GENETIC MARKER ASSISTED SELECTION FOR COMMON BUNT RESISTANCE (TILLETIA SP.) IN SOME WHEAT LINES

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Abstract: In view of the molecular marker assisted selection for common bunt resistance, wheat lines in segregation have been used, as well as stabilized dihaploid wheat lines – resulted from different hybrid combinations in which parental forms resistant to bunt were used. To identify the polymorphisms between the resistant and sensitive forms, a pair of specific primers for the common bunt resistant gene (Bt-10) were used, as well as a number of twenty non-specific RAPD (Random Amplified Polymorphic DNA) primers. The main objective was finding some polymorphic markers for common bunt resistance in view of the selection of these lines on the basis of the markers. Such a marker were obtained (1450 bp) with specific primers, a marker linked to Bt-10 gene, and with non-specific primers more polymorphic fragments were obtained, present in the resistant forms and absent in the sensitive forms, possibly linked to common bunt resistant genes. In perspective, it is stipulated the conversion of these RAPD molecular markers to SCAR (Sequence Characterized Amplified Region) specific markers.

INTRODUCTION

Common bunt of wheat caused by fungi type Tilletia (T. tritici and T. foetida) is one of the most prevalent diseases of common bunt of wheat, causing yield losses and diminishing crop quality both in common wheat (Triticum aestivum L.) and in durum wheat (Tr. Durum Desf.), (Goates et al., 1996). Common bunt resistance is controlled by a number of 15 genes (Bt genes), present in some varieties.

In the classical breeding process for common bunt resistance, the individual selection assumes testing under conditions of natural or artificial infection, selection that is sometimes inefficient because of the influence of environmental conditions.

The molecular marker assisted selection (MAS), markers linked to the interest gene, is advantageous given the fact that these ones are not influenced by the environmental conditions. (Mohan et al., 1997). Identifying the molecular markers that transmit themselves in linkage to the resistance gene will facilitate the rapid and early selection of the resistant wheat lines, guaranteeing the reduction of breeding cost.

The RAPD technique (Random Amplified Polymorphic DNA), (Williams et al., 1990) has been used for the identification of molecular markers linked to resistance genes to common bunt (Demeke et al., 1996, Lintott et al., 1998) and to other wheat diseases (Devos and Gale, 1992). This technique relies on random amplification of DNA fragments using PCR technique with a single oligonucleotide primer (10-mer) and the analysis of the molecular polymorphism that assumes the presence of amplification products in resistant individuals and their absence in sensitive individuals.
Microsatellite or SSR markers relies on the amplification of a DNA microsatellite sequence (Simple Sequence Repeats), using a pair of specific primers to the sequences that flank it. These ones ensure a high polymorphism at the allelic level and can be codominant or dominant.

We put forward in this paper to present the results of testing some specific SSR primers and RAPD non-specific primers at wheat lines in view of the selection on the basis of markers for common bunt resistance.

MATERIAL AND METHOD

The biological material used was represented by 13 wheat lines belonging to some segregant generations, out of which 5 manifested field resistance to *Tilletia* (marked from 23 to 27) and 8 varieties sensitive to this disease, parental forms that had been used in crossings (marked from 15 to 22). Also, a number of 69 dihaploid lines (DH) were used, that derive from two hybrid combinations, 28 lines from the combination GP 369 (P99419G4-1AI1-1/00356G8-1), (marked from 9.1 to 9.28) and 41 lines from the combination GP 384 (99419G4-1AI1-1/98047G14-2INC1), (marked from 4.1 to 4.4). The first number represents the combination and the second indicates the line number within the combination.

The DNA extraction was made from leaves using the CTAB method (Rogers and Benedich, 1994), and the determination of DNA quality and purity was made spectrophotometrically.

The microsatellite sequences was analysed with the pair of specific primers FSD and RSA, that are specific for the resistance gene *Bt-10* (Laroche et al., 2000). Because, after the amplification, an electrophoretic profile with more bands was obtained, similar to RAPD markers – the removing of non-specific bands was attempted, by increasing the annealing temperature gradient of the primers, with two grades per cycle, from the level of 42°C until the level to which amplification products were not obtained anymore.

The RAPD amplification was performed testing a number of twenty decamer primers. Out of these, five emphasised the polymorphic fragments to resistant forms (table 1).

<table>
<thead>
<tr>
<th>Primers</th>
<th>Nucleotidic sequence of primers</th>
<th>Tm</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPA 16</td>
<td>5'-AGCCAGCGAA-3'</td>
<td>32°C</td>
</tr>
<tr>
<td>Mic07</td>
<td>5'-TGTCTGGGTG-3'</td>
<td>32°C</td>
</tr>
<tr>
<td>Mic13</td>
<td>5'-TTCCCCCCAG-3'</td>
<td>34°C</td>
</tr>
<tr>
<td>Mic14</td>
<td>5'-TGAGTGGGGTG-3'</td>
<td>32°C</td>
</tr>
<tr>
<td>UBC 570</td>
<td>5'-GGCCGCTAAT-3'</td>
<td>32°C</td>
</tr>
</tbody>
</table>

Tm- primer melting temperature.

