Indian J. Genet., **66**(4): 332-334 (2006)

Short Communication

Pyramiding of leaf rust resistance genes *Lr9* and *Lr24* through molecular marker assisted selection in wheat (*Triticum aestivum* L.)

D. Datta, M. Prashar and S. C. Bhardwaj

Directorate of Wheat Research, Regional Station, Flowerdale, Shimla 171 002

(Received: April 2006; Revised: October 2006; Accepted: November 2006)

Leaf rust (*Puccinia triticina* Eriks.) occurs throughout the wheat (*Triticum aestivum* L.) growing regions of the world. Cultivation of resistant varieties is the most effective, eco-friendly and economically viable method of combating the rust diseases. It is important that the genetic base of rust resistance in the cultivated varieties be widened and also develop lines with more than one effective resistance gene. It is, however, difficult to ascertain successful incorporation of two or more genes through conventional techniques of Host Pathogen Interaction. The presence of such gene, in the absence of differential interaction, can be detected through molecular markers. In the ensuing work, molecular marker reported for *Lr9*, which was already validated in the parents, was utilized and genetic stock with combined resistance of *Lr9* + *Lr24* was developed through combination of Host Pathogen Interaction and molecular marker.

Molecular marker [1] was used as tool for selecting leaf rust resistance gene *Lr9* in the segregating generations of wheat whereas incorporation of *Lr24* gene in the genetic stock was authenticated through a molecular marker [2]. F_2 and F_3 generations from the cross and HP 1633/HP1776, were screened for leaf rust resistance as well as *Lr9* marker. The F_2 and F_3 seedlings were raised in the aluminum bread pan trays comprising ten rows with the 7th row of each tray as a susceptible check, Agra local. Seven days old seedlings with fully expanded primary leaves were inoculated with uredospores suspended in light weight, non-phytotoxic isoparaffmic oil (soltrol). The inoculated seedlings were kept in a saturated humid glass chamber for 48 hours. The seedlings were then transferred to glass house benches at about 22°C. Infection types (IT) were recorded 14 days after inoculation and were classified according to standard method [3]. The IT's o; (naught fleck), ; (fleck), ;-(fleck minus), ;1 (fleck one), 1 (one), 2 (two) and X (mesothetic) were classified as resistant reactions whereas IT's 3 (three) and 3+ (three plus) were classified as susceptible. DNA was extracted by cTAB method [4] with minor modifications. Amplifications were performed in PTC-200 Thermal cycler (MJ Research, Waltham, MA). PCR products of *Lr9* and *Lr24* were analyzed in 1.5% agarose gel in 0.5 × TAB buffer. The PCR conditions for different molecular markers are presented in Table 1.

Table 1. Profile of molecular markers for the genes *Lr9* and *Lr24*

Genes	Components	Cycles	Reference
Lr9 (SCAR)	2mMMgCl$_2$, 100uMdNTP, 40Nm primer, 0.5U Taq Polymerase, 5ng DNA	1 × 94°C 6' 40 × 94°C 1'; 62°C l'; 72°C 2'; 1 × 72° C4'	Schachermayr *et al.*, 1994
Lr24 (SCAR)	2mMMgCl$_2$, 200uMdNTP, 0.6uM primer, 1.0U Taq Polymerase, 50ng DNA	1 × 94°C0.5'; 38 × 94°C 1.5'; 68°C 2'; 72°C	Dedryver *et al.*, 1996

Selection strategy: The F_2 generated from individual F_1s were tested with pathotype 121R127 which is virulent on *Lr9*. Fifty resistant seedlings were transplanted in the field and DNA was isolated from each of the resistant plants which were subjected to molecular marker analysis for the detection of *Lr9* gene. The plants positive for the molecular marker were selected and advanced to next generation. The F_3 progenies were tested at the seedling stage with leaf rust pathotype 121R127 and the non-segregating families were transplanted in the field. Twelve plants from each of the non-segregating FS progenies were tagged randomly and DNA was isolated from the tagged plants for detecting families homozygous for *Lr9* marker. The F_3 families that did not segregate for molecular marker were advanced to the next generation. Incorporation of the other specific leaf rust resistance gene (*Lr24*) was confirmed in the F_4 generation through progeny testing of 30-40 individuals per family with pathotype 121R127.

Once the validity of the *Lr9* and *Lr24* markers were confirmed in the parental lines of the anticipatory pre-breeding programme (unpublished data), in the next

