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DEVELOPMENT OF RAPD-BASED MOLECULAR MARKERS FOR CHROMOSOME 5A OF COMMON WHEAT

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Abstract

During the present study, 6 deletion lines (del 5AS-3, del5AS-7, del5AS-10, del5AL-10, del5AL-12 and del5AL-23) of chromosomes 5A of common wheat were used to identify RAPD (Randomly Amplified Polymorphic DNA) based molecular markers specific for short and/or long arm of chromosome 5A and one deletion line (5DS-1) used as a positive control. Out of the 7 RAPD primers used, OPA-07 showed useful polymorphism for long arm deletion line del5AL-23. By comparing the C-banding karyotype of the deletion lines, it is inferred that the primer (OPA-07) anneals to the distal half of the long arm of chromosome 5A.

Introduction

Common wheat (*Triticum aestivum* L.) belongs to family Gramineae. Genomically it is an allohexaploid having three genomes, AABBDD, 2n = 6x = 42 chromosomes. It is the world's most important cereal crop. In Pakistan it is grown on about 18, 00,000 hectares with an annual production of 19.1 million tones giving an average yield of 2.4 tones per ha (Anon., 2005).

Wheat grown in the world belongs to three main classes, (i) diploid wheat, 2n=2x=14, mainly used for fodder e.g., *Triticum monococcum*, (ii) tetraploid or durum wheat, 2n = 4x = 28, e.g., *Triticum dicoccoides*, mainly used to make biscuits, pastries, noodle and pasta and (iii) hexaploid or common/bread wheat, 2n = 6x = 42 which is used to make bread, nan, chapatti etc. Hexaploid wheat is the most common class of wheat grown all over the world. About 95% of wheat crop grown in the world is hexaploid. In Pakistan almost all the wheat grown is of hexaploid type. It is grown as a Rabi season crop all over the country, generally sown in end of October and harvested in May.

Recent development of molecular biology has revolutionized Marker Assisted Selection (MAS) and its utilization in breeding for important crops like wheat (Paterson *et al.*, 1991). Many kinds of molecular markers e.g., Restriction Fragment Length Polymorphism (RFLP), Polymerase Chain Reaction (PCR), Simple Sequence Repeats (SSR) and Amplified Fragment Length polymorphism (AFLP) are being used in present day breeding programs (Karp, 1997; Vos, *et al.*, 1995). Among these markers, Randomly Amplified Polymorphic DNA (RAPD) is especially useful and users friendly as it does not require any sequence information (Dos Santos, *et. al.*, 1994; Thorman, *et. al.*, 1994; Link, *et. al.*, 1995). RAPD have been used widely to study genetic diversity, genome structure and gene tagging in various crops of commercial importance.

Common wheat (*Triticum aestivum* L.) is the most important researched crop in field of cytogenetics and molecular biotechnology. Genetic stock, C-banding karyotypes and genetic and physical map of wheat chromosomes have been developed (Sears, 1981; Gill, *et al.*, 1996). Recent developments regarding construction of deletion lines of common wheat (Endo & Gill, 1996) have greatly enhanced physical mapping in wheat. These physical maps help in isolation, cloning and utilization of useful genes in wheat and related species.

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Although lots of mapping has been done for wheat genome (A, B and D) and genetic maps for all the homoeologous group of wheat chromosomes have been developed, relatively low progress in high density mapping of wheat genome has been made as compared to the diploid species e.g., rice, maize, barley. Main reason behind slow progress in wheat mapping are triplication of genomes (A, B and D), larger genome size (16 billion bp) and high amount of repetitive sequences (Bennett & Smith, 1976). On the other hand availability of genetically compensating nullisomic–tetrasomic and ditelosomic lines developed by late E. R. Sears (Sears, 1956; 1966; 1981; Sears & Sears, 1978) and Bacterial Artificial Chromosomes (BAC) libraries (Yan, *et al.,* 2004) has made tremendous progress in mapping of a numbers of useful genes (for example *Vrn1, Vrn2*) in wheat genome.

Present research was undertaken to map Randomly Amplified Polymorphic DNA (RAPD) on chromosome 5A of common wheat using a set of deletion lines (Endo & Gill, 1996) involving both short and long arms of chromosomes 5A.

