploid specific to *Th. bessarabicum*. Only 13 primers gave amplification products that were easily detectable. For the seven primers shown in Fig. 1, there were some amplification products in the amphiploid that were common to wheat and *Th. bessarabicum*. The amplification products present in the amphiploid specific to *Th. bessarabicum* are marked with arrows (Fig. 1). Although the DNA of the amphiploid contains the genomic DNA of both Chinese Spring and *Th. bessar-*

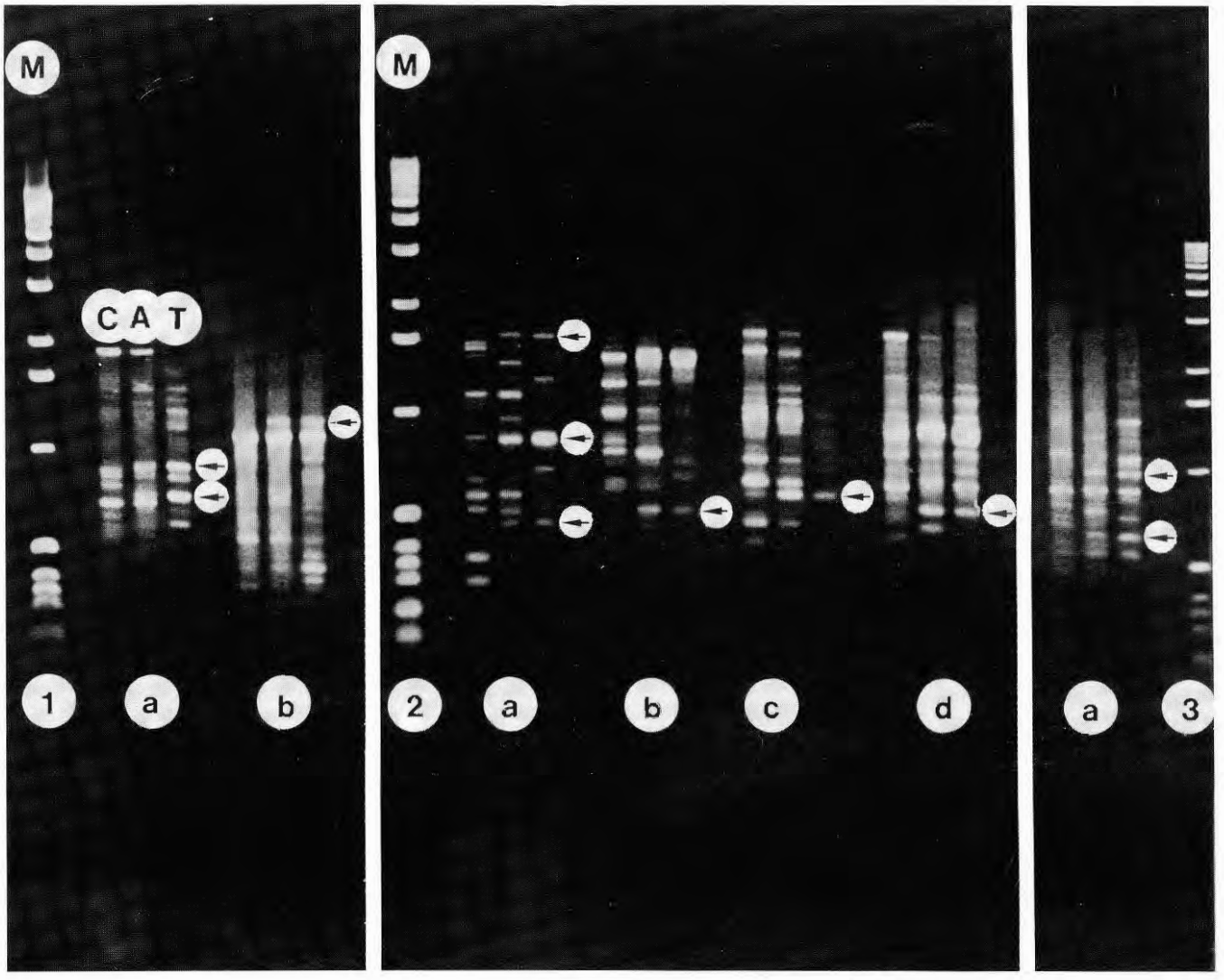

FIGURE 1 - Amplification of genomic DNA (20 ng) of *Triticum aestivum* cv. Chinese Spring (C), the amphiploid of CS/*Thinopyrum bessarabicum* (A) and *Th. bessarabicum* (T). The molecular weight marker tracks are designated as (M).
The primers used (left to right) were L20 (1a), L16 (1b), N1 (2a), N3 (2b), N5 (2c), L5 (2d) and L18 (3a). The marker bands observed in *Th. bessarabicum* and present in the amphiploid are indicated by arrows.

abicum, there were cases where the amphiploid did not show the complete profiles of the amplification products of both parents. The amphiploid, however, did not show any amplification products not present in the parents.

