Pak. J. Bot., 39(1): 239-243, 2007.

DNA MARKER FOR LEAF RUST DISEASE IN WHEAT

TANWIR AHMAD MALIK, AZHAR IQBAL, MUHAMMAD ASLAM CHOWDHRY, MUHAMMAD KASHIF AND SAJID-UR-RAHMAN

Department of Plant Breeding & Genetics,
University of Agriculture, Faisalabad, Pakistan.

Abstract

Leaf (Brown) rust is major disease of wheat in Pakistan as well as in other countries. The objective of the study was to find DNA marker(s) linked to leaf rust resistance trait for use in tailoring disease resistant wheat cultivar. PCR technique and bulked segregant analysis was used to identify DNA marker linked to leaf rust resistant gene in F_2 segregating population. The F_2 population derived from the cross involving leaf rust susceptible, SK-7 and resistant parent, PBG-8881 with Lr29 resistant gene was scored for disease resistance. The F_2 population segregated into resistant and susceptible plants in a ratio of 3:1 showing monogenic inheritance. The primer 60-5 amplified a polymorphic molecules of 1100 base pairs from the genomic DNA of resistant plant. This DNA molecule can be used as DNA marker to identify leaf rust resistant plants in a breeding programme for developing rust resistant wheat cultivars.

Introduction

Wheat (*Triticum aestivum* L.) is the most important cereal crop of the world. Leaf rust (*Puccinia triticina*) disease causes significant reduction in wheat yield and poses a constant threat to sustainable wheat production in all wheat growing countries including Pakistan (Sing & Julio, 2006). It is the most wide spread and regular accruing rust on wheat (Kolmer, 1996). Genetic resistant is most economical method of reducing yield losses due to leaf rust. A large number of leaf rust races and resistant genes have been described. The spread of races of leaf rust depends upon humidity, temperature and other environmental variations. The expression of resistant genes depend on the genetics of host parasite interaction as well as the environmental conditions. Phenotypic selection of plants resistant to leaf rust may not be very precise. It has been observed that newly developed strains get leaf rust at the latest stages of experimental testing. This may be some times due to the evolution of a new race but mostly it is due to dependence on the phenotypic selection in breeding (Chowdhry, personal communications). Over the last twenty years DNA marker techniques have been developed which helps selection of plants on the basis of genotype so environmental variables may not affect the selection of plants for a particular trait. DNA marker closely associated with disease resistant genes have potential to be used in maker assisted selection for durable leaf rust resistant cultivars (Xu *et al*. 2005). These markers can help pyramiding resistant genes into single cultivars for durable resistant.

RAPD (Random Amplified Polymorphic DNA) technique is easy to perform, requires small amount of genomic DNA and does not require radiolabeling (Michelmore *et al*., 1991). This technique is based on amplification of random DNA segments of genomic DNA by polymerase chain reaction (PCR), using primers of arbitrary sequence. RAPD markers have been used for tagging genes for disease resistance (Welsh & McClleland, 1990, Klein-Lankhorst *et al*., 1991, Paran, 1991, Martin *et al*., 1991, Park *et al.*, 2004 and Milla *et al*., 2005) and for other traits.

Near isogenic lines may be used to identify DNA marker linked to a gene however, bulked segregant analysis using segregating population is more successful and quick

(Michelmore *et al*., 1991). Bulked segregant analysis has been extensively used for the identification of RAPD markers linked to various genes (Poulsen *et al.* 1995, Warburton *et al.* 1996, Amir *et al*., 2002). The objective of the present study was to identify DNA marker for leaf rust resistant gene Lr 29 in wheat. RAPD technique was used to identify DNA markers for leaf rust resistance following bulked segregant analysis. This gene provides resistant against leaf rust races prevalent in the country (Hussain *et al.*, 2006).

