

Full Length Research Paper

Mapping of randomly amplified polymorphic DNA primer (RAPD) on chromosome 2A of common wheat

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Randomly amplified polymorphic DNA primers (RAPDs) were mapped on chromosomes 2A of wheat genotypes using “Chinese Spring” nullisomic-tetrasomic lines. One particular pair of chromosome was absent and another homologous pair was present in the extra dose. Genomic DNA was isolated from two genetic stocks viz; NT1D1B and NT2A2B. Polymerase chain reaction (PCR) were conducted using RAPD primers GLC-07 and GLB-11. PCR amplification using primer GLC-07 produced single band of approximately 1100 bp in both the genetic stocks, indicating that the primer was annealed to a loci other than chromosome 2A. RAPD primer GLB-11 amplified a polymorphic allele of approximately 500 bp which was present in NT1D1B but was absent in NT2A2B, indicating that the locus was present on chromosome 2A of common wheat. Hence, this marker (GLB-11) can reliably be used to keep track of chromosome 2A of wheat.

Key words: Wheat, randomly amplified polymorphic DNA primer (RAPD), mapping, chromosomes, homologous, genomes.

INTRODUCTION

Wheat is a staple food of 180 million Pakistanis. It is one of the most widely cultivated food crops and is the staple food in more than 40 countries of over 35% of the global population (Williams, 1993). By 2020, world demand for wheat is expected to be 40% higher than the present production (Rosegrant, 1997). It is the cheapest source and supplies 72% of the calories and protein in the average diet, principally in the form of chapattis, roti, nans, breads, cakes, biscuits, porridges and other products (Heyne, 1987). It is the universal cereals of the old and new World agriculture and belongs to the world's most important crop plants (Feldman et al., 1995; Nevo et al., 2002). Modern wheat cultivars belong primarily to three species; hexaploid (bread) wheat (*Triticum aestivum* L., $2n = 6x = 42$), tetraploid wheat (*Triticum turgidum* L., $2n = 4x = 28$) and diploid wheat (*Triticum monococcum* $2n = 2x = 14$). Bread/common (hexaploid) wheat is most important

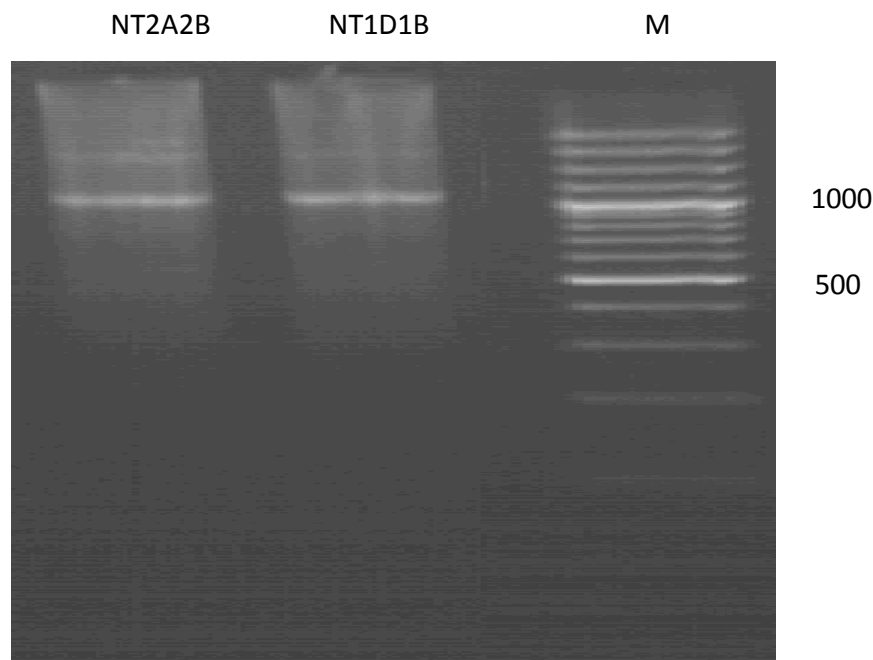
among all the species of *Triticum* and contains three genomes A, B and D (Sear, 1969).

