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Full Length Research Paper

Development of RAPD based markers for wheat rust resistance gene cluster (*Lr37-Sr38-Yr17*) derived from *Triticum ventricosum* L.

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Rust diseases are the major cause of low yield of wheat in Pakistan. Wheat breeders all over the world as well as in Pakistan are deriving rust resistance genes from alien species like *Triticum ventricosum* and introducing them in common wheat (*Triticum aestivum*). One such example is the introgression of rust resistance gene cluster *Lr37-Sr38-Yr17* derived from *T. ventricosum* chromosome 2NS into the common wheat. A basic prerequisite to introduce alien rust resistance gene (like those present on 2NS segment) in locally adapted varieties is availability of a suitable marker system which can be used to keep track of presence of newly added gene in the old background. In this present study, one hundred and fifty Randomly Amplified Polymorphic DNA (RAPD) primers were used to detect polymorphism between two near isogenic lines NILs (Anza and Anza+2NS) of wheat and to develop RAPD based molecular markers for rust resistance gene cluster derived from *T. ventricosum*. Polymerase chain reactions were carried out using standard protocols. All the amplification products were in the range of 250 to 1000 bp. Thirteen molecular markers (RAPDs) out of a total of 150 (approximately 8.6%) were developed for rust resistance gene cluster *Lr37-Sr38-Yr17* and recommendations have been made to utilize these markers in Pakistani wheat breeding programs aimed at establishing rust resistant germplasm.

Key words: Wheat, RAPD markers, rusts resistance genes.

INTRODUCTION

One of the reasons of reduced yield of wheat in Pakistan is rust diseases (Hussain et al., 1980). Rusts are the most destructive and widespread diseases of wheat. Wheat rust pathogens belong to genus, *puccinia*; family, *pucciniaceae*; order, *uredinales* and class, *basidomycetes*. In wheat, rusts are of three types; (1) leaf rust or brown rust, (2) stem rust or black rust and (3) stripe rust or yellow rust. Losses due to leaf rust over large areas are generally light to moderate, 1 - 20%. Individual fields can be destroyed when the disease is severe prior to heading. In stem rust, losses are often severe (50 - 70%). Losses of 40% can be caused by yellow rust with some fields totally destroyed. Severe losses result when spikes are infected (USDA, 2006). To control these diseases, the best strategy is to use rust resistance genes (*Lr, Sr, Yr*) and introduce them into the local bread wheat varieties. There are many rust resistance genes in world wheat germplasm (McIntosh et al., 1995). Many rust resistance genes (e.g., *Lr47, Yr15, Yr5 etc.*) have been derived from wild relatives of wheat. An important rust resistance gene cluster (*Lr37-Sr38-Yr17*) is located within a segment of short arm of chromosome 2N of *T. ventricosum*. This 2NS chromosome segment was translocated to the short arm of bread wheat chromosome 2A (Helguera et al., 2003). The *Lr37-Sr38-Yr17* gene cluster confers resistance in wheat against, leaf rust (caused by *Puccinia triticina*), stem rust (caused by *Puccinia graminis*) and stripe rust (caused by *Puccinia striiformis*), respectively (Dyck and Lukow, 1988; McIntosh et al., 1995; Robert et al., 1999; Seah et al., 2000).

To benefit from the rust resistance gene cluster derived from 2NS, the recombinant 2AS-2NS chromosome has to

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be incorporated in Pakistani wheat varieties which are well adapted to the local environmental conditions. To keep track of *Lr37- Sr38-Yr17* cluster of rust resistance genes in segregating populations, it is essential to have markers linked to the gene cluster. In the past, various kinds of markers like morphological markers, cytological markers and biochemical markers were used to detect alien genes incorporated in wheat. These markers, though successful in many cases, were not considered suitable for large scale utilization mainly because these markers were limited in number, expensive to use and time consuming.

Recent introduction of DNA technology has generated a large number of molecular markers. Molecular markers have significant value in breeding programs to characterrize and evaluate genetic variability in germplasms and to identify varieties (Chao, 2006).