Materials and Methods

Material used during present study included 7 deletion lines of hexaploid wheat cultivar Chinese Spring (del5DS-1, del5AS-3, del5AS-7, del 5AS-10, del5AL-10, del 5AL-12 and del5AL-23). These lines developed by Endo & Gill (1996) were kindly provided by Dr. John Raupp, Wheat Genetic Resources Center, Plant Pathology Department, Kansas State University, USA.

Leaf samples were used as a source for isolation of total genomic DNA using protocol of Weinning & Langridge (1991). For removing RNA, DNA was treated with 2µl RNA-ase at 37^{O}C for 2 hours and then DNA samples were stored at 4^{0}C. For the use of PCR 1:4 dilution of DNA was made in double distilled, deionized and autoclaved water. Seven Randomly Amplified polymorphic DNA primers (OPA-01, OPA-02, OPA-03, OPA-05, OPA-06, OPA-O7 and OPA-17obtained from Operon technologies USA) were used to amplify the genomic DNA. PCR reactions were carried out in 25µl reaction using standard protocols (Devos & Gale, 1992). Amplification conditions involved an initial denaturation step of 4 min., at 94ºC followed by 40 cycles each consisting of denaturation step 1 min., at 94ºC followed by annealing step of 1 min., at 34ºC and an extension step of 2 min at 72ºC. All amplification reactions were performed using the Gene Amp PCR system 2700 (Applied Biosystem). The amplification products were electrophoresed on 2.0% agarose/TBE gels and visualized by staining with Ethidium bromide and viewed under UV light (Sambrook *et al.,* 1989).

Results and Discussion

During the present study a set of deletion lines of wheat having deletion on various segment of chromosome 5A were used in an attempt to identify chromosome/ chromosome arm/sub arm chromosome segment using Randomly Amplified Polymorphic DNA Primers.

Seven decamer (10bp) RAPD primers were used to amplify genomic DNA from six deletion lines involving deletion on short (3 deletion lines) and long (3 deletion lines) arms of chromosome 5A. Most of the primers (except OPA-07) showed complete homozygosity for the loci detected in the genetic stocks. The only useful polymorphism (missing band (s) in deletion line as compared to positive control (having intact chromosome 5A) was observed using RAPD primer OPA-07 where a double band of high molecular weight was missing in deletion line del5AL-23 (Fig. 1). It is therefore concluded that OPA-07 can be used as a molecular marker for chromosome 5A

(especially for long arm of chromosome 5A) of common wheat. C Banding karyotyping of deletion lines (www.graingenes.org) showed that deletion was on the distal half of 5AL. It is suggested that OPA-07 can be used as a marker for distal half of 5AL.

Because of smaller number of deletion lines and less numbers of RAPD primers used during present studies, it is recommended that more work should be carried out in this area for better understanding of deletion mapping and ultimately better understanding of genome structure of common wheat.

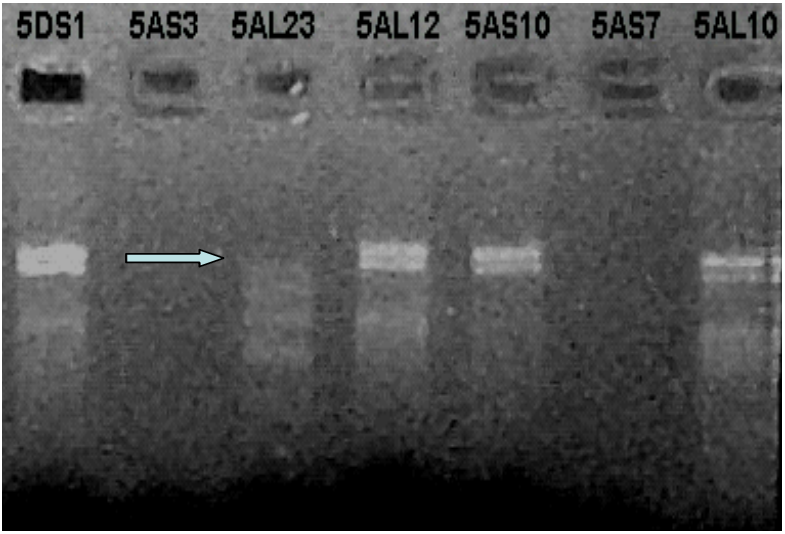

Fig. 1. PCR amplification profile of 7 deletion lines using RAPD primer OPA-07. Del5DS-1 was used as a positive control. The other 6 deletion lines for long and short arm of chromosome 5A of common wheat. Arrow indicates missing bands in del5Al-23.

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