Acta Phytopathologica et Entomologica Hungarica 38 (3–4), pp. 245–249 (2003)

Detection of Leaf Rust Resistance Genes *Lr9* and *Lr10* in Wheat *(Triticum aestivum)* by PCR Based STS Markers

R. SINGH[1]*,
R. TIWARI[1] and D. DATTA[2]

[1]Directorate of Wheat Research, Karnal-132 001, India
[2]Regional Station, Directorate of Wheat Research, Flowerdale, Shimla, India

Leaf rust resistance genes *Lr9* and *Lr10* were detected in wheat *(Triticum aestivum)* genotypes by PCR based STS markers. Out of sixty-two elite wheat genotypes, screened for the presence of leaf rust resistance gene *Lr10,* nineteen genotypes revealed the presence through PCR analysis with the primers specific to *Lr10* gene. Validation of the marker for *Lr9* gene in the parental lines followed by successful detection of the gene in F_4 lines out of cross HP1633 *(Lr9)* X HP1776, was also done. Usefulness of molecular markers for the detection of rust resistance genes in different genotypes is discussed.

Keywords: Molecular markers, leaf rust resistance gene, *Triticum aestivum.*

Successful wheat production in the rust areas of the world continues to depend on the use of rust resistant cultivars. Leaf rust caused by *Puccinia recondita* f. sp. *tritici* is considered to be one of the most important diseases of wheat. The rapid changes that occur in the virulence characteristics of populations, raises a continuous threat to the effectiveness of existing resistant varieties. Breeding for durable resistance against this disease is based on the combination of different leaf rust *(Lr)* resistance genes in one cultivar (Van Ginkel and Rajaram, 1993). Bringing more than one gene together into a single elite variety by conventional means is very laborious and time consuming. In some cases it is not achievable because screening for one resistance gene interferes with the ability to screen for another, a frequent problem in disease resistance breeding, while in certain cases the virulent isolates for the resistant genes are not available. In recent years, DNA-based markers have shown great promise in expediting plant breeding procedures. Molecular markers have been reported that are closely linked to *Lr* genes either of alien origin viz., *Lr9, Lr19, Lr24, Lr25, Lr29* (Schachermayr et al., 1994, 1997; Procunier et al., 1995; Dedryver et al., 1996), or of wheat origin viz., *Lr1, Lr10, Lr34* (Feuillet et al., 1995; Schachermayr et al., 1997; William et al., 1997). The objective of this research activity was to study the correspondence between the molecular markers and Host-Pathogen Interaction (HPI) test based detection of *Lr10* gene in some genotypes. Molecular markers were also used to identify *Lr9* resistance gene in F_4 population developed with the objective of having genotypes with more than one resistance genes.

*E-mail: rajenderkhokhar@yahoo.com

0238–1249/2003/$ 20.00 © 2003 Akadémiai Kiadó, Budapest

Materials and Methods

Plant material

Sixty-two elite wheat genotypes comprising of Advanced Varietal Trial (AVT) final year entries and checks of the year 2001–2002 were used for PCR based detection of *Lr10* gene. Detection of *Lr9* gene was done in F_4 lines developed out of the cross HP 1633 *(Lr9)* X HP 1776 *(Lr24).*

DNA isolation and PCR amplification

Genomic DNA was extracted from 100 mg fresh leaves (Dellaporta et al., 1983). Polymerase chain reaction (PCR) amplifications were carried out using a PTC-200 thermocycler (MJ Research). Reactions were performed in a final volume of 25 µl using 1 unit of *Taq* DNA polymerase. The sequence of primers, and the quantities of other components of the reaction mixture and the PCR conditions used to amplify were specific for each gene *(Table 1).* PCR products were analysed by electrophoresis using a 1.5% agarose gel in TAE buffer followed by staining with ethidium bromide.