The PCR based assays are relatively easier, cheaper than RFLPs/AFLPs and are more user's friendly, as they do not involve any hybridization with radioactive material and can be used to screen larger segregating populations which are commonly handled in routine wheat breeding programs to generate new sustainable varieties (Qi et al., 1996). Among PCR based assays, Randomly Amplified Polymorphic DNA (RAPDs) are more effective and easier than specific PCR based assays because they do not require sequence information. Since the RAPD technique does not require any previous knowledge of the target genome, and is relatively simple and rapid to carry out, RAPD markers have been extensively used in population genetic analysis of biodiversity, and studies of relationships among species at different levels (Thormann and Osborn, 1992; Williams et al., 1993; Heun et al., 1994). In cereal crops such as wheat (Cao et al., 1999), rice (Yu and Nguyen, 1994) and Barley (Tinker et al., 1993; Marillia and Scoles, 1996; Fernandez et al., 2002), the technique has been applied to identify cultivars and revealing phylogenetic relationships among them.

This present study was carried out to analyze near isogenic lines of wheat (Anza and Anza+2NS) without and with rust resistance gene cluster (*Lr37-Sr38-Yr17*) introgressed into common wheat from *T. ventricosum*. RAPD primers were used to develop molecular markers for the alien chromatin (2NS) carrying rust resistance gene cluster. The RAPD based markers developed during present study can be used to keep track of 2NS segment (*Lr37-Sr38-Yr17*) in local wheat breeding populations during hybridization and selection procedures.

MATERIALS AND METHODS

Plant material

Seeds of near isogenic lines of wheat variety "Anza" (without rust resistance gene cluster) and "Anza + 2NS" (with rust resistance gene cluster *Lr37-Sr38-Yr17*) were obtained from Prof. J. Dubcovsky's lab at the Department of Agronomy and Range Science, University of California Davis, USA. The seeds were grown at the green house of the Institute of Biotechnology and Genetic Engineering, NWFP Agricultural University, Peshawar, Pakistan during growing sea-son 2005-2006. Recommended cultural practices were carried out.

DNA extraction

Approximately 10 cm, young and fresh leaves were collected from plants in the glass house and put in eppendorf tubes and subsequently dropped in liquid Nitrogen to freeze the plant material. The plant material was crushed with knitting needle to a fine powder. Five hundred µl DNA extraction buffer (1%SDS, 100 mM NaCl, 100 mM tris base, 100 mM Na_2EDTA, pH 8.5 by HCl) was added to each eppendorf tube containing the crushed leaf material and mixed well with the knitting needle. Equal volume (500 µl) of phenol : chloroform : isoamylalcohol (ratio of 25:24:1) was added to the tubes and tubes were shaken until a homogenous mixture was obtained. These tubes were centrifuged at 5000 rpm for 5 min. The aqueous phase was transferred to fresh tubes. One-tenth volume (50 µl) of 3 M sodium acetate (pH 4.8) and equal volume (500 µl) isopropanol was added in the tubes and mixed gently to precipitate the DNA. The samples were centrifuged at 5000 rpm for 5 min to make the DNA pellet. After pouring the supernatant, the pellet was washed with 70% ethyl alcohol and was dried at room temperature for an hour and re-suspended in 40 µl TE buffer (10 mM Tris, 1mM EDTA, pH 8.0; Weining and Langridege, 1991). DNA was treated with 40 µg RNAse A at 37°C for 1 h to remove RNA and was stored at 4°C. A 1:4 dilution of DNA was made in doubled distilled, deionized and autoclaved water to use in PCR.

