Development of RAPD Based Markers for Characterizing Disomic Chromosome Addition Lines of *Thinopyrum bessarabicum* in Wheat (*Triticum aestivum* L.)

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Introduction

Genetic markers play an important diagnostic role in the development of alien addition or substitution lines. Morphological, cytological, and biochemical markers are traditionally used to facilitate the characterization of alien genetic material. Recent evidence indicates that markers based upon restriction fragment length polymorphisms (RFLPs) may also be significant. Randomly amplified polymorphic DNA sequences (RAPDs) are another potential set of markers (Mullis and Faloona, 1987; Welsh and McClelland, 1990; Williams *et al.*, 1990).

Thinopyrum bessarabicum (2n=2x=14) is a self-fertile maritime grass, genomically designated as JJ, with a high level of tolerance to drought and salinity (Dewey, 1984) that makes it a suitable alien source of genetic variability for wheat improvement. Here we report the development of RAPD based markers for the identification of disomic addition lines of *Th. bessarabicum* in a wheat background. The disomic addition lines used in this study possess distinct biochemical markers and are being cytologically characterized.

Materials and Methods

The amphiploid of *Triticum aestivum* cv. Chinese Spring / *Thinopyrum bessarabicum* (2n=8x=56; AABBDDJJ) and the disomic addition lines produced by us in CIMMYT, Mexico, were used in the study. The disomic *Th. bessarabicum* addition lines were characterized using proteins / isozymes and contain distinct biochemical markers (Table 1, Mujeeb-Kazi *et al.*, 1992). *Triticum aestivum* cv. Chinese Spring and Genaro-81 were utilized as controls because they were used as parents in the production of the disomic addition lines.

Isolation of genomic DNA was according to the protocols described by Hoisington (1992). A total of 80 decamer primers (Operon Inc.) from Kits A, L, O, and N were evaluated against the two parents and the amphiploid. When polymorphisms were detected, those primers were used on a set of disomic addition lines together with the wheat parents, *Th. bessarabicum*, and the amphiploid.

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, 0.001% gelatin, 2.0 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% triton X-100, 1.0 units of *Taq* polymerase (Promega) and 15 ng of primer. Reactions were performed in an ERICOMP thermocycler using one 2-min cycle at 93° C, 45 1-min cycles at 92° C, 1 min at 36° C, and 1 min at 72° C. At the end of 45 cycles, a temperature of 72° C was maintained for 5 min. The reaction mixtures were overlaid with 30 μl of mineral oil. Approximately 10 μl of the reactions were

Table 1. Biochemical markers, homoeology with wheat chromosomes, and the addition line number assigned to the disomic addition lines of *Thinopyrum bessarabicum* (2n=2x=14; JJ).

Biochemical marker	Homoeology with wheat chromosomes	Addition line
HMW-Glutenin	Group 1	1J
SOD	Group 2	2J
EST-5	Group 3	3J
PGM	Group 4	4J
b-AMY	Group 5	5J
GOT-2	Group 6	6J
a-Amy-2	Group 7	7J

separated on 3.0% agarose gel (Nusieve 3:1) in TAE buffer at 50 mAmp for 5.0 h, stained with ethidium bromide, and photographed.

Results and Discussion

Figure 1 shows the electrophoretic separation of amplification products when different random primers were used to amplify the genomic DNA. For all six primers (Figure 1), there were some amplification products in the amphiploid common to wheat and *Th. bessarabicum*. Most primers produced polymorphic amplification products between wheat and *Th. bessarabicum*. However, the amphiploid did not always show all the amplification products of the two parents. In RAPDs, primer binding to a complementary sequence is a random process. In the case of the amphiploid, due to the larger genome size, the availability of a particular sequence in 20 ng DNA is less compared to that of the two parents. It is also possible that primer binding occurs with

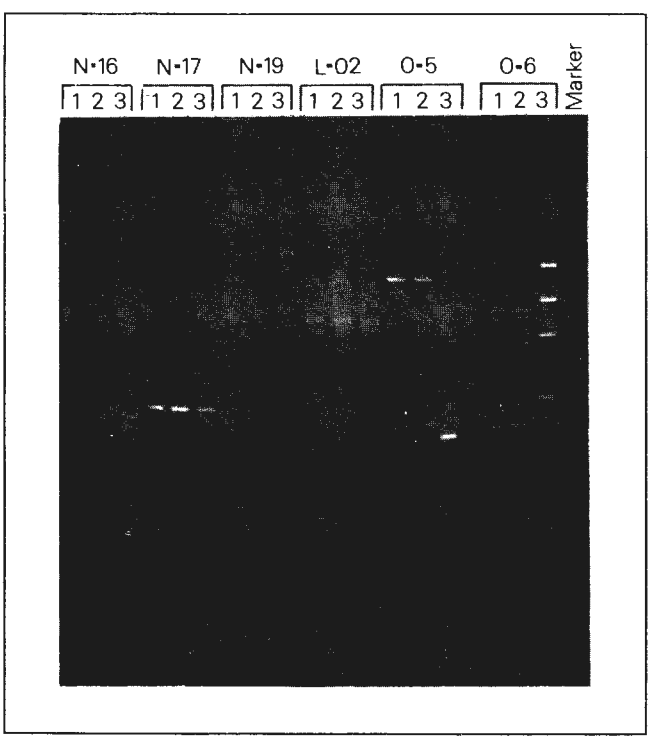

Figure 1. Amplification patterns of genomic DNA of *Triticum aestivum* cv. Chinese Spring (1), amphiploid of Chinese Spring/*Thinopyrum bessarabicum* (2), and *Th. bessarabicum* (3) with primers N-16, N-17, N-19, L-02, O-5, and O-6, respectively.

incomplete homoeology, thus explaining the absence of some amplification products in the amphiploid. However, the consistency in the amplification products for a given primer indicates that the marker fragments common to the alien species and the amphiploid could be used as markers, although the amphiploid did not contain all the amplified fragments specific to *Th. bessarabicum*.

