Genetic analysis of leaf rust resistance in three common wheat cultivars MP 3288, HI 784 and HI 1418 under field conditions

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Abstract

This study aimed to investigate the genetic basis of leaf rust resistance in three bread wheat cultivars viz. MP 3288, HI 1418 and HI 784 which have been maintaining high levels of resistance to leaf rust since their release in 2011, 2000, and 1983, respectively. These cultivars also possess leaf tip necrosis phenotype. These were crossed with a susceptible bread wheat cultivar Lal Bahadur and also among themselves in non-reciprocal manner. The F₁, F₂, and F₃ populations were raised and the inheritance of leaf rust resistance was studied using prevalent and highly virulent Puccinia triticina pathotype 77-5 (121R63-1) during 2014-17. These studies showed that the field (adult-plant) resistance of these cultivars is governed by two dominant genes each. Closely linked molecular markers L34DINT9F and L34PLUSR revealed the presence of non-race specific adult-plant leaf rust resistance gene Lr34 in all cultivars of present study. Absence of the other documented race non-specific APR genes viz., Lr46, Lr67 and Lr68 was indicated in all the three test cultivars based on genotyping with closely linked molecular markers WMC44, CFD71 and csgs, respectively. The other dominant gene appears to be an all-stage resistance gene since all the three cultivars displayed high levels of seedling resistance to the test pathotype. Stable resistance of these cultivars could be due to synergistic/additive or complementary effects resulting from the combination of Lr34 and the all-stage resistance gene.

Key words: Adult-plant resistance, Lr34, all-stage resistance, inheritance study, leaf rust resistance, leaf tip necrosis

Introduction

Wheat is one of the most important cereal crops in the world with a production of 749 million tonnes (FAO STAT 2016). Wheat yield is affected by many biotic and abiotic factors in different production environments of the world. Leaf rust, caused by Puccinia triticina Erikss., is most common among the three wheat rusts and is one of the most important diseases affecting wheat production worldwide. To date, 76 leaf rust resistance genes in wheat have been mapped to different chromosome locations and given gene designations (McIntosh et al. 2015). The durability of most of these genes depends on the rapidity with which new virulent races are emerging against their large scale deployment. Varieties carrying single resistance genes often become susceptible in a relatively short span of time (5-8 years) due to emergence of new rust races (Tomar et al. 2014). Therefore, introgression of wheat with different leaf rust resistance genes at rapid pace is very important in order to ward off leaf rust epidemics. Though majority of the rust resistance genes can be detected in seedlings, they remain effective throughout the plant life and hence, are described as overall or all-stage resistance genes. Genes like Lr1, Lr10, and Lr21 are examples of race specific leaf rust resistance genes which are effective throughout the plant life (Dyck and Kerber 1985). These seedling resistance genes are characterized by very low infection types with hypersensitive flecks with/without minute uredinia surrounded by necrosis and/or chlorosis to avirulent leaf rust pathotypes. These race specific (resistant to a number of rust pathotypes but susceptible to others) seedling resistance genes are highly vulnerable as they increase the selection advantage to the pathogen populations leading to ‘breakdown’ of resistance due to evolution of new virulent races. Several wheat varieties which were resistant when they were released owing to seedling resistance genes, become susceptible once leaf rust
races with matching virulences evolved. Certain leaf rust resistance genes were shown to provide partial resistance, manifested by reduced uredinial numbers and size accompanied by varying degree of chlorosis (Caldwell, 1968). These genes express in adult plants and are ineffective in the seedling stage. They confer non-race specific resistance which is equally effective against all the rust pathotypes, and also sometimes provide resistance to other diseases as well. Genes like Lr34, Lr46, Lr67 and Lr68 are known examples of non-race specific adult plant resistance (APR) genes. These genes are also associated with leaf tip necrosis phenotype (ltn). Even though these APR genes do not provide complete resistance singly which is characterized by hypersensitive infection types, they are known to have provided long-term durable non-specific resistance. Durability of resistance and their ltn phenotype prompted us to study the genetic basis of leaf rust resistance in Indian bread wheat cultivars MP 3288, HI 784 (Swati) and HI 1418 (Naveen Chandausi) which had shown resistance to leaf rust since their release in 2011, 1983 and 2000, respectively.