Table 1

Sequence primers and PCR cycling conditions for amplification of specific genes of leaf rust *(Lr)*

Genes	Forward and reverse primers	PCR components	PCR cycling	Reference
Lr9	5' CCACACTACCCCAAAGAGACG 3' 5' TCCTTTTATTCCGCACGCCGG 3'	1X PCR buffer 100 µM / dNTPs 20 ng each primer 20 ng template	1x 94 ºC 4' 40x 94 ºC 1'– 62 ºC 1'– 72 ºC 2' 1x 72ºC 5'	(Schachermayr et al., 1994)
Lr10	5' GAAGCCCTTCGTCTCATCTG 3' 5' TTGATTCATTGCAGATGAGATCACG 3'	1X PCR buffer 100 µM/ dNTPs 40 ng each primer 20 ng template	1x 94 ºC 4' 30x 94 ºC 1'– 60 ºC 1'– 72 ºC 2' 1x 72 ºC 5'	(Schachermayr et al., 1997)

Results and Discussion

Out of sixty-two elite wheat genotypes, screened for the presence of leaf rust resistance gene *Lr10,* nineteen genotypes revealed the presence *Lr10* by PCR analysis with the primers specific to *Lr10* gene *(Table 2).* The genotypes having *Lr10* amplified the desired fragment of 282 bp *(Fig. 1).* Out of these nineteen genotypes showing presence of the gene, information through HPI test confirms the presence of *Lr10* in six genotypes viz.,

Table 2

Genotypes (with pedigrees) showing presence of *Lr10* gene with the molecular markers

Genotypes	Pedigree
HS 420	KAJ 3320 // CMH 73A-497 / 3*CNO 79
HS 422	PRL / VEE # 6 // STAR /3/ IRENA
PBW 492	WH485 / PBW343 / RAJ 1482
PBW 493	**PBW 154** / PBW343 / WH 542
PBW 500	PBW 351 / W 4387
WH 736	CMH 81.137/CMH 81.580
K-9943	HUW 243 / **HD 2402**
HD 2329	HD 1962//E 4870/ K65/3/ HD 1553/ UP 262
PBW 343	ND/VG1944// KAL/ **BB** / YACO'5'/ 4/ VEE # 5 '5'
NW 2026	KT / BAGE
HI 977	GLL/AVSTII-61.157// CNO/ NO/ 3/ Y50E/3*KAL
RAJ 3765	**HD 2402** / VL 639
PBW 373	ND /VA 1944 / KAL **/BB/** 3 / YACO "S" /4 / VEE# 5 "S"
VL 738	NS 12.07 / LIRA 'S' /VEE 'S'
VL 804	CPAN 3018 / CPAN 3004 // **PBW65**
HS 240	AU/ KAL/ **BB**/ 3/BOW / **PVN 'S'**
HD 2790	ATTILA /3/HE 1/ 3* CNO79/2*SERI#5
GW 326	DL 270 7/**J431**
GW 273	CPAN 2084/ VW 205

Note: Genotypes in bold letters represent the genotypes in which *Lr10* was reported through HPI.

HS 420, HD 2329, HI 977, Raj 3765, GW 326 and GW 273 (Nayar et al., 1994). Detection of *Lr10* gene in the genotypes PBW 493, K 9943, PBW 343, PBW 373, VL 804 and HS 240 could be accrued to one or the other parents possessing *Lr10* gene in the pedigree of these genotypes. For rest of the seven genotypes, where marker detected *Lr10* gene, validation through other means is a matter of further investigation.

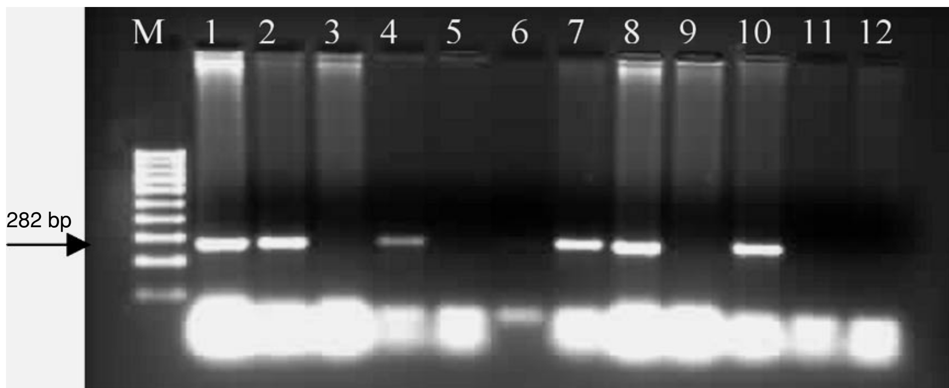

Fig. 1. PCR based detection of *Lr10* gene in AVT 2nd year entries
(M-mol. Wt. Marker (100 bp ladder), 1-HD 2329, 2-Raj 3765, 3-HS 418, 4-HS 420, 5-VL 818, 6-VL 822, 7-PBW 343, 8-K 9943, 9-PBW 498, 10-VL 804, 11-Sonalika, 12-HD 2815)

