Screening of Pakistani wheat landraces for stripe rust resistance using molecular markers

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Abstract

Wheat crop is cultivated throughout the world and it is most valuable food grain and dietary mainstay for all human beings, it provides one half of human food calories. Many biotic and abiotic stresses including a number of diseases badly affect the production of wheat. Among them yellow rust or stripe rust caused by Puccinia striiformis Westend. f. sp. tritici Eriks, is one of the most detrimental disease in cool, moist temperate regions of the world, including Asia, Europe, North America, South America, Middle East and Africa. In the present study 50 Pakistani wheat germplasm were evaluated for stripe rust. For molecular study, four specific markers were used for the amplification of DNA to detect Yr18, YrCN19, Yr24, and Yr32 genes in the DNA samples. Molecular screening for four stripe rust genes revealed that Yr18 gene was not detected in all forty eight germplasm, YrCN19 was found in 05 genotypes namely Rawal-87, GA-2002, Kaghan-93, Faisalabad-85 and Shalimar-88, Yr24 was found in 11 genotypes viz: KSK, Wadanak-85, Raskoh, Haider-2002, Punjab-76, GA-2002, Pari-73, Kaghan-93, AS-2002, Khyber-83 and Fakhri-sarhad. Yr32 was found in 04 genotypes viz: Haider 2002, Rawal 87, Soghat 90 and Chakwal 86.

Keywords: Agarose, PCR, Puccinia, SSR markers, stripe rust

INTRODUCTION

Wheat crop is cultivated throughout the world and it is most valuable food grain and dietary mainstay for all human beings it provides one half of human food calories (Anjum and Walker, 1991). The production of wheat all over the world from an area of 220 million hectares was approximately 572 million metric tons (Anonymous 2006). Many biotic and abiotic stresses including a number of diseases badly affect the production of wheat. Among them yellow rust or stripe rust caused by Puccinia striiformis Westend. f. sp. tritici Eriks, is one of the most detrimental disease around the world (Stubbs, 1988; Line, 2002). The stripe rust is privileged by long cool springs and mild winters its symptoms includes the appearance of citron yellow Uredia (spore masses) on the host, and produces long stripes over the surfaces of leaf (Smiley and Cynthia, 2003). DNA technology has improved due to recent developments so to control of these stresses plant breeders search for resistant varieties. Molecular markers like SSR (microsatellites) based markers were used for detection of fungal resistant genes. Stripe rust resistant genes which have been reported so far are more than 70 (Karuparty et al, 2007).

Stripe (yellow) rust of wheat, caused by Puccinia striiformis Westend. f. sp. tritici Eriks., is one of the most damaging diseases of wheat in many areas around the world (29,30). Stripe rust has traditionally been associated with wheat production in the cool, temperate regions of the world, including Asia, Europe, North...
Table 1: Molecular markers and primer sequences used for Yr18, YrCN19, Yr24 and Yr32.

<table>
<thead>
<tr>
<th>Locus name</th>
<th>Location (Chromosome number)</th>
<th>SSR markers</th>
<th>Primer sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yr18</td>
<td>7D</td>
<td>Xgwm120</td>
<td>GTGAAGCAGACCCAACAC</td>
<td>Singh, (1992)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GACGGCTGCGACGTAGAG</td>
<td></td>
</tr>
<tr>
<td>YrCN19</td>
<td>2B</td>
<td>Xgwm410</td>
<td>GCTTGAGACGCCACATG</td>
<td>Luo et al. (2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CGGACCTTGAGAGGCTAGA</td>
<td></td>
</tr>
<tr>
<td>Yr24</td>
<td>1B</td>
<td>Xgwm498</td>
<td>GTGGTAGGCCTTGAGTCTAGA</td>
<td>Li et al. (2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CGGGAGGAGTAAAGAAAGG</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TTGAAGTGCTATTGGCT</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: PCR conditions used in the present research for Yr18, YrCN19, Yr24, and Yr32 genes.

<table>
<thead>
<tr>
<th></th>
<th>Locus name</th>
<th>PCR conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yr18</td>
<td>94°C-3min, 45 cycles (94°C – 1 min, 60°C – 1 min, 72°C – 2 min), 72°C – 10 min and 4°C – hold</td>
</tr>
<tr>
<td>2</td>
<td>YrCN19</td>
<td>94°C – 3 min, 45 cycles (94°C–1min, 55°C–1min, 72°C – 2min), 72°C – 10 min and 4°C – hold</td>
</tr>
<tr>
<td>3</td>
<td>Yr24</td>
<td>95°C – 3 min, 35 cycles (94°C – 1 min, 60°C – 1 min, 72°C – 1.5 min), 72°C – 10 min and 4°C – hold</td>
</tr>
<tr>
<td>4</td>
<td>Yr32</td>
<td>95°C-3min, 35 cycles (94°C-10s, 51°C - 30s, 72°C – 30 s), 72°C – 10 min and 4°C – hold</td>
</tr>
</tbody>
</table>

MATERIAL AND METHOD

Fourty eight wheat germplasm were screened by using molecular markers (microsatellites). The germplasm was kindly provided by NARC Islamabad. Morphological study was also done for 15 parameters/ traits. Wheat germplasm was planted in three replications in randomized complete block design (RCBD). The rows were kept with a space of 30cm from each other. Observations were recorded at appropriate stage for morphological traits. Small scale DNA protocol of Weining and landgridge 1992 was used to isolate the DNA.

Gel Electrophoresis

The quality and quantity of the DNA isolated was run checked on 1% agarose /TBE gel. 100 ml Tris Boric acid – EDTA (TBE) buffer was taken and 1g agarose was dissolved in it and then boiled. Then the gel was cooled for some time and 7 µl ethedium bromide was added to it. Two µl 6X loading dye was mixed with the five µl DNA and then loaded in the wells. Then a constant voltage of 70 volts was given to the gel. The gel was then photographed with the help of Uvitch gel documentation system.

DISCUSSION

Pakistan economy depends upon agriculture but still there is a deficiency in the production of wheat. The most damaging diseases of wheat throughout the world one fungal especially stripe rust (Sumikova and Heanzalova, 2010) Marker based selections is the technique through which environmental factors has no influence (Mago etal; 2005) Present research work was about the 48 genotypes for the molecular study of stripe rust resistance genes. Stripe rust resistant genes Yr18, YrCN19, Yr24 and Yr32 were screened using SSR markers (microsatellites). Each gene was optimized carefully and separately for the PCR polymerase chain.
reaction. The Yr18 gene was not found in any of the 48 genotypes. The YrCN19 was found in 11 genotypes namely Rawal 87, GA-2002, Kaghan 93, Yr+5 and Shalimar 88. Yr24 was found in 11 genotypes namely Merco 2007, KSK, Wadanak 85, Raskoh, Haider 2002, Punjab 76, GA-2002, Pari 73, Kaghan 93, AS-2002, Rawal87, Soghat 90 and Chakwal 86 out of 48 genotypes. The study of comparative yield performance showed that yield can be improved by improving their associated characters. In the light of the above study it is clear that using molecular markers is more reliable and quicker method for identifying individual genes than applying traditional genetic methods.

REFERENCES