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Development of Molecular Markers for Leaf Rust Resistance Genes Incorporated from Alien Species into Common Wheat

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Abstract: National average yield of wheat in Pakistan is only 2.54 tons per hectare. One of the main reason for low yield of wheat in Pakistan is leaf rust disease. During present study, RAPD based molecular markers were developed for three leaf rust resistance genes (*Lr47*, *Lr37* and *Lr51*) incorporated to wheat from wild relatives. Three sets of near isogenic lines (Anza, Anza+*Lr37,* Kern, kern+*Lr47,* Yecora Rojo and Yecora Rojo+*Lr51*) of wheat were used. A total of 12 RAPD primers were used to identify alien rust resistant genes. One (OPA07), three (OPA12, OPA15, OPB11) and two (OPA11, OPB11) RAPD primers were identified which produced specific bands for alien rust resistant genes *Lr47*, *Lr51* and *Lr37*, respectively.

Key words: Alien genes, common wheat, *Lr37*, *Lr47*, *Lr51*, RAPD, Rust

INTRODUCTION

Common (Bread) wheat (*Triticum aestivum* L.) belongs to family gramineae. Genomically it is an allohexaploid, AABBDD, having 2n = 6x-42 chromosomes. Planned scientific breeding of wheat has resulted in the production of high yielding pure line varieties. But it eroded genetic variability of land races (Frankel, 1970; Sears, 1993). Wild relatives of wheat including *Triticum speltoides*, *Triticum ventricosum*, *Triticum monococcum*, *Secale cereal*, *Hordeum vulgare* etc. are rich sources of a number of valuable genes, especially those responsible for resistance to a-biotic (cold, drought and salt) and biotic (fungus and inset pests) stresses and can be utilized for wheat improvement (McIntosh *et al*., 1995).

Wheat rust disease (caused by fungus *Puccinia triticinaea*) is the most important disease of wheat all over the world including Pakistan. There are 3 types of rust (i) leaf rust or brown rust (caused by *Puccinia recondita*) (ii) stem rust or black rust (caused by *Puccinia graminis* and (iii) stripe rust or yellow rust (caused by *Puccinia striiformis*). Rust epidemics can cause considerable damage (upto 80%) to wheat yield in Pakistan (Hussain *et al*., 1987). Presently wheat breeders and geneticists are extracting rust resistant genes from wild relatives of wheat and utilizing them for wheat improvement. A leaf rust resistance gene *Lr47* was derived from short arm of chromosome 7S of *Triticum Speltoides* and translocated onto short arm of chromosome 7A of wheat (Heulgara *et al*., 2000). Similarly a rust resistant gene *Lr37* has been introgreessed into common wheat from short arm of chromosome 2N of *Triticum ventricosum* (Helguera *et al*., 2003). Another leaf rust resistance gene *Lr51* has been transferred from *Triticum speltoides* to common wheat (Helguera *et al*., 2005). During present study RAPD based molecular markers were developed for alien rust resistance genes incorporated in wheat from *T. speltoides* (*Lr47*, *Lr51)* and *T. ventricosum* (*Lr37*).

MATERIALS AND METHODS

Three set of near isogenic lines (with and without alien gene responsible for rust resistance) of common wheat were obtained from Professor Jorge Dubcovsky, Department of Agronomy and Range Science, University of California, Davis, USA (Chicaiza *et al*., 2006).

1 = Yecroa Rojo, 2 = Yecra Rojo + *Lr51*, 3 = Anza, 4 = Anza+*Lr37*, 5 = Kern and 6 = Kern+*Lr47*

Small scale DNA isolation protocol described by Weining and Langridge (1991) was used to isolate total genomic DNA from young and fresh leaves of the plants. Plants were grown in pots at department of Botany, Hazara University, Mansehra during 2008. About 14 days after germination, 0.5 g fresh leaves were collected in 1.5 mL eppendorf tubes which were placed immediately in liquid Nitrogen. Leaf material was crushed with a knitting needle to a fine powder. Five hundred micro liter DNA extraction buffer was added and mixed well. Five hundred µL of Phenol:Chlorofom:Isoamyalcohol (in ratio of 25:24:1) was added and vortex until homogenous mixture was obtained. The tubes were then centrifuged at 5000 rpm for 5 min. Aqueous phase were transferred to a fresh