Ten base pair RAPD primers were obtained from Genelink Inc., New York, USA. PCR reaction was carried out in 25 µl reaction containing 1 µl total genomic DNA solution, 0.25 µM of primer, 200 µM of each dATP, dGTP, dCTP, dTTP, 50 mM KCl, 10mM Tris, 1.5 mM $MgCl_2$ and 2.5 units of Taq DNA polymerase (Devos and Gale, 1992). Amplification conditions were an initial denaturation step of 4 min at 94°C followed by 40 cycles each consisting of a denaturation step of 1 min at 94°C, annealing step of 1 min at 34°C, and an extension step of 2 min at 72°C. The last cycle was followed by 10 min extension at 72°C. All amplification reactions were performed using Gene Amp PCR System 2700 (Applied Biosystem) programmable thermocycler. The amplification products were electrophoressed on 1.5 - 2.0% agarose/TBE gel and visualized by staining with ethidium bromide under ultra-violet (U.V.) light. The gels were photographed using computer programme "UVitech".

A total of 150 Randomly Amplified Polymorphic DNA (RAPD) primers (obtained from Gene Link Inc., NY 10532, USA) were used during present study to detect polymorphism between Anza and Anza+2NS.

RESULTS AND DISCUSSION

Near isogenic lines of Anza and Anza+*Lr37-Sr38-Yr17* were used to develop PCR based molecular markers for rust resistance gene cluster (*Lr37-Sr38-Yr17*) derived from short arm of chromosome 2N of *T. ventricosum*. Quality of DNA isolated from the two test entries using mini-prep "DNA isolation procedure" was checked using 1% agarose/TBE gel. Two samples from each test entry were isolated. Different amplification patterns were observed using different primers. In general, amplification products ranged in size (estimated by using 1 Kb ladder molecular size marker, Gene Link) from 250 bp – approximately 1000 bp. For genetic analysis and molecular

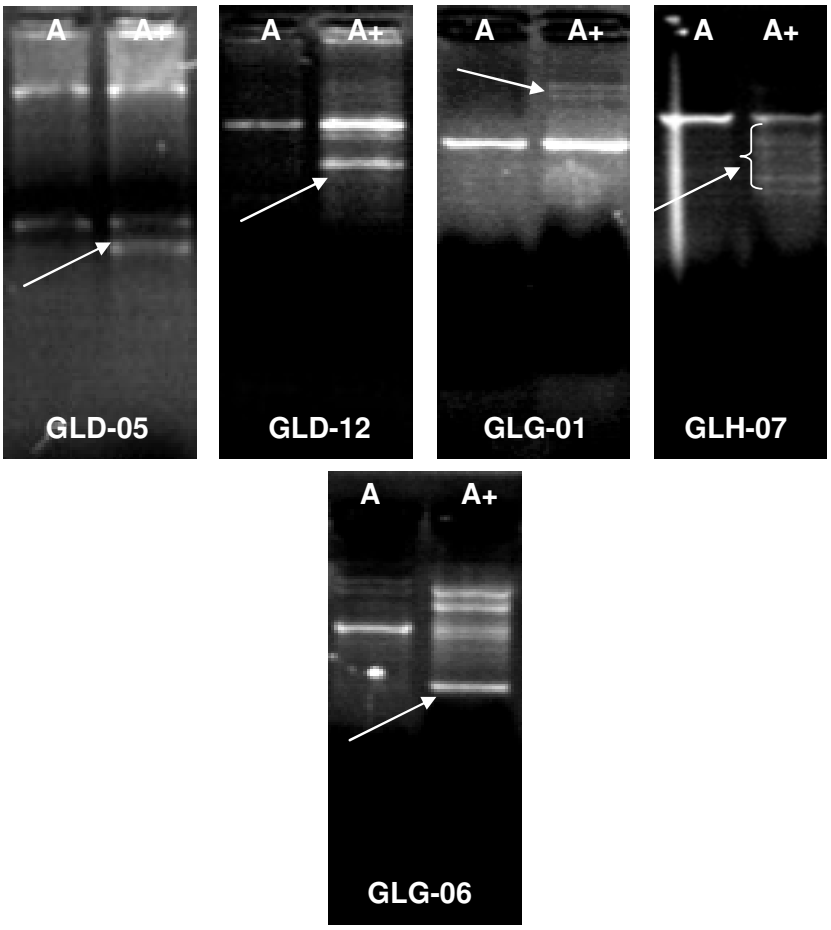

Figure 1. PCR amplification of near isogenic lines Anza and Anza+*Lr37-Sr38-Yr17* using rust resistance gene cluster (*Lr37-Sr38-Yr17*) specific primers.
A = Anza, A+ = Anza+ *Lr37-Sr38-Yr17*, Arrow indicates *Lr37-Sr38-Yr17* Specific bands