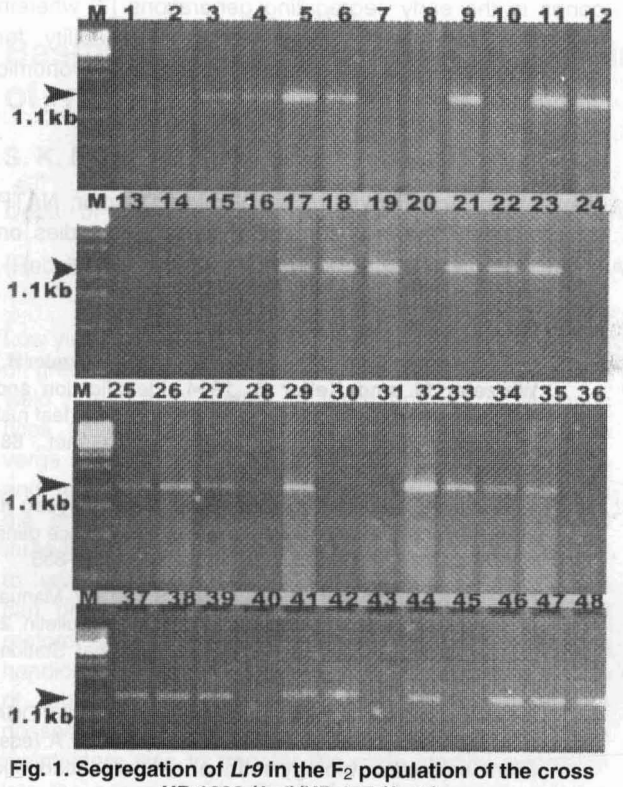


Fig. 1. Segregation of *Lr9* in the F_2 population of the cross HP 1633 (*Lr9*)/HP 177 (*Lr24*)

phase a comprehensive breeding strategy, as described in materials and methods, was laid down for their utilization in marker assisted selection. In the F_2, out of fifty seedlings resistant to 121R127, 30 plants were positive for the presence *Lr9* (Fig. 1). The F_3 progenies derived from the F_2 plants carrying *Lr9*, detected through molecular marker, were tested with 121R127 and the non-segregating lines were picked up thus enabling the fixation of *Lr24*. Marker analysis was applied on the nine fixed lines of *Lr24* (Fig. 2). Two F_3 lines were non-segregating for *Lr9* specific marker (Fig. 2). Plants selected from these two F_3 families were homozygous for the presence of *Lr9* marker and also resistant to pathotype 121R127 in a population of 30-40 individuals per family in the p4 generation which confirmed the fixation of *Lr9* and *Lr24* through molecular marker analysis and host pathogen interaction, respectively. It was absolutely necessary to test the segregation of marker in the F_4 since only 12 plants per F_3 family were tested for marker analysis. The derived genetic stock (FLW6) was finally tested for presence of specific markers of *Lr9* and *Lr24* which authenticated successful incorporation of these genes in the stock (Fig. 3). Among these two markers, though the marker for *Lr24* was not consistent in the white grain carrier lines but it was never amplified in the lines without *Lr24* gene (unpublished data and Fig. 3), therefore, presence of specific band was considered as the confirmatory proof of the presence of *Lr24*.

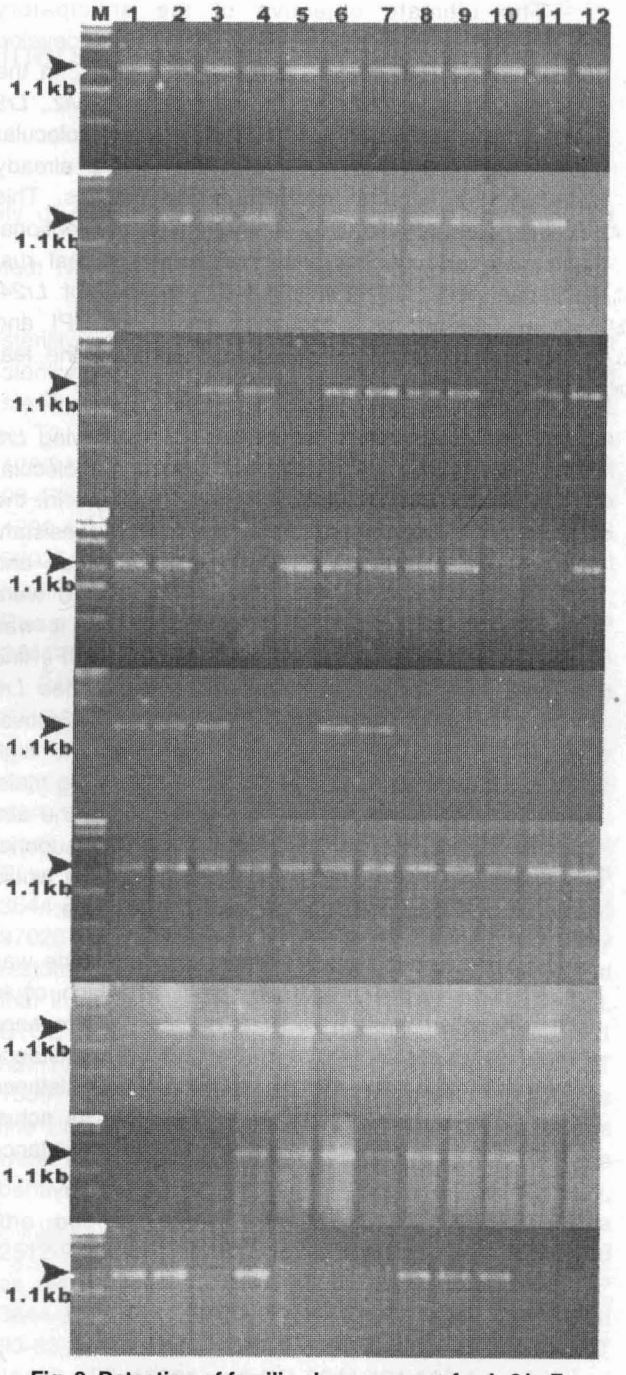
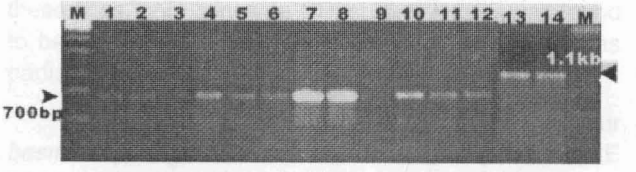


