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Identification of Rapd Primers Linked to Leaf Rust Resistant Gene *Lr51* Introgressed from *Triticum speltoides* to Common Wheat (*Triticum aestivum* L.)

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Abstract: Leaf rust is the most important disease of wheat all over the world. Geneticists and breeder are now extracting new leaf rust resistant genes from wild relatives of wheat. An important leaf rust resistant gene *Lr51* has been transferred from *Triticum speltoides* to common wheat. Present research was carried out to develop Randomly Amplified Polymorphic DNA (RAPD) based marker for the leaf rust resistant gene *Lr51*. Out of seven RAPD primers used, one RAPD prime viz; GLA-09 was found which could be used reliably to keep track of *Lr51* in wheat background. This primer (GLA-09) amplified a polymorphic DNA fragment of approximately 1100 bp in the wheat lines having the leaf rust resistant gene *Lr51*.

Key words: Leaf rust, *Lr51*, molecular marker, PCR, RAPD, *Triticum aestivum*, *Triticum speltoides*

INTRODUCTION

Common wheat (*Triticum aestivum* L.) is a segmental hexaploid having 2n = 6x = 42 chromosomes which belong to three different genomes A, B and D. These genomes are homoeologous to each other (Sears, 1966). In Pakistan wheat is grown as a rabi (cold) season crop on an area of approximately 8 million hectares with a total production of approximately 21 million tones giving an average yield of 2.6 tons / hectare (MINFAL, 2007). Average yield of wheat in Pakistan is 3 times less than national average yield of wheat in developed countries like USA, Australia, Canada etc. (Anonymous, 2007). Main reason behind low production of wheat in the country is rust disease caused by fungus Puccinia. Three main types of rust effect wheat crop all over the world viz; (i) leaf rust caused by *Puccinia triticina,* (ii) stem rust caused by *Puccinia graminis* and (iii) stripe or yellow rust caused by *Puccinia striitiformis* (McIntosch *et al*., 1995). Planed scientific breeding over the past 100 years has produced high yielding uniform lines of wheat but eroded natural genetic variability once existed in landraces of wheat (Sears, 1981). Wheat breeders all over the world are extracting useful rust resistant genes from wild relatives of wheat including *Triticum speltoides*. A leaf rust resistant gene *Lr51* has been transferred from chromosome 1S of *Triticum Speltoides* (2n = 2x = 14, genomically SS) to chromosome 1B of common wheat. This gene (*Lr51*) is present on an interstitial segment (approximately 14-32 cM long) translocated to long arm of chromosome 1B of common wheat and offers high level of resistance against new races of leaf rust pathogen (Helguera *et al*., 2005). To facilitate transfer of *Lr51* in Pakistani germplasm, it is important to develop an easy, cheap and reliably scoreable marker for the alien segment carrying the *Lr51* gene. During present study, seven Randomly Amplified Polymorphic DNA (RAPD) primers were screened to find a suitable marker for *Lr51*, which can be routinely used to keep track of *Lr51* in Pakistani wheat breeding programs.

MATERIALS AND METHODS

Seeds of near isogenic lines of common wheat cultivar Yecora Rojo and Yecora Rojo + *Lr51* were kindly provided by Dr. J. Dubcovsky, University of California, Davis. Plants were grown in pots at the Department of Genetics, Hazara University during 2008, using recommended agricultural practices. At 3-4 leaf stage, approximately 10 cm long fresh leaves (0.5 g) were used to isolate total genomic DNA using small scale DNA isolation procedure described by Weining and Langridge (1991). Polymerase Chain Reactions using Randomly Amplified Polymorphic primers (obtained from Gene Link, 1052, NY, Inc, USA) were carried out following standard procedure (Khan *et al*., 2000). PCR products were separated on 2% agarose/TBE gels. Gels were stained with ethedium bromide and visualized under UV light using "Uvitech" gel documentation system. Considering general concern regarding reproducibility of RAPDs, all the reactions were repeated twice with independently isolated DNA samples.