Materials and methods

The investigations were carried out at ICAR-Indian Agricultural Research Institute, Regional Station, Indore during 2015-2017. Wheat cultivars MP 3288, HI 784 and HI 1418 were crossed (five ear heads per each cross) with Lal Bahadur (susceptible female parent) (Lal Bahadur/MP3288; Lal Bahadur/HI784; Lal Bahadur/HI1418) and were also inter crossed among themselves without reciprocals (MP3288/HI784; HI784/ HI1418 and HI1418/MP3288).

Leaf rust pathotype used

Seedling and adult plant tests were conducted, respectively, in a glasshouse and isolated field nurseries, with virulent, aggressive, and widely prevalent Puccinia triticina pathotype 77-5 (121R63-1, North American nomenclature THTTS) (Bhardwaj et al., 2010). The avirulence/virulence formula for this pathotype based on the seedling tests is given as under:

**Avirulent:** Lr9, Lr18, Lr19, Lr24, Lr25, Lr28, Lr29, Lr32, Lr39, Lr41, Lr42 and Lr45; **Virulent:** Lr1, Lr2a, Lr2b, Lr2c, Lr2d, Lr3a, Lr3ka, Lr10, Lr11, Lr12, Lr13, Lr14a, Lr14b, Lr14ab, Lr15, Lr16, Lr17, Lr20, Lr21, Lr22a, Lr22b, Lr23, Lr26, Lr27+Lr31, Lr30, Lr33, Lr34, Lr35, Lr36, Lr43, Lr44, Lr46, Lr48 and Lr49.

Population studies

Seeds of parental wheat cultivars (MP 3288, HI 784, HI 1418 and Lal Bahadur), 'check' RL 6058 (Thatcher + Lr34 with ltn phenotype), F<sub>1</sub> and F<sub>2</sub> populations were space planted 10 cm apart in 2.0 m rows with 30 cm spacing. Parental cultivars were sown in two replications among the hybrid populations. About 350 F<sub>2</sub> seeds from each cross with Lal Bahadur and about 1200 from each of the crosses among the three varieties were sown. Seed from individual F<sub>1</sub> plants was sown separately. Rust spreader rows consisting of mixtures of highly susceptible wheat lines were planted after every 20 test rows and also all around the experimental plot. Leaf rust inoculation was done using hypodermic syringes and also by several rounds of spraying aqueous suspensions of urediniospores 50-60 days after planting. Leaf rust scores on flag and flag-minus-one leaves was recorded based on the modified Cobb scale (Peterson et al. 1948) and host response (Roelfs et al. 1992). F<sub>2</sub> plants were grouped into resistant and susceptible classes to determine ratios. F<sub>3</sub> families derived from individual F<sub>2</sub> plants were progeny-tested to confirm genotypes. Fifty seeds from F<sub>2</sub> plants were sown in 2.5 m rows spaced 30 cm apart. F<sub>3</sub> families were classified as homozygous resistant, segregating or homozygous susceptible, and chi-squared tests were used to test the goodness-of-fit of observed F<sub>2</sub> and F<sub>3</sub> ratios to expected Mendelian segregation ratios. The seedling tests of parental wheat cultivars (MP 3288, HI 784, HI 1418 and Lal Bahadur) and 'check' RL 6058 (Thatcher + Lr34) were conducted at 20-25°C ± 2°C using standard glasshouse procedures.

Seedling tests

The test seedlings were raised in 10 cm clay pots and 8-10 days old seedlings (primary leaf fully expanded and second leaf just emerged) were inoculated by spraying freshly collected urediniospores of P. triticina pathotype 77-5 (121R63-1). After inoculation, the pots were incubated in moist chambers for 12-18 h, and were then transferred on to glasshouse benches. Infection types (ITs) of the parental lines along with check were recorded 12-14 days after inoculation (Nayar et al. 1997).