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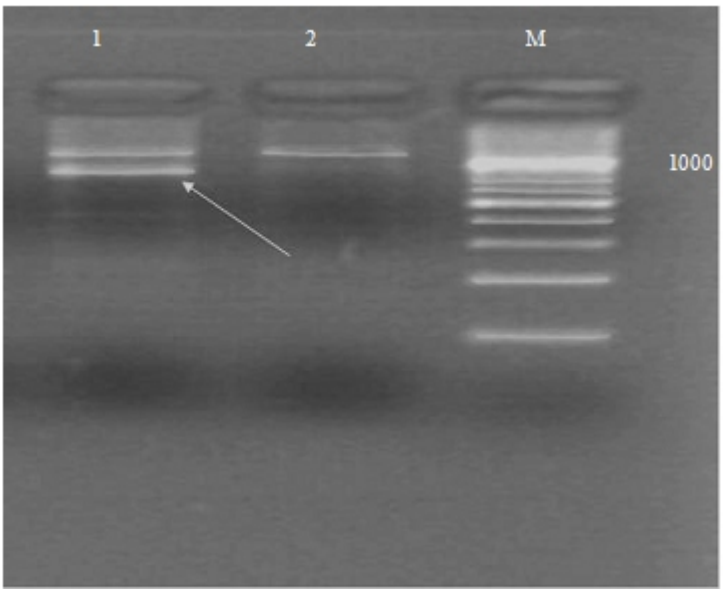

Fig. 1: PCR amplification profile of near isogenic lines of wheat [(Kern+*Lr47* (lane 1) and Kern (lane 2)] using RAPD primer OPD-3. Arrow indicates *Lr47* specific alleles

tube and 50 µL 3M Sodium Acetate (pH = 4.8) and 500 µL Isopropanol was added and mix gently. Tubes were centrifuged at 5000 rpm for 5 min to make the DNA pallet. Supernatant was discarded and DNA pallet was dissolved in 40 µL TE (Tris-EDTA buffer). Quality and quantity of DNA was checked on 1% Agarose/TBE gel. Gels were stained with Ethedium Bromide and visualized under UV light using "Uvitech" gel documentation system. Polymerase Chain Reaction (PCR) was carried out using standard technique (Devos and Gale, 1992). Seventeen Randomly Amplified Polymorphic DNA primers viz; OP -A2, -A3, -A6, -A7, -A9, -A11, -A12, -A14, -A15, -B11, -C3 and -D3 (obtained from Operon Technologies, USA) were used during present study.

RESULTS AND DISCUSSION

During present research, Randomly Amplified Polymorphic DNA (RAPD) primers were used to tag rust resistance genes incorporated from alien species (*Triticum speltoides* and *Triticum ventricosum*) into homoeologous chromosomes of common wheat (*T. aestivum* L.). A total of 12 RAPD primers (OPA02, OPA03, OPA06, OPA07, OPA09, OPA11, OPA12, OPA14, OPA15, OPB11, OPC03, OPD03) were used to identify rust resistant genes introgressed in common wheat. An example of PCR amplification of near isogenic lines Kern and Kern+*Lr47* using RAPD primer OPA-7 is presented in Fig. 1. A very strong diagnostic band of approximately 1000 bp was amplified in the near Isogenic line Kern+*Lr47* (indicated by an arrow in Fig. 1. This band was absent in the susceptible parent "Kern" and can be used reliably as marker for the presence of leaf rust resistance gene *Lr47* incorporated to short arm of chromosome 7A of common wheat from short arm of chromosome 7S of *Triticum speltoides*.

One (OPA07), three (OPA12, OPA15, OPB11) and two (OPA11, OPB11) RAPD primers were identified which produced specific bans for alien rust resistant genes *Lr47, Lr51* and *Lr37*, respectively. While 4 RAPD primers (OPA07, OPD03, OPA09 and OPC03) produced wheat homoeologous chromosomes specific bands. These wheat specific primers (OPA07 and OPD03 for chromosome 7A, OPA09 and OPC03 for chromosome 2A of wheat) cannot be used for identification of alien rust resistant genes in wheat background but can be used for mapping wheat homoeologous chromosomes. For 3 RAPD primers OPA06 and OPA02 no useful polymorphism was detected in the sets of near isogenic lines used during present study.

Present findings further strengthened previous reports (Khan, 2000; Khan and Shapherd, 1995; Autrique *et al*., 1995, Heulgara *et al*., 2000, 2003, 2005) where various PCR based markers (RAPDs, ASA, CAPS etc.) were used to map rust resistant genes incorporated from alien species into wheat chromosomes.

CONCLUSION

The RAPD markers developed during present study will also be useful in future to reduce the translocated alien segment in wheat background. In this regards it is

also suggested that more work should be done to identify more molecular markers for the resistant genes, so that more precise shortening of alien segments can be carried out. This shortening of alien segment can be done by using second round of *ph1b* induced homoeologous recombination and presently developed RAPD markers.

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