In RAPDs, primer binding to a complementary sequence is a random process. In the case, of the amphiploid due to an increase in the genome size, the availability of a particular sequence in 20 ng of DNA is less compared to that of the parents. Further, in RAPDs, primers may bind where there is incomplete homology. It is also possible that greater similarity between a primer and the template of one of the parental genomes results in preferential amplification of this sequence in the amphiploid. This may explain why the amphiploid did not possess all the amplification products present in Chinese Spring and *Th. bessarabicum*. The consistency in the amplification products for a given primer indicates that the marker fragments specific to the alien species could be effectively utilized to diagnose the presence of chromatin from that species, even though the amphiploid may not contain all the products specific to that species.

To establish the repeatibility of the amplification products the same primer was used at least thrice with no differences observed in the patterns of amplification products of the repeated runs. Further, to evaluate whether there was polymorphism in the amplification products among

different plants, DNA was isolated from five individual seedlings of Chinese Spring and amplified using two different primers, N-1 and N-3. The amplification products (Fig. 2) demonstrate a lack of polymorphism in the DNA extracts from individual seedlings of Chinese Spring for the fragments that amplify with high intensity for these two primers.

Use of PCR is a newer technique compared to the use of more conventional markers such as morphological traits or proteins/isozymes for further characterizing alien addition and substitution lines in a wheat background. The technique does not allow for establishing alien/wheat chromosomal homoeologies. However, since polymorphisms generated by PCR are most probably based on short repetitive DNA sequences (DEVOS and GALE, 1992), it may be possible to track the small chromosomal interchanges using PCR techniques more effectively after the alien/wheat chromosomal homoeologies are established.

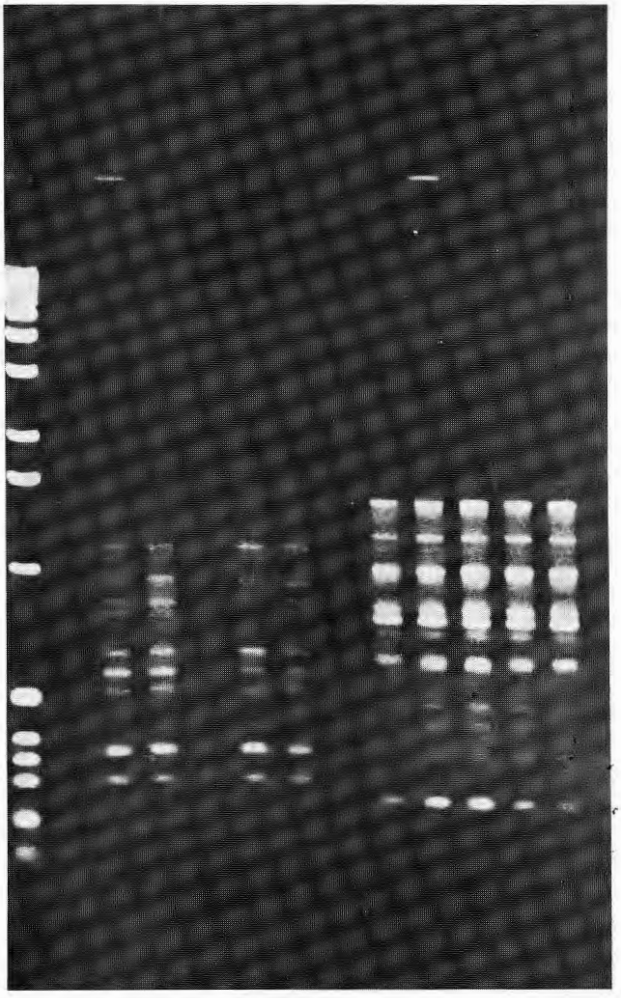

FIGURE 2 - Amplification of genomic DNA of *Triticum aestivum* cv. Chinese Spring (20 ng) using primers N-1 (left) and N-3 (right). Each track indicates amplification products from DNA extracts from different seedlings of Chinese Spring.

Several disomic addition lines of *Th. bessarabicum* ($2n = 6x = 42 + 2$) have been produced in the wheat wide-crossing program in CIMMYT. These addition lines are in an agronomically superior plant type, possess high fertility and are cytologically stable with a high frequency of 22 bivalents at meiosis. These additions are being characterized by isozyme and cytological markers (MUJEEB-KAZI, 1992). Although the use of isozyme/protein and cytological markers may be convenient in identifying the disomic addition lines, they have limited use in tracking small segments of alien chromatin in a wheat background. We propose that once a disomic addition line has been characterized by other means, RAPD markers be used to identify alien introgressions involving small segments of alien chromatin in a wheat background. This initial study shows the presence of randomly amplified DNA specific to *Th. bessarabicum* in the amphiploid. Chromosomal location of these marker bands is being undertaken with the use of disomic addition lines.

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