DNA extraction and PCR analysis

DNA was isolated from 15-day-old seedlings of the wheat genotypes MP 3288, HI 784 and HI 1418 by the CTAB method. The molecular markers used for detection of APR genes associated with ltn phenotype...
were L34DINT9F and L34PLUSR for \( Lr34 \) gene (Lagudah et al. 2009), WMC44 for \( Lr46 \), CFD71 for \( Lr67 \) (Hiebert et al. 2010) and csGS for \( Lr68 \) (Herrera-Foessel et al. 2012). Amplifications were carried out in 10 µl reaction mixtures containing 0.8 µl of dNTPs (Merck, India), 0.1 µl of Taq polymerase (1 U) (Xcelris Labs, India), 1.5 µl each of forward and reverse primers (10 pmol) (Xcelris Labs, India), 1.5 µl of 10X PCR buffer and 2.5µl of template DNA. PCR was performed in a Bioer Gen pro thermo cycler. PCR conditions included initial denaturing at 94°C for 5 min, followed by 30 cycles of 94°C for 45 s, annealing at 48°C (\( Lr34 \)) or 60°C (\( Lr46, Lr67 \) and \( Lr68 \)) for 45 s and 72°C for 45 s and final extension of 72°C for 15 min (modified from http://maswheat.ucdavis.edu/protocols). PCR products were separated on 2.5% high-resolution agarose gel (Amresco) and visualized by ethidium bromide staining in a GELDOC system (SYNGENE).

Results

Seedling and adult plant resistance tests

The resistant parental lines HI 1418, HI 784 and MP 3288 along with the susceptible parental line Lal Bahadur and 'check' RL 6058 were screened against \( P. triticina \) pathotype 77-5 for two years. The three resistant parental lines as well as the F1s from their crosses with Lal Bahadur were highly resistant (Free to 5MR) to leaf rust pathotype 77-5. RL 6058 showed a much lower level of leaf rust resistance (up to 60MSS), compared to the three resistant parental lines, but higher level of resistance compared to Lal Bahadur (Table 1).

<table>
<thead>
<tr>
<th>Parental lines</th>
<th>Field responsea to Pt. 77-5</th>
<th>Seedling ITs to Pt. 77-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP 3288</td>
<td>0</td>
<td>TMR 0;</td>
</tr>
<tr>
<td>HI 1418</td>
<td>TR</td>
<td>TMR 0;</td>
</tr>
<tr>
<td>HI 784</td>
<td>TR</td>
<td>5MR 12</td>
</tr>
<tr>
<td>Lal Bahadur</td>
<td>100S</td>
<td>100S 4</td>
</tr>
<tr>
<td>RL 6058 (Thatcher + ( Lr34 ))</td>
<td>40MSS 60MSS 4</td>
<td>4</td>
</tr>
</tbody>
</table>

aField responses were recorded as per modified Cobb scale (Peterson et al. 1948) and host response (Roelfs et al. 1992)b Seedling ITs were recorded as per scale given by Nayar et al. 1997

Table 2. Segregation for adult-plant resistance to \( P. triticina \) pathotype 77-5 in \( F_2 \) populations

<table>
<thead>
<tr>
<th>Crosses</th>
<th>( F_2 )</th>
<th>( \chi^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lal Bahadur/MP 3288</td>
<td>304</td>
<td>14</td>
<td>1.85(15:1)</td>
</tr>
<tr>
<td>Lal Bahadur/HI 784</td>
<td>311</td>
<td>17</td>
<td>0.64(15:1)</td>
</tr>
<tr>
<td>Lal Bahadur/HI 1418</td>
<td>285</td>
<td>14</td>
<td>1.25(15:1)</td>
</tr>
<tr>
<td>MP 3288/HI 784</td>
<td>1183</td>
<td>0</td>
<td>4.64(255:1)</td>
</tr>
<tr>
<td>HI 784/ HI 1418</td>
<td>1142</td>
<td>0</td>
<td>4.48(255:1)</td>
</tr>
<tr>
<td>HI 1418/ MP 3288</td>
<td>1173</td>
<td>0</td>
<td>4.6(255:1)</td>
</tr>
</tbody>
</table>

R, resistant; S, susceptible; Chi-square value for significance at \( P = 0.05 \) and 1 df is 3.84 and \(^a\)Homozygous susceptibility was confirmed by progeny tests

Population studies

When the \( F_2 \) ratios of susceptible parent x resistant parent crosses based on their progeny tests involving \( F_3 \) families were analyzed, the data well to 15 resistant (R) : 1 susceptible (S) plants (Table 2). This indicated the presence of two dominant genes governing resistance in each of the three genotypes studied. In resistant x resistant parents crosses, there was no segregation for any susceptible plants in the \( F_2 \) populations indicating that the three genotypes had at least one major gene in common. These genotypes exhibited seedling resistance also as low infection types (ITs) were observed in their seedling tests with the \( P. triticina \) pathotype 77-5 (Table 1).