tagging of rust resistance gene cluster, each individual band was considered as a single locus/allele. Alleles were scored as present (1) or absent (0). Out of 150 primers used during present study, only 25 showed polymorphic bands specific to either 2NS (carrying *Lr37-Sr38-Yr17*) or wheat 2AS specific alleles. Among the 25 polymorphic primers, 13 produced poly-morphic bands for the chromosome segment carrying (*Lr37-Sr38-Yr17*) rust resistance gene cluster while 20 primers amplified wheat specific bands (Figure 1).

Common wheat (*Triticum aestivum* L.) is the world's most important food crop and ranks number one among food crops in Pakistan. Almost all of the wheat grown in Pakistan is of hexaploid type (2n = 6 x = 42) which is used to make bread, *chapati* and *nan*. On world basis, only 5% wheat grown is of durum type (2n = 4x = 28) which is mostly used to produce pasta, noodles and biscuits etc (FAO, 2003). Annual production of wheat in Pakistan is around 20 million tones while annual need of country is around 26 million tones (MINFAL, 2004, 2005). Every year Pakistan has to import approximately 5-6 million tones of wheat. One of the main reasons behind low production of wheat in Pakistan is rust diseases which may cause reduction up to 70% in grain yield of wheat (Hussain et al., 1980). Due to continuous evolution in pathogen fungus (*Puccinia*), addition of new rust resistance genes in wheat cultivars/germplasm is of primary importance. There are number of leaf (*Lr*), stem (*Sr*) and stripe (*Yr*) rust resistance genes available in global wheat germplasm which have been widely used in improvement of wheat over the past years. Some breeders/geneticists (Frankle 1970; Sears, 1981) believed that natural genetic diversity once present in wheat is now reducing due to replacement of highly variable but low yielding land races with high yielding but pure line varieties. Wheat breeders are now looking towards wild relatives of wheat to extract new useful genes especially those conferring resistance to wheat rusts (McIntosh et al., 1995). In such an attempt, Helguera et al. (2003) utilized a chromosome segment from short arm of chromosome 2N of *T. ventricosum*

(2NS) which carries 3 rust resistant genes viz., *Lr37* (confers resistance to leaf rust), *Sr38* (confers resistance to stem rust) and *Yr17* (confers resistance to stripe/yellow rust). The three rust resistance genes present on 2NS segment have been found effective against a wide range of rust pathotypes and also carries no significant penalty to grain yield (Heluegra et al., 2003).

Our ultimate aim is to introduce chromosome segment 2NS (carrying *Lr37-Sr38-Yr17* rust resistance gene cluster) in Pakistani wheat varieties so that rust resistant germplasm would be generated which can be used for the development of locally adapted, high yielding rusts resistant varieties for Pakistan agricultural system. A basic prerequisite to introduce alien rust resistance gene (like those present on chromosome 2NS segment) in locally adapted varieties is availability of a suitable marker system which can be used to keep track of presence/absence of newly added alien gene in the local background. Previously, morphological, cytological and biochemical markers were used to identify the alien genes in wheat background (for review see Islam and Shepherd, 1991). These markers although successful in many cases (Koebner and Shepherd, 1985; 1986) were not considered suitable mainly because of absence of such markers over a large part of wheat and/or alien genome. Development in molecular biology, especially development of practically unlimited number of molecular markers has transformed the opportunities of utilizing DNA based markers for introgression of useful alien genes in wheat background (Paterson et al., 1991). Various kinds of PCR based assays have been used to detect alien genes in wheat background e.g. RAPD (Randomly Amplified Polymorphic DNA; Williams et al., 1993), SSR (Simple Sequence Repeats; Plaschke et al., 1995), ASA (Allele Specific Amplification), ISJ (Interon Splice Junction Primers; Weining and Langridge, 1991) and STS (Sequence Tag Sites; Kleinhofs et al., 1993), etc. Random Amplified Polymorphic DNA primers (RAPDs) are much easier than specific primer sets because in case of RAPDs sequence information is not required and hence they can be used routinely to screen larger segregating/breeding populations which are commonly used in wheat breeding programs.