Fig. 2. Detection of families homozygous for *Lr9* in F_3 progeny of the cross HP 1633 (*Lr9*)/HP1776(*Lr24*)



Figs. 3. (1-12: Amplification product of *Lr24* marker; 13-14: amplification product of *Lr9* marker) M: Marker lane: 1-6: Advanced lines of the cross HP1633/HP1776 (white seed), 7-8: Catalogued lines of *Lr24* (red seed), 9-12: Catalogued lines of *Lr24* (white seed) 13-14: Advanced lines of the cross HP1633/HP1776

The ultimate objective of the anticipatory pre-breeding programme at Flowerdale is to develop genetic stocks with combined resistance genes. In the initial stage the following genes were targeted *viz.*, *Lr9* + *Lr24*, *Lr19* + *Lr26* and *Lr24* + *Lr26* because molecular markers for *Lr9*, *Lr19*, *Lr24* and *Lr26* were already tested in the parents involved in the crosses. This objective was not attainable solely through conventional techniques because the phenotypic effect of leaf rust resistance gene *Lr9* is masked in presence of *Lr24*. However, through the combined efforts of HPI and molecular marker it was possible to combine the leaf resistance genes *Lr9* and *Lr24*.

One of the two homozygous F_3 line carrying *Lr9* + *Lr24*, selected with the help of HPI and molecular marker, had not lost the *Sr* genes present in the parents, HP 1633 and HP 1776. The F_4 lines resistant to stem rust pathotypes 62G29-1 were selected and thus the *Sr* gene from HP 1633 and HP 1776 were also fixed in the later generations. Though it was possible to recover *Sr* genes from one of the F_3 line that was fixed for two leaf rust resistance genes *Lr9* + *Lr24*, however, this was not enough to recover outstanding genotypes that would withstand the high selection pressure and fit into the station breeding trials. However, it did not affect our objectives since the aim was not to generate breeding lines but to obtain superior pre-breeding lines with combined resistance that would serve as the parent in the crossing programme.

Similar to our study, presence of *Yr17* gene was confirmed with DNA marker in the lines which could not be identified through HPI test because of presence of other genes [5]. Molecular markers for rust resistance had been used mainly for detecting the hidden resistance genes [6] in advanced lines or cultivars but its actual potential lies in the selection of multiple resistance genes in the early segregating generations [7] wherein it would facilitate to retain sufficient variability for exercising selection of desirable traits of agronomic importance and yield attributes.

Acknowledgement

The authors are thankful to the Project Director, NATP for providing financial support to carry out studies on wheat rust genetics through team of excellence.

References

1. **Schachermayr G. M., 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.
2. **Dedryver F., Jubier M. F., Thouvenin J. and Goyeau H.** 1996. Molecular marker linked to leaf rust resistance gene *Lr24* in different wheat cultivars. Genome **39**: 860-835.
3. **Nayar S. K., Prashar M. and Bhardwaj S. C.** 1997. Manual of current techniques in wheat rusts. Research Bulletin, **2**: 18. Directorate of Wheat Research, Regional Station, Flowerdale, Shimla 171 002.
4. **Rogers S. O. and Bendich A. J.** 1988.extraction of DNA from plant tissues. *In*: Gelvin S. B. and Shilperoot R. A. (eds) Plant molecular biology manual. Kluwer Academic publishers, Dordrecht, pp I-10.
5. **Robert O., Dedryver F., Leconte M., Rolland B. and De Vallavieille-Pope.** 2000. Combination of resistance tests and molecular tests to postulate yellow rust resistance gene *Yr17* in bread wheat lines. Plant Breed., **119**: 467-472.
6. **Singh R., Tiwari R. and Datta D.** 2003. Detection of leaf rust resistance genes *Lr9* and *Lr10* in wheat (*Triticum aestivum*) by PCR based STS markers. Acta Phytologica et Entomologica Hungarica, **38**: 245-249.
7. **Singh R., Datta D., Priyamvada Singh S. and Tiwari R.** 2004. Marker-assisted selection for leaf rust resistance genes *Lr19* and *Lr24* in wheat (*Triticum aestivum* L.) J. Appl. Genet., **45**: 399-403.