To establish the repeatability of the banding patterns, the same primer was used at least thrice with no difference observed in the amplification products of the repeated runs. Further, to evaluate whether polymorphisms existed in the amplification products among different plants, DNA was isolated from three individual seedlings of *T. aestivum* cv. Chinese Spring and three different *Th. bessarabicum* accessions and amplified with three different primers in two different thermocyclers. The amplification patterns showed a lack of polymorphism in the DNA extracts from individual Chinese Spring seedlings and from different *Th. bessarabicum* accessions for the major amplification products (those with highest intensity). There was some ambiguity in the low intensity amplification products. Therefore, it may be better to consider the amplification products that amplify consistently and with highest intensity in RAPD analysis.

Figure 2 indicates the amplification patterns of the disomic addition lines together with the parents and the amphiploid with primers O-5 and O-6, respectively. An amplified fragment of *Th. bessarabicum* with O-5 is present in disomic addition line 5 (homoeologous with group 5 chromosomes of wheat), whereas primer O-6 has two amplification products in *Th. bessarabicum* that are present in addition lines 2 and 6 (homoeologous with group 2 and 6 chromosomes of wheat, respectively). Primer N-17 had two amplification products that were also present in the amphiploid, one of which could be assigned to addition line 1 with homoeology to group 1 chromosomes of wheat (not shown). It has been possible to allocate RAPD markers to four disomic addition lines using single arbitrary decamers as primers.

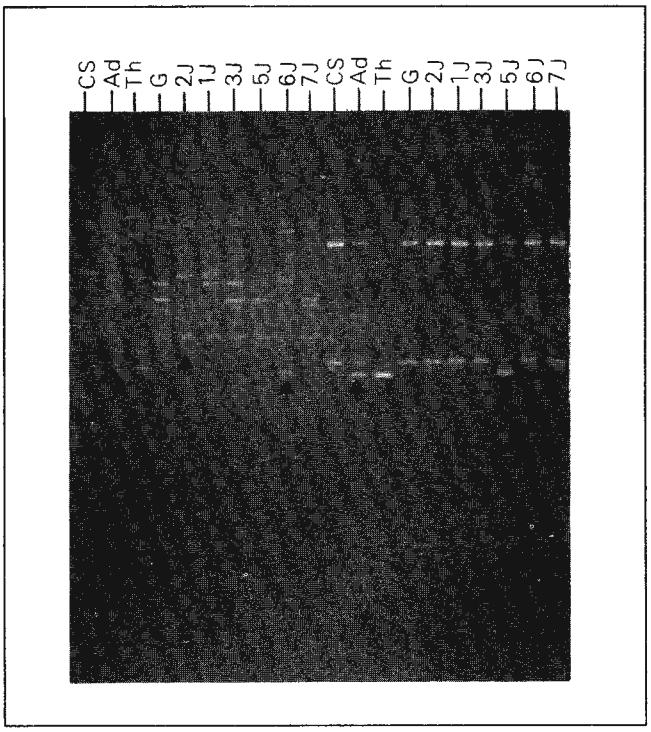

Figure 2. Amplification patterns of genomic DNA of *Triticum aestivum* cv. Chinese Spring, Genaro-81 (G), with the amphiploid of Chinese Spring/*Thinopyrum bessarabicum* (Ad), *Th. bessarabicum* (Th), and six of the seven disomic addition lines with primers O-6 and O-5, respectively. The 45 addition line had no marker and was not included.Marker fragments in the disomic additions are indicated by arrow.

We have been able to observe adequate polymorphisms between bread wheat and *Th. bessarabicum* using single decamer primers. This is not unexpected, becuase the probability of detecting polymorphisms among amplified products is greater when alien species are involved. Since compared to the more conventional markers (morphological and biochemical) PCR is a novel technique for identifying alien genetic material, it may as yet not be possible to locate any marker bands to specific chromosomes or chromosome arms, unless the material used for PCR has already been characterized using conventional techniques. However, when a given addition or substitution line has been identified conventionally, RAPD markers can be used to further characterize the line and establish additional markers. Although at this stage the nature of the amplified sequences is inconclusive, it is more likely that repetitive sequences will be amplified when decamers are used as primers, as suggested by Devos and Gale (1992).

Although the use of isozyme/protein and cytological markers may be convenient in identifying disomic addition lines, their use in tracking small segments of alien chromatin in a wheat background is limited. We propose that once disomic addition lines have been characterized by other means, RAPD markers be used to further characterize the addition lines. If these amplified sequences (observed as RAPD markers) are dispersed along chromosomes, in the future they may be useful for detecting subtle introgressions involving alien chromatin in a wheat background.

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