During the present study, one hundred and fifty RAPD primers were used to detect polymorphism between two near isogenic lines (NILs) (Anza and Anza+2NS) of wheat. Two kinds of polymorphism were detected (i) where specific allele(s) on short arm(s) of homologous group 2 chromosome of wheat were amplified and (ii) where introgressed segment of chromosome arm 2NS was amplified (for example 2NS specific amplification using RAPD primer GLD-12). 25 out of a total of 150 primes were found polymorphic between Anza and Anza+2NS. Among these 25 polymorphic primers 13 produced 2NS specific fragments and 20 produced wheat specific fragments. The RAPD primers yielding 2NS specific amplification were GLC-16, GLD-03, GLD-05, GLD-12, GLD-20, GLG-01, GLG-05, GLG-06, GLG-13, GLG-19, GLH-07, GLH-17 and GLH-18. Wheat specific amplification were yielded by RAPD primers GLB-11, GLC-16, GLC-19, GLD-02, GLD-03, GLD-20, GLE-05, GLE-08, GLF-02, GLG-02, GLG-04, GLG-05, GLG-06, GLG-10 GLG-19,GLH-03, GLH-14, GLH-15, GLH-17 and GLH-18.

The development of 13 out of 150 (approximately 8.6%) RAPD based markers for a sub-arm introgression indicated the potential and value of RAPD primers for detection of useful alien genes in wheat background. Any of these 13 useful polymorphic primers (polymorphic for 2NS segment) can be used in routine wheat breeding program to detect presence of 2NS segment carrying *Lr37-Sr38-Yr17* rust resistance genes.

Pakistani wheat varieties continuously need incorporation of new rust resistance genes. This can be achieved by traditional hybridization and selection procedures. The newly developed DNA based markers can be employed effectively to screen segregating populations which are basic material for development of new and improved germplasm.

REFERENCES

Cao W, Scoles G, Hucl P (1999). The use of RAPD analysis to classify *Triticum* accessions. Theor. Appl. Genet. 98: 602-607.

Chao S (2006). Application of molecular marker technologies on cereal crops improvement. American Oat Workers Conference, July 23-25, Fargo, ND.

Devos KM, Gale MD (1992). The use of Randomly Amplified Polymorphic DNA markers in wheat. Theor. Appl. Genet. 84: 567-572.

Dyck PL, Lukow OM (1988). The genetic analysis of two interspecific sources of leaf rust resistance and their effect on the quality of common wheat. Can. J. Plant Sci. 68: 633-639.

FAO (2003). Statistical databases. http://apps.fao.org/.

Fernandez ME, Figueiras AA, Benito C (2002). The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origi. Theor. Appl. Genet. 104: 845-851.

Frankle OH (1970) Genetic conversion in perspective. In: Frankel OH, Bennet E (eds) genetic resources in planta-their exploitation and conversion. Blackwell Scientific Pupl., Oxford, pp. 469-489.

Helguera M, Khan IA, Kolmer J, Lijavetzky D, Zhong-qi L, Dubcovsky J (2003). PCR Assays for the *Lr37-Yr17-Sr38* cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. Crop Sci. 43: 1839-1847.

Heun M, Murphy JP, Phillips TD (1994). A comparison of RAPD and isozyme analysis for determining the genetic relationships among *Avena sterlis* L. accessionc. Theor. Appl. Genet. 87: 689-696.

Hussain M, Hassan SF, Kirmani MAS (1980). Virulences in *puccinia recondita* in Pakistan during 1978 and 1979. In proceedings of the 5th European and Mediterranean Cereal Rusts Conference, Bari, Italy. p: 21.