A study revealed the presence of *Lr10* gene in wheat lines HI 977, HD 2402 and PBW 154 when postulated with the pathotypes in Mexico. But this gene was not detected in these lines when postulated with pathotypes of India and Australia (Nayar et al., 1994). Similarly *Lr10* could not be postulated in cvs. Hereward and Encore by Australian pathotypes (Singh et al., 2001) but recently both HPI and molecular marker studies confirmed the presence of *Lr10* gene in these cultivars (Blazkova et al., 2002). The *Lr10* is not widely effective on its own. However, it is suggested to play a role in leaf rust resistance in combination with other *Lr* genes in most areas except Australia (McIntosh et al., 1995).

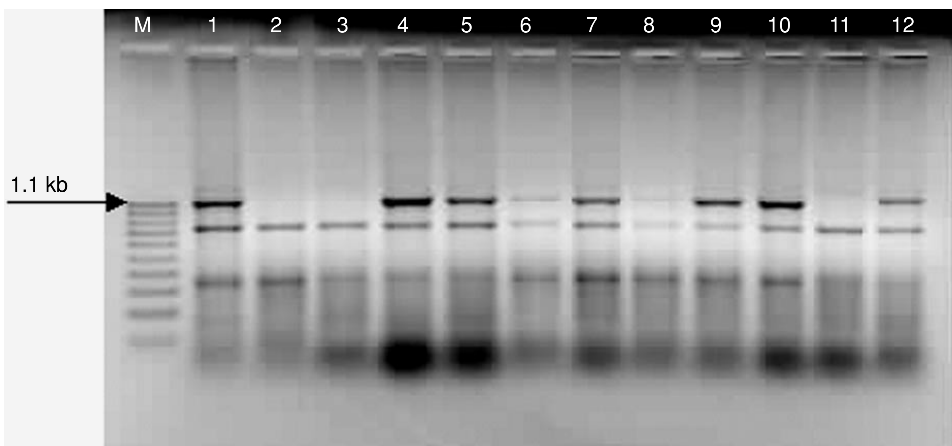

Fig. 2. PCR based detection of *Lr9* gene in F_4 lines of cross HP 1633 × HP 1776 (M-mol. Wt. Marker (100 bp ladder), 1-HP 1633, 2-HP 1776, 3-12-F4 lines of cross HP 1633 × HP 1776)

For the detection of *Lr9* gene in the F_4 lines of cross HP 1633 *(Lr9)* × HP 1776 *(Lr24),* initially DNA of the parental lines were amplified with the primers specific to *Lr9* gene and it was found that 1.1 kb fragment specific for *Lr9* gene was amplified only in HP1633 and not in HP1776. These primers were also utilized to see amplification in Tc**Lr9,* Agent and HW 3012 genotypes and the expected 1.1 kb fragment got amplified only in Tc**Lr9* and not in Agent and HW 3012 genotypes (both the genotypes are devoid of *Lr9* gene). Twenty-two F_4 lines were subsequently screened with the molecular marker in which thirteen lines amplified the desired fragment. Thus molecular marker for determination of *Lr9* proved to be a useful tool for confirming the presence of *Lr9* in this study. This kind of study paves the way for marker aided selection of the rust resistance genes. The utility of such kind of studies is further authenticated by studies by Robert et al. (2000) where presence of *Yr17* gene was confirmed with molecular marker in lines which could not be identified through HPI test because of the presence of other genes.

Once developed, the molecular tests are more convenient, less time consuming and could therefore be used to identify different rust resistance genes in lines or genotypes in

early stages of selection. Marker-aided selection (MAS) is potentially useful for breeding for disease resistance in at least four ways: as a substitute for a disease screen, to accelerate the return to the genotype of the recurrent parent during backcrossing, to reduce linkage drag of linked deleterious genes, and to select for disease resistance QTLs. Thus with the help of molecular markers, the combination of different leaf rust resistance genes which are active at the seedling and/or the adult stage should facilitate more efficient breeding for durable resistance.