In the world, wheat is grown on approximately 220 million hectares with a total production of 564.6 million metric tones (FAO, 2006). In Pakistan, it is grown on approximately 18, 00,000 hectares with an annual production of 19.1 million tones giving an average yield of 2.4 tones per hectare (MINFAL, 2006). Genetic stocks of wheat cultivar “Chinese Spring” were developed (Sears, 1966; Sears and Sears, 1978). These stocks included Nullisomic-tetrasomic and ditellosomic lines. These lines have been widely used for mapping of wheat chromosomes. Morphological, biochemical and cytological markers were used to map the wheat chromosomes (Islam and Shepherd, 1992). But these markers were not considered suitable for large scale mapping. With the recent introduction of molecular biology, DNA based markers including polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), single nucleotide polymorphisms (SNPs), etc are now being used for the construction of genetic maps of commercially

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Table 1. Sequence of randomly amplified polymorphic DNA (RAPD) primers used during the present study.

S/N	Oligo name	Sequence (5'-3')	Molecular weight	GC (%)
1	GLB-11	GTAGACCCGT	3.028	60
2	GLC-07	GTCCCCACGA	3.018	70

**Figure 1.** PCR amplification profile of the two genetic stocks of common wheat, NT2A2B and NT1D1B using RAPD primer GLC-07. M = Molecular size marker (100 bp DNA ladder, Gene Link, USA).

important crops species including wheat (*T. aestivum* L.). Among the DNA markers, randomly amplified polymorphic DNA primers (RAPDs) are easier, cheaper and quicker and are being used widely for mapping of wheat chromosomes (Williams et al., 1990).

The present study was carried out with the following objectives: optimization of polymerase chain reaction protocol for RAPD analysis and genome specific mapping of wheat chromosome 2A using RAPDs.

MATERIALS AND METHODS

Chinese Spring Nullisomic-tetrasomic NT2A2B and NT1D1B were used in the present study. Plants were grown in pots at the Department of Genetics, Hazara University, Mansehra, using recommended agricultural practices.

Leaf samples were used as a source for isolation of total genomic DNA using protocol of Weining and Langridge (1991). For the degradation of RNA, DNA was treated with 2 μ l RNAs A at 37°C for 2 h and then DNA samples were stored at 4°C. During the present research, two RAPD primers, GLC-07 and GLB-11 (Table 1) (Gene Link, Inc, 10532, NY, USA) were used to amplify genomic DNA isolated from the Nullisomic-tetrasomic lines NT2A2B and NT1D1B of common wheat. PCR reactions were assembled using

standard protocol (Devos and Gale, 1993). Amplification conditions involved an initial denaturation step of 4 min at 94°C followed by 40 cycles each consisting of denaturation step of 1 min at 94°C followed by annealing step of 1 min at 34°C and an extension step of 2 min at 72°C. All amplification reactions were performed using the Gene Amp PCR system 2700 (Applied Biosystem). The PCR products were electrophoresed on 1% Agarose gel with ethidium bromide staining and visualized under ultraviolet (UV) light using Uvitech gel documentation system.

RESULTS AND DISCUSSION

Polymerase chain reactions were carried out using RAPD markers for mapping individual wheat chromosome. In the present research work, two genomic DNA samples (NT2A2B and NT1D1B) were used as template DNA. The line NT2A2B was used to map RAPD primer on chromosome 2A of common wheat. The NT1D1B was used as positive control because it has a complete 2A chromosome. PCR amplification using RAPD primer GLC-07 produced a band of 1100 bp in both the lines, indicating that the primer annealed at a locus other than chromosome 2A (Figure 1). In finding useful polymorphism