Materials and Methods

An F_2 population derived from the cross between disease resistant parent PBG-8881, developed at the Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan and disease susceptible parent SK-7 (an exotic genotype from Kazakistan) was planted alongwith the parents during the month of November (normal planting time of the crop), 2003. The experiment was conducted in a triplicate randomized complete block design. The plants were sown in 4.5 meter long rows with plant to plant distance of 15 cm and row to row distance of 30cm. A total of 1395 population of the F_2 and 93 each of the parents and the F_1 was maintained. During the month of February and March, 2004 plants were screened for disease resistance. Chi-square test was used to test the ratio of resistant and susceptible plants.

Spores collected from different leaf susceptible genotypes available in the gene pool were used for artificial inoculation of the experimental population. Out of the total F_2 population 20 resistant and 20 susceptible plants were selected for DNA extraction. DNA was isolated from the leaves of selected plants following the CTAB method (Doyle & Doyle, 1987). Two DNA pools contrasting for resistant and susceptible trait were formed by mixing equal amount of DNA from individual samples. One pool or bulk consisted of equal amounts of DNA of 20 F_2 plant samples showing resistant trait and the other was similarly formed from DNA of 20 F_2 plant samples with susceptible trait. These two DNA bulks were studied by PCR for polymorphism. In total 370 oligonucleotide 10-base primers from Operon Technologies, USA and Genosys Biotechnlogy, UK were used for PCR. The enzyme *Taq* DNA polymerase, PCR buffer, $MgCl_2$ and dNTP's were from Sigma. Amplification products were analyzed by 1.2% agarose gel electrophoresis run in 0.5% TAE buffer and detected by staining the gel with ethidium bromide. Marker of known molecular weight was used to calculate the size of different amplified DNA fragments. Samples were electrophoresed for about 2 hours, at 50 volts. After electrophoresis, the amplified products were viewed and photographed using gel documentation system. Data was scored from good quality photographs of each amplification reaction. The left lane of the gel was considered as lane-1. Amplified fragment were scored by starting from the top of the lane to its bottom. All visible and unambiguously scorable fragments amplified by primers were scored under the heading of total scorable fragments. The fragments that were repeatedly present in one bulk and absent in the other, were scored as polymorphic fragments.

Results and Discussion

The F_1 plants were resistant to leaf rust. In the F_2 population leaf rust resistant plants and susceptible plants segregated into a ratio of 3:1 showing monogenic inheritance of disease with dominance of leaf rust resistance (Table 1). Similar findings have been reported by Brown-Guedira *et al*., (1997). The genotype PBG-8881 having Lr 29 leaf resistant gene was used as resistant parent in the cross. Hussain *et al*., (2006) has reported that Lr 29 is resistant to prevalent leaf rust races in Pakistan.

Table 1. Chi-squared values and probabilities of goodness of fit in F_2 generation phenotypes resulting from cross of resistant and susceptible parents against leaf rust in wheat.

Cross	Number of plants		X^2-value	P-range
	Resistant	Susceptible		
SK-7 x PBG-8881	1034	361	0.528	0.50 – 0.25

In the present study, 360 primers were used which amplified upto nine bands with an average of 5.6 per primer in RAPD reactions. Haley *et al.*, (1993) reported 5.4 bands per primer in bean. Miklas *et al*., (1993) observed 5.6 bands per primer ranging between 100 to 2000 bases. The results of the present study suggest that a relationship exists between the GC composition of a primer and the success of amplifying the genomic DNA. The primers with 70% GC base composition gave a higher number of RAPD bands per primer compared to the primers with 60% GC base composition.

Bulked segregant analysis provides a rapid and technically simple way for identifying markers linked to specific genes. The only pre-requisite is the existence of a population resulting from a cross that segregates for the gene of interest. However, the success of the approach depends on the genetic divergence between the parents in the target region. In a large number of earlier studies bulked segregant analysis has been successfully used to find RAPD markers(s) linked to various traits. It has been indicated that when bulks are constructed using reasonable number of individuals, the bulked segregant method is sufficiently robust to cope with a low level of phenotypic misclassification. In the present studies, a polymorphic band of 1100 bases from the primer 60-5 was observed between the resistant and susceptible bulk (Fig. 1).