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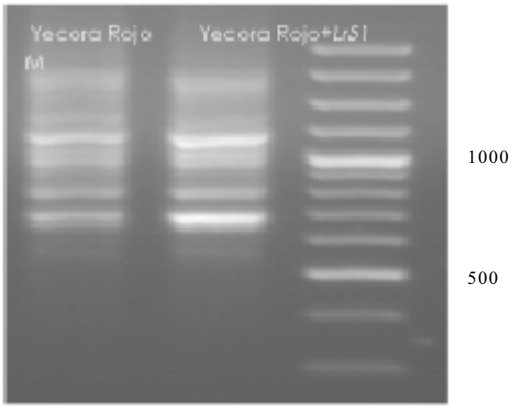

Fig. 1: PCR amplification profile of near isogenic lines of wheat (Yecora Rojo and Yecora Rojo+*Lr51*) using RAPD primer GLA-20. M= Molecular size marker (100 bp DNA ladder, Gene Link). Molecular weight (in bp) is presented on right

RESULTS AND DISCUSSION

Seven RAPD primers viz; GLA -07, GLA-09, GLA-20, GLB -05, GLC-12, GLC-16 and GLC-20 were used to amplify total genomic DNA isolated from two near isogenic lines of wheat viz; Yecora Rojo and Yecora Rojo+ *Lr51*. Examples of PCR amplification profiles using RAPD primers GLA-20 and GLA-09 are presented in Fig 1 and 2, respectively. DNA fragments of various size (ranging from 400-1300 bp) were amplified using seven RAPD primers. On an average 4.3 alleles per genotype were amplified during present study. No useful polymorphism was detected using GLA -07, GLA-20, GLB -05, GLC-12, GLC-16 and GLC-20 RAPD primers (an example is presented in Fig. 1). RAPD primers GLA-09 amplified a polymorphic DNA fragment of approximately 1100 bp (size of fragment estimated using 100 bp DNA ladder, Gene Link) specific for near isogenic line Yecora Rojo+*Lr51*. This specific band (arrowed in Fig. 2) was absent in near isogenic line Yecora Rojo which suggested that the RAPD primer GLA-09 can be used to keep track of rust resistant gene *Lr51* introgressed from *Triticum speltoides* into hexaploid wheat background.

In the past, *Lr51* and other alien genes introgressed in common wheat were mostly tagged using Restriction Fragment Length Polymorphism (RFLP) based assay (Dubcovsky *et al*., 1998). The technique though is highly reliable but comparatively more expensive, time consuming and potentially hazardous than Polymerase Chain Reaction (PCR). PCR markers using specific primers were previously used to develop markers for various rust resistance genes (Autrique *et al*., 1995). An added advantage of using RAPD primers during present

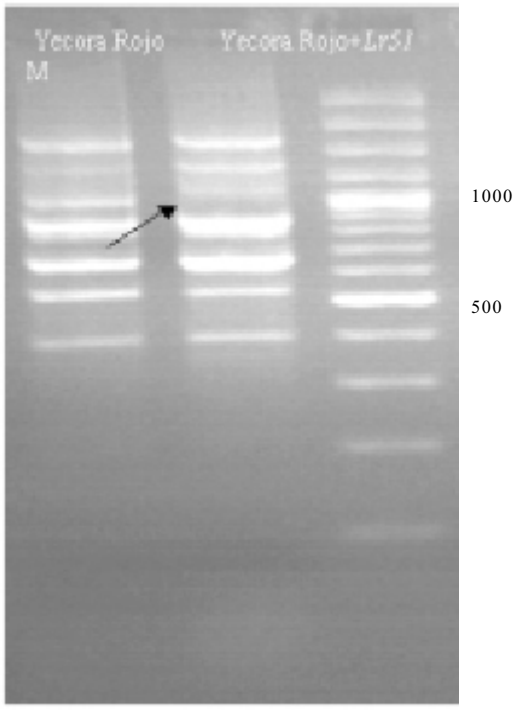

Fig. 2: PCR amplification profile of near isogenic lines of wheat (Yecora Rojo and Yecora Rojo+*Lr51)* using RAPD primer GLA-09. M= Molecular size marker (100 bp DNA ladder, Gene Link). Molecular weight (in bp) is presented on righht. Arrow indicate *Lr51* specific DNA fragment

work was that RAPDs do not require any sequence information on the target DNA (Rafalski *et al*., 1996). Identification of 1 out of 7 primers used during present work indicated that RAPDs can be used reliably for tagging of commercially important genes and giving due consideration to cheaper cost per assay, it is possible to use these RAPD markers to screen larger segregating populations of local wheat breeding programs.

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