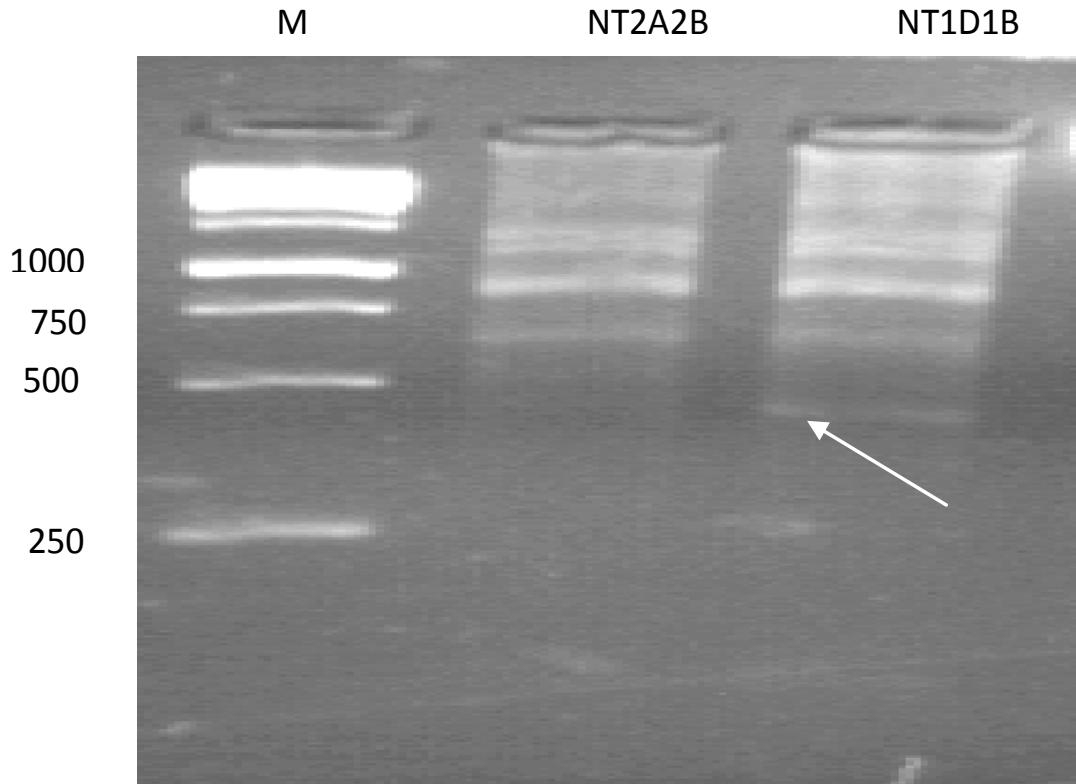


Figure 2. PCR amplification profile of the two genetic stocks, NT2A2B and NT1D1B using RAPD primer GLB-11. M = Molecular size marker (1 kb DNA ladder, Gene Link, USA). The diagnostic band (approximately 500 bp) for chromosome 2A of common wheat is indicated by an arrow).

for chromosome 2A of common wheat, another primer (GLB-11) was used to amplify the genomic DNA isolated from the two genetic stocks. PCR amplification with GLB-11 produced a diagnostic band of 500 bp in NT1D1B, but the fragment was absent in the NT2A2B line which indicate that the locus is present on the chromosome 2A of common wheat (Figure 2). This DNA fragment can be used as a reliable marker to keep track of chromosome 2A of common wheat (*T. aestivum*). Previously, in most of the cases, RFLP based assays were used to map the wheat chromosomes (Chao et al., 1989; Blanco et al., 1998; Dubcovsky et al., 1996; Gill et al., 1993, 1996); it is an expensive, time consuming and technically difficult technique (Rafalski, 1986). More recently, cheaper, quicker and more user friendly technique of polymerase chain reaction using randomly applied polymorphic DNA primers has been used for mapping wheat chromosomes (Williams et al., 1990, Ali et al., 2009).

The present research is therefore a step for the construction of RAPD based map of wheat chromosomes which will be more useful for underdeveloped countries like Pakistan, where it is not feasible to work with expensive technique like RFLP. In addition, marker developed during the present research (GLB-11) can be used to transfer useful genes in chromosome 2A of common wheat.

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