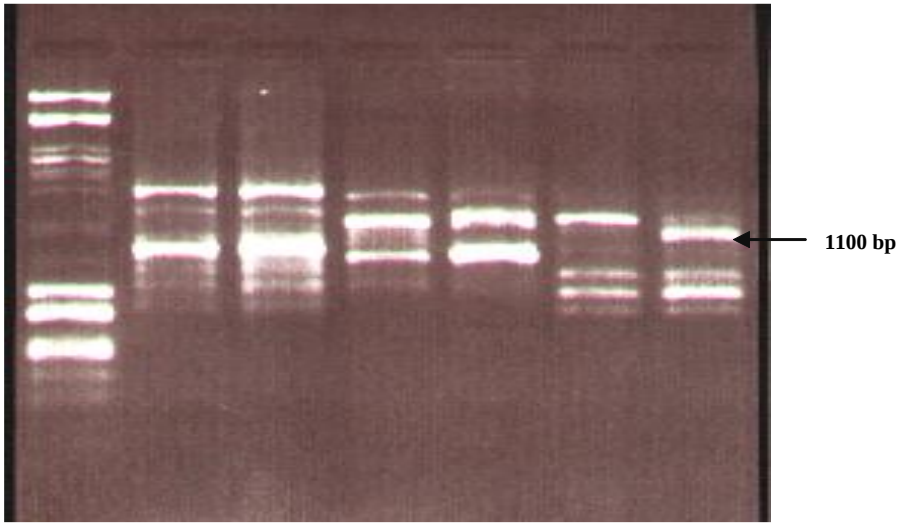

Fig. 1. Gel picture showing bulked segregant analysis of the DNA of resistant (R) and susceptible (S) wheat bulk involving three primers 60-3, 60-4, and 60-5. First lane marked M is molecular weight marker. The polymorphic DNA of 1100 bp amplified by the primer 60-5 in the resistant bulk is linked to the disease resistant gene, Lr29.

The polymorphic molecule of 1100 bases amplified from 19 individual samples of the DNA of 20 resistant plants and was absent in the amplification reaction containing the DNA of 20 susceptible plant. So the disease resistant and susceptible plants can be identified using this DNA marker. Penner *et al*., (1993) observed ten polymorphic fragments between the bulks constructed for crown rust resistance but only one of the polymorphic DNA fragment was found to be tightly linked to the crown rust resistance gene. Similarly, Miklas *et al*., (1993) identified one RAPD marker named OPA-14_{1100} linked to rust resistance in common bean. Poulsen *et al*., (1995) identified a 2.7 kb RAPD marker, linked to leaf rust resistance gene in barley generated by the primer OPU-02. Results of earlier studies and the present study indicate that, the RAPD markers linked to trait of interest can easily be identified using bulked segregant analysis and may be used effectively in breeding programmes. Using DNA marker, plant breeders may select plants on the basis of their genotype. Hence DNA marker assisted selection provides precision in genotypic selection of plants in a breeding programme.

Acknowledgement

The authors are thankful to the management of the Centre of Agricultural Biochemistry and Biotechnology, University of Agriculture, Faisalabad, Pakistan for providing equipments. The financial support of the Pak-Kazakistan project is also highly appreciated.

References

Amir, A.H., T.A. Malik, H. Raza and Mehboob-ur-Rehman. 2002. DNA markers studies for leaf nectarines in upland cotton. *Asian J. Pl Sci*., 1(4): 395-396.

Brown-Guedira, G.L., B.S. Gill, T.S. Cox and S. Leath. 1997. Transfer of disease resistance genes from *Triticum araraticum* to common wheat. *Rev. Plant Path*., 76(11):

Doyle, J.J. and J.I. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11-15.