Islam AKMR, Shepherd KW (1991). Alien genetic variation in wheat improvement. In: Gupta PK, Tsuchiya T (eds) Chromosome engineering in plants. Genetics, Breeding and Evolution. Vol. A Elsevier Science Publ., Amsterdam, pp. 291-312.

Kleinhofs A, Killan A, Saghai-Maroof MA, Biyashev RM, Hayes P, Chen FQ, Lapitan N, Fenwick A, Black TK, Kanazin V, Ananiev E, Dhaleen L, Kudrna D, Bollinger J, Knapp SJ, Liu B, Sorrells M, Heun M, Franckowiak JD, Hoffman D, Skadsen R, Steffenson BJ (1993). A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. Theor. Appl. Genet. 86: 705-712.

Koebner RMD, Shepherd KW (1985). Controlled introgression to wheat of genes from rye chromosome arm 1RS by induction of allosyndesis. 1. Isolation of recombinanats. Theor. Appl. Genet. 73: 197-208.

Koebner RMD, Shephered KW (1986). induction of recombination between rye chromosome IRL and wheat chromosomes. Theor. Appl. Genet. 71: 208-215.

Marillia EF, Scoles GJ (1996). The use of RAPD marker in *Hordeum* Phylogeny. Genome. 39: 646-654.

McIntosh RA, Wellings CR, Park RF (1995). Wheat rusts, an atlas of resistance genes. CSIRO, Australia.

MINFAL (2004). Agricultural Statistics of Pakistan. Ministry of Food, Agriculture and Livestock. Islamabad, Pakistan. pp: 5-6.

MINFAL (2005). Agricultural Statistics of Pakistan. Ministry of Food, Agriculture and Livestock. Islamabad, Pakistan. pp: 5-6.

Paterson AH, Tanksley SD, Sorrells ME (1991). DNA markers in plant improvement. Ad Agron. 46: 39-90.

Plaschke J, MW Ganal, MS Roder (1995). detection of genetic diversity in closely related bread wheat using microsatellite marker. Theor. Appl. Genet. 91: 1001-1007.

Qi L, Cao M, Chen P, Li W, Liu D (1996). Identification, mapping and application of polymorphic DNA associated with resistance gene *pm21* of wheat. Genome. 39: 191-197.

Robert O, Abelard C, Dedryver F (1999). Identification of molecular markers for the detection of the yellow rust resistance gene *Yr17* in wheat. Mol. Breed. 5(2): 167-175.

Seah S, Spielmeyer W, Jahier J, Sivasithamparam K, Lagudah ES (2000). Resistance gene analogs within an intrrogressed chromosomal segment derived from *Triticum Ventricosum* that confers resistance to nematode and rust pathogens in wheat. Mol. Plant. Microb. Interac. 13: 334-341.

Sears ER (1981). Transfer of alien genetic material to wheat. In: Evans LT, Peacock WJ (eds), Wheat Science: today and tomarrow. Cambridge University Press. Cambridge, pp. 75-89.

Thormann CE, Osborn TC (1992). Use of RAPD and RFLP markers for germplasm evaluation. Application of RAPD technology to plant breeding, Crop Sci., Madison, Wisconsin, pp. 9-11.

Tinker NA, Fortin MG, Mather DE (1993). Random amplified polymorphic DNA and pedigree relationships in spring barley. Theor. Appl. Genet. 85: 976-984.

USDA, (2006). http://www.ars.usda.gov/main/main.htm.

Weining S, Langridge P (1991). Identification and mapping of polymorphism in cereals based on Polymerase Chain Reaction. Theor. Appl. Genet. 82: 209-216.

Williams JGK, Hanafey MK, Rafalski J (1993). Genetic analysis using random amplified polymorphic DNA markers. Method Enzymol. 218: 704-740

Yu LX, Nguyen HT (1994). Genetic variation detected with RAPD markers among upland and lowland rice cultivars (*Oryza sativa* L.) Theor. App. Genet. 87: 668-672.