Asian Journal of Agricultural Sciences 2(2): 51-52, 2010
ISSN: 2041-3890

Submitted Date: December 22, 2009 Accepted Date: January 11, 2010 Published Date: April 10, 2010

Identification of RAPD Marker for Chromosome 1D of Common Wheat

[1]Shaista Bibi, [1]Habib Ahmad, [2]Khaist Begum, [2]Sajid ul Ghafoor and [3]Imtiaz Ahmad Khan
[1]Department of Botany, Hazara University, Mansehra, Pakistan
[2]Department of Genetics, Hazara University, Mansehra, Pakistan
[3]Department of Genetics, Karachi University, Pakistan

Abstract: Development of genetically compensating nullisomic-tetrasomic and ditelosomic lines of common wheat (*Triticum aestivum* L.) have been widely used to construct high density genetic maps of homoeologous wheat chromosomes. During present research, easier, cheaper and quicker procedure of Polymerase Chain Reaction (PCR) was used to map Randomly Amplified Polymorphic DNA primers on chromosome 1D of common wheat. Genomic DNA was isolated from two genetic stocks of wheat cultivar Chinese Spring viz; NT-1D1B and NT-2A2B. PCR were conducted using RAPD primers GLC-07 and GLC-11. RAPD primer GLC-11 amplified a polymorphic allele of approximately 500 bp, which was present in NT-2A2B (used as positive control) but was absent in NT-1D1B indicating that the locus is present on chromosome 1D of common wheat. Hence this marker (GLC-11) can reliably be used to keep track of chromosome 1D of hexaploid wheat.

Key words: Biotechnology, chromosome mapping, DNA, molecular genetics, molecular marker, nullisomic tetrasomic lines, PCR, randomly amplified polymorphic DNA, triticum aestivum, wheat genetics

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) belongs to family Poeacea (gramineae) genus *Triticum*. Globally wheat is cultivated on more than 200 million ha with a total production of approximately 600 million tones (FAO, 2008). In Pakistan, wheat is cultivated on 8.303 million hectares with a total production of approximately 21 million tones with an average yield of 2.5 tonnes per ha. (MINFAL, 2007). Genetically bread /common wheat is an allohexaploid (AABBDD) having 2n = 6x = 42 chromosomes. These chromosomes belong to three different genomes A, B and D. Each genome has 7 pairs of chromosomes (1A-7A, 1B-7B and 1D-7D) (Sears, 1966).

Like any other crop of commercial importance, quality and quantity of wheat has to be increased continuously to support ever growing human population. This requires continuous breeding and release of new improved and high yielding varieties. A prerequisite of developing new varieties is the information regarding genetic maps of the wheat chromosomes. Sears (1966) developed compensating types of nullisomic-tetrasomic lines of wheat in Chinese Spring background. These lines have extensively been used for mapping of wheat chromosomes using various kinds of markers including morphological, cytological and biochemical markers (Islam and Shepherd, 1992). Recently DNA based markers including PCR, Amplified Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Single nucleotide Polymorphism (SNP) are being used for mapping of wheat chromosomes (Tsujimoto *et al*., 2000). Among these markers systems, Polymerase Chain Reaction based assays are cheaper, easier and faster. Among various PCR based assays, Randomly Amplified Polymorphic DNA primers (RAPD) are more important because they do not need any sequence information (Shiran *et al*., 2006). Previously expensive, time consuming and technically difficult procedure of RFLP were used to map wheat chromosomes. During present study, RAPD primers were mapped on wheat chromosome 1D using nullisomic-tetrasomic lines.

MATERIALS AND METHODS

Cytogenetic stock of wheat cultivar Chinese Spring (CS) viz; nullisomic-tetrasomic 1D1B (NT-1D1B) developed by Sears (1966) were kindly provided by Professor Jorge Dubcovsky, Department of Agronomy and Range Science, University of California, Davis, USA. Seeds of nullitetra line 2A2B was kindly supplied by Dr. John Raupp, Wheat Genetics and Genomics Research Centre, Kansas Sate University, USA. This line NT-2A2B was used as positive check for chromosome 1D because it had a complete intact chromosome 1D. Plants were grown in pots at the Department of Botany, Hazara University, Mansehra, during winter 2008 using recommended agricultural practices.