Literature

Blazkova, V., Bartos, P., Park, R. F. and Goyeau, H. (2002): Verifying the presence of leaf rust resistance gene *Lr10* in sixteen wheat cultivars by use of a PCR-based STS marker. Cereal Res. Comm. 30, 9–16.

Dedryver, F., Jubier, M. F., Thouvenin, J. and Goyeau, H. (1996): Molecular markers linked to the leaf rust resistance gene *Lr24* in different wheat cultivars. Genome 39, 830–835.

Dellaporta, S. L., Wood, J. and Hicks, J. B. (1983): A plant DNA minipreparation: Version II. Plant Mol. Bio. Rep. 1, 19–21.

Feuillet, C., Messmer, M., Schachermayr, G. and Keller, B. (1995): Genetic and physical characterization of the *Lr1* leaf rust resistance locus in wheat (*Triticum aestivum* L.). Mol. Gen. Genet. 248, 553–562.

McIntosh, R. A., Wellings, C. R. and Park, R. F. (1995): Wheat Rusts: An Atlas of Resistance Genes. CSIRO Australia, Melbourne, and Kluwer Academic Publisher, Dordrecht, the Netherlands.

Nayar, S. K., Nagarajan, S., Prashar, M., Bhardwaj, S. C., Jain, S. K. and Datta, D. (1994): Revised catalogue of genes that accord resistance to Puccinia species in wheat. Research Bulletin No. 3, 48 pp. Directorate of Wheat Research, Regional Station, Flowerdale, Shimla, India.

Nayar, S. K., Tandon, J. P., Kumar, J., Prashar, M., Bhardwaj, S. C., Goel, L. B. and Nagarajan, S. (1994): Basis of rust resistance in Indian wheats. Research Bulletin No. 1, 32 pp. Regional Station, Directorate of Wheat Research, Flowerdale, Shimla, India.

Prins, R., Groenewald, J. Z., Marais, G. F., Snape, J. W. and Koebner, R. M. D. (2001): AFLP and STS tagging of *Lr19,* a gene conferring resistance to leaf rust in wheat. Theor. Appl. Genet. 103, 618–624.

Procunier, J. D., Townley-Smith, T. F., Fox, S., Prashar, S., Gray, M., Kim, W. K., Czarnecki, E., Dyck, P. L. (1995): PCR-based RAPD/DGGE markers linked to leaf rust resistance genes *Lr29* and *Lr25* in wheat (*Triticum aestivum* L.). J. Genet. Breed. 49, 87–92.

Robert, O., Dedryver, F., Leconte, M., Rolland, B. and De Vallavieille-Pope, C. (2000): Combination of resistance tests and molecular tests to postulate the yellow rust resistance gene *Yr17* in bread wheat lines. Plant Breed. 119, 467–472.

Schachermayr, G., Feuillet, C. and Keller, B. (1997): Molecular markers for the detection of the wheat leaf rust resistance gene *Lr10* in diverse genetic backgrounds. Mol. Breed. 3, 65–74.

Schachermayr, G., Siedler, H., Gale, M. D., Winzeler, H., Winzeler, M. and Keller, B. (1994): Identification and localization of molecular markers linked to the *Lr9* leaf rust resistance gene of wheat. Theor. Appl. Genet. 88, 110–115.

Singh, D., Park, R. F. and Mclntosh, R. A. (2001): Postulation of leaf (brown) rust resistance genes in 70 wheat cultivars grown in the United Kingdom. Euphytica 120, 205–218.

Van Ginkel, M. and Rajaram, S. (1993): Breeding for durable resistance to diseases in wheat: An international perspective. In: T. Jacobs and J. E. Parlevliet (eds): Durability of Disease Resistance, pp. 259–272. Kluwer Academic Publisher, Dordrecht.

William, H. W., Hooisington, D., Singh, R. P. and Gonzalen-de-Leon, D. (1997): Detection of quantitative trait loci associated with leaf rust resistance in bread wheat. Genome 40, 253–260.