Haley, S.D., P.N. Miklas, J.R. Stavely, J. Byrum and J.D. Kelly. 1993. Identification of RAPD markers linked to a major rust resistance gene in common bean. *Theor. Appl. Genet*. 86(4): 505-512.

Hussain, M., M.A. Khan, M. Hussain, F. Muhammad, A. Rahman and M. Younis. 2006. Shifts and prevalence of leaf rust virulence in Pakistan during 1991-2005. In the Abstracts of " International Wheat Seminar" held at the Wheat Research Institute, ARRI, Faisalabad, Pakistan.

Klein-Lankhorst, R.M., A. Vermunt, R. Weide, T. Liharska and P. Zabel. 1991. Isolation of molecular markers for tomato (*L. esculentum*) using Random Amplified Polymorhpic DNA (RAPD). *Theor. Appl. Genet*., 83(1): 108-114.

Kolmer, J.A. 1996. Genetics of resistant to wheat leaf rust. *Ann. Rev. Phytopathology*, 34: 435-455.

Martin, G.B., T.J.K. William and S.D. Tanksby. 1991. Rapid identification of markers linked to a *Pseudomonas* resistance genes in tomato by using random primers and near isogenic lines. *Proc. Natl. Acad. Sci*., 88: 2336-2340.

Michelmore, R.W., I. Paan and R.V. Kesseli. 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genome regions using segregating population. *Proc. Natl. Acad. Sci*., 88: 9828-9832.

Miklas, P.N., J.R. Stavely and J.D. Kelly. 1993. Identification and potential use of a molecular marker for rust resistance in common bean. *Theor. Appl. Genet*., 85(6-7): 745-749.

Milla, S.R., J.S. Levin, R.S. Lewis and R.C. Rufty. 2005. RAPD and SCAR markers linked to an introgressed gene conditioning resistance to *Peronospora tabacina* in Tobacco. *Crop Sci*., 45: 2346-2354.

Paran, I., R. Kesseli and R. Michelmore. 1991. Identification of restriction fragment length polymorphism and random amplified polymorphic DNA markers linked to downy mildew resistance genes in lettuce, using near-isogenic lines. *Genome*, 34: 1021-1027.

Park, S.O., D.P. Coyne, J.R. Steadman, K.M. Crosby and M.A. Brick. 2004. RAPD and SCAR markers linked to the Ur-6 andean gene controlling specific rust resistance in common bean. *Crop Sci*., 44: 1799-1807.

Penner, G.A., J. Chong, C.P. Wight, S.J. Molnar and G. Fedak. 1993. Identification of RAPD marker for the crown rust resistance gene Pc68 in oats. *Genome*, 36: 818-820.

Poulsen, D.M.E., R.J. Henry, R.P. Johnston, J.A.G. Irwin and R.G. Rees. 1995. The use of bulk segregant analysis to identify a RAPD marker linked to leaf rust resistance in barley. *Theor. Appl. Genet*., 91(2): 270-273.

Sing R.P. and H.E Julio. 2006. Rust Dynamics and Challenges to Wheat Breeding. In the Abstracts of " International Wheat Seminar" held at the Wheat Research Institute, ARRI, Faisalabad, Pakistan.

Warburton, M.L., V.L. Becerra-Velasquez, J.C. Goffreda and F.A. Bliss. 1996. Utility of RAPD markers in identifying genetic linkages to genes of economic interest in peach. *Theor. Appl. Genet*., 93(5-6): 920-925.

Welsh, J. and M. McClleland. 1990. Fingerprinting genome using PCR with arbitrary primers. *Nucleic Acid Res*., 18: 7213-7218.

Xu, X., G. Bi, B.F. Carver, G.E. Shaner and R.M. Hunber. 2005. Molecular characterization of slow leaf-rusting resistance in wheat. *Crop Sci*., 45: 758-765.

(Received for publication 26 December 2006)