The amplification program included the following thermal cycles were 3.0 min at 94 C, 1 min at 94 C, 1 min at 32 C, 2 min at 72 C and 7 min at 72 C, a total of 45 cycles.

The amplification products were separated by agarose gel electrophoresis (1.4 %) with TBE (Tris–borat-EDTA) buffer and colored with ethidium bromide. Their visualizing was made in UV light, and the images were captured by a TV camera.
RESULTS AND DISCUSSIONS

Analysis with PCR specific primers
After the gradient amplification with FSD/RSA primers in case of lines DH 9.1-9.6, at primers annealing temperature of 48°C, only two well-marked bands were obtained, one of 1000 bp, present in the sensitive form too, and one of 1450 bp, absent in the sensitive form (figure 1). The 275 pb band mentioned by Laroche and his collaborators (2000) as a marker for the Bt-10 gene – present in our case only at lower annealing temperature of primers, practically disappeared. Therefore, we estimate that the 1450 bp band might be considered marker for the Bt-10 gene. In case of lines from a GP 384 hybrid combination, the respective band was absent only in lines 4.26 and 4.28, considered sensitive (figure 2).

Figure 1. PCR (SSR) amplification products with the specific primers FSD/RSA using the temperature gradient profile (a-42°C, b-44°C, c-46 °C, d- 48°C), in the dihaploid lines resulted from the GP 369 hybrid combination (9.1-9.6) and the reference line (17). L-100 bp molecular marker.

Figure 2. PCR (SSR) amplification products with specific primers FSD/RSA in the dihaploid lines resulted from the GP 384 hybrid combination (4.1-4.28) and the reference lines (17, 21). L-100 bp molecular marker.

With the help of specific molecular markers, defined by FSD and RSA primers, the presence of resistance genes has been ascertained, at the majority of the analyzed DH lines.
Analysis with non-specific RAPD primers

In case of non-specific molecular markers, the assignment of molecular markers to the resistant genes represents a first step, on the basis of the analysis of molecular polymorphism, obtained comparatively to sensitive forms. Polymorphic bands, specific to resistance genes, were obtained in case of primers OPA 16 and Mic14.

The primer OPA 16 showed a polymorphic band of 900 bp in line 24, considered resistant and absent in sensitive parental forms (15-20), (figure 4,a).

A similar situation was also ascertained in case of primer Mic 14, the polymorphic band of approximately 630 bp being present in line 23, considered resistant and absent in sensitive parental forms (figure 4,b).

Because with the two primers were obtained different polymorphic bands in different lines, considered resistant, we can assume that the respective bands might be considered markers of different resistance genes.

The polymorphic bands resulted from the two primers were also obtained in the dihaploid lines (figure 5 and 6).
For the amplification of DNA in dihaploid lines, lines that had not been tested in field for resistance to *Tilletia*, other three decamer primers (UBC 570, Mic 07 and Mic 13) were used, for which a very high polymorphism was obtained, comparatively to the reference sensitive forms. (figures 8, 9, 10). It is estimated that at least a part of the polymorphic bands might be associated with resistance genes to *Tilletia*. This can be confirmed after testing the lines regarding their resistance to *Tilletia* in conditions of artificial infection.
Figure 9 (a). RAPD amplification products with the Mic 13 decamer primer in the dihaploid lines resulted from the GP 384 hybrid combination (4.1-4.28) and the reference lines (17). L-100 bp molecular marker.

Figure 10 (b). RAPD amplification products with the Mic 07 decamer primer in the dihaploid lines resulted from the GP 369 hybrid combination (9.1-9.28) and the reference lines (17). L-100 bp molecular marker.

CONCLUSIONS

• For the selection of wheat dihaploid lines, bearing the resistance genes to *Septoria*, the marking with SSR was successfully used, with specific primers, complementary to some unique sequences which flank microsatellite DNA sequences.

• By amplification in temperature gradient – in the case of the FSD/RSA specific primers – a marker of 1450 bp was identified, possibly linked to the *Bt-10* resistance gene.

• With the help of this marker, the presence of the resistance genes was ascertained at the majority of the analyzed DH lines.

• In case of RAPD marking of wheat lines tested for field resistance to *Tilletia*, OPA 16 and Mic 14 decamer primers showed each one a polymorphic band of 900 bp and respectively 630 bp, bands that probably mark resistance genes to *Tilletia*.

• In case of RAPD marking of dihaploid lines that were not tested yet for field resistance, a very high polymorphism was obtained from the used primers (UBC 570, Mic 07 and Mic 13), the polymorphic bands, absent in the sensitive forms, might be linked to some resistance genes to *Tilletia*.

REFERENCES