PCR analysis

L34DINT9F and L34PLUSR markers are known to amplify a 517-bp allele on chromosome 7DS in lines carrying the APR gene \( Lr34 \). This allele was detected in HI 1418, HI 784 and MP 3288 showing the presence of the gene \( Lr34 \) in each one of them (Fig. 1). The other markers viz., WMC44, CFD71 and csGS used to detect \( Lr46, Lr67 \) and \( Lr68 \), respectively, did not amplify any of the expected products indicating the absence of these genes in the test genotypes (Fig. 2, 3 and 4). As the field (adult-plant) resistance of the three cultivars studied is based on two dominant genes, an all-stage resistance gene (expressed in seedlings and effective throughout the plant life) appears to be present in each of them in addition to the APR gene \( Lr34 \).

Discussion

The three bread wheat cultivars studied viz., MP 3288,
Brazilian cultivar ‘Frontana’ maintaining leaf rust resistance since its release in 1934 (Roelfs 1988). No virulence to any of the lines carrying \( \text{Lr34} \) has been reported anywhere (Krattinger et al. 2009). This gene while reducing uredinial size increases the latent period (Singh and Huerta-Espino, 2003).

Although this gene has been providing durable resistance to leaf rust, level of resistance is greatly influenced by inoculum load and environmental factors, particularly temperature, and hence, singly, it is not able to provide adequate resistance under heavy inoculum load and warmer temperatures. However, several studies have reported enhanced levels of leaf...
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The leaf rust resistance was also reported to be enhanced when Lr34 gene was combined with Lr13 and perhaps Lr12 suggesting that these were the probable sources of durable resistance (Roelfs 1988). A near immune response was observed when Lr34 was present with Lr37 due to their synergistic action (Kloppers and Pretorius 1997). The soft red winter wheat cultivars and breeding lines which had seedling resistance genes Lr2a, Lr9, and Lr26 combined with adult plant resistance genes like Lr34 and Lr12 were highly resistant to leaf rust. Lines carrying seedling resistance genes Lr1, Lr10, Lr11, and Lr18 along with adult plant resistance genes displayed higher levels of leaf rust resistance (Kolmer 2009). Saharan et al. (2012) identified the presence of QTL along with Lr34 gene in HD2189 X Agra Local population which is responsible for leaf rust resistance in HD 2189 cultivar. Based on the leaf rust responses of several lines carrying different gene combinations. Sawhney (1992) reported the likelihood that Lr34 interacted with the complementary genes Lr27 and Lr31 to produce enhanced resistance under conditions of high disease incidence. Kumar et al. (2018) reported that presence of Lr34 gene will also improve spot blotch resistance as both leaf rust and spot blotch resistance genes are closely linked. The Lr34 gene has been cloned and found to encode an ATP-binding cassette (ABC) transporter (Krattinger et al. 2009) which obstructs the invasion and spread of wheat pathogens. When this gene was transferred to tetraploid durum wheat, the transgenic durum lines showed elevated gene expression levels, compared to the endogenous hexaploid background gene, and high levels of seedling resistance to wheat leaf rust, stripe rust and powdery mildew diseases (Rinaldo et al. 2017). The gene Lr34 is common among the three genotypes as revealed by genotyping with closely linked molecular markers, Lr34 DINT9F and Lr34PLUSR. However, allelic relationship of the second gene among the three genotypes is not known based on the available data and need to be studied. High levels of stable leaf rust resistance manifested by the three cultivars studied appear to be based on the synergistic/additive or complementary effects resulting from the combination of Lr34 and the all-stage resistance gene. These cultivars can be utilized in bread wheat crop improvement programme as they combine high levels of leaf rust resistance with desired agronomic traits.

Authors’ contribution

Conceptualization of research (TLP, ANM, SC); Designing of the experiments (TLP, ANM, SC); Contribution of experimental materials (JBS, SVS); Execution of field/lab experiments and data collection (TLP, KSS, JBS); Analysis of data and interpretation (TLP, ANM); Preparation of manuscript (TLP, ANM, SC, SVS).

Declaration

The authors declare no conflict of interest.

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References