A small scale DNA isolation procedure developed by Weining and Langridge (1991) was used with minor

Corresponding Author: Imtiaz Ahmad Khan, Department of Genetics, Karachi University, Pakistan

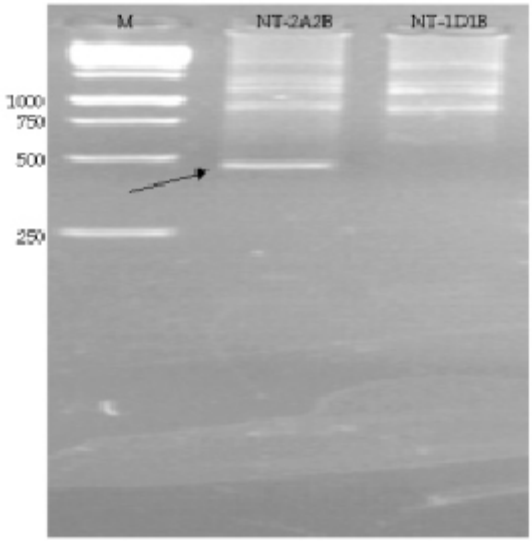

Fig. 1: PCR amplification profile of two genetic stocks NT-1D1B and NT-2A2B using RAPD primer GLC-11. M = Molecular size marker (1kb DNA ladder, Gene Link, USA). Size of DNA fragments (in bp) are presented on left. An arrow indicates the diagnostic band for chromosome 1D of common wheat

modifications to isolate total genomic DNA from the leaves. Quality and quantity of the DNA was checked on 1% agarose/TBE gel. Randomly Amplified Polymorphic DNA (RAPD) primers GLC-07 and GLC-11 (obtained from Gene Link, Inc, 10532, NY, USA) were used to amplify genomic DNA isolated from the two nullisomic-tetrasomic. PCR reactions were carried out using standard procedure (Devos and Gale, 1993). The PCR Products were electrophoretically separated on 2% Agarose gel and visualized under UV light by staining with Ethedium Bromide and documenting using "Uvitech" gel documentation system.

RESULTS AND DISCUSSION

Initially RAPD primer GLC-07 was used to amplify genomic DNA isolated from the two genetic stocks. A single band of 1100 bp was observed in both the lines indicating that the primer annealed at a locus, which was not present on chromosome 1D. The next RAPD primer used to obtain useful polymorphism for chromosome 1D was GLC-11 (Fig. 1). One useful (polymorphic) band of approximately 500 bp was amplified in NT-2A2B (arrowed in Fig. 1) but the fragment was absent in the NT-1D1B line, which indicated that the locus is present on the chromosome 1D of common wheat. This DNA fragment can be used as a reliable marker to keep track of chromosome 1D of *Triticum aestivum*. Present research supported earlier work where PCR based assays were used to map/tag genes of agronomic importance in wheat (Tsujimoto *et al*., 2000, Uauy *et al*., 2005)

It is recommended that work similar to the one reported in this paper should be conducted on large scale so that more RPAD primers can be mapped on wheat homoeologous chromosomes, which will ultimately help in construction of high-density genetic map of wheat genome based on RAPDs. Giving due consideration to comparatively easier, cheaper and faster nature of PCR based assay, it is assumed that the approach will be more help full for developing countries like Pakistan where it is not feasible to work with more difficult, expensive and time consuming technique of RFLP.

REFERENCES

Devos, K. and M.D. Gale, 1993. The genetic maps of wheat and their potential in plant breeding. J. Agr., 22: 93-99.

FAO, 2008. Retrieved from: http://faostat.fao.org/site/567/DesktopDefault. aspx?PageID=567#ancor

Islam, A.K.M.R. and K.W. Shepherd, 1992. Production of wheat-Barley recombinant chromosomes through induced homoeologous pairing, 1. Isolation of recombinants involving barley arms 3HL and 6HL. Theor. Appl. Genet., 83: 489-494.

MINFAL, 2007. Agricultural Statistics of Pakistan. Ministry of Food Agriculture and Livestock, Islamabad, Pakistan.

Sears, E.R., 1966. Nullisomic-Tetrasomic Combinations in Hexaploid Wheat. In: Riley, R. and K.R. Lewis, (Eds.), Chromosome Manipulation and Plant Genetics. Oliver and Boyd, Edinburgh, pp: 29-45.

Shiran, B., R. Azimkhani, S. Mohammadi and M.R. Ahmadi, 2006. Potential use of random amplified polymorphic DNA marker in assessment of genetic diversity and identification of rapeseed (*Brasssica napus L.)* cultivars. Iran. Biot., 5(2): 153-159.

Tsujimoto, H., K. Mochida and K. Kishii, 2000. Expansion of Available Genetic Resources for Wheat Improvement by Super Wide Hybridization. In: Oono, K.L., (Ed.), Integration of Biodiversity and Genome Technology for Crop Improvement. National institute of Agrrobiology Resources, Tsukuba, Japan, 181: 100-102.

Uauy, C., J.C. Brevis, X. Chen, I.A. Khan, L. Jackson, O. Chicaiza, A. Distelfeld, T. Fahima and J. Dubcovsky, 2005. High-Temperature Adult Plant (HTAP) stripe rust resistance gene *Yr-36* from *Triticum turgidum* ssp. *dicoccoides* is closely linked to the grain protein content locus *Gpc-B1*. Theor. Appl. Genet., 112: 97-105.

Weining, S. and P. Langridge, 1991. Identification and mapping of polymorphism in cereals base on polymerase chain reaction. Theor. Appl. Genet., 82: 209-216.