PRELIMINARY RESULTS ON MARKER ASSISTED SELECTION FOR DISEASE RESISTANCE IN COMMON WHEAT (*Triticum aestivum*)

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Abstract: Our attempts to find RAPD markers linked with resistance genes to *Septoria* and *Tilletia* relying on co-segregation and to test the SSR markers, identified in literature, in order to select bread wheat resistant to *Septoria* and *Tilletia* are presented. Several candidate RAPD markers possibly linked with *Septoria* resistance genes and fewer linked with *Tilletia* resistance genes were identified. Some RAPD and SSR markers were specific for some resistant lines. These markers, probably, marked different resistance genes in different lines. It seems that some *Septoria* resistance genes came from rye genome because these genes were marked with some primers specific for rye genome. We found segregation for these markers in several lines, indicating that the analyzed lines were not completely stabilized. The ISSR markers used could not discriminate the resistant from susceptible genotypes to *Septoria* and *Tilletia*.

INTRODUCTION

Marker assisted selection (MAS) supposes achievements of an indirect selection by molecular markers strongly linked with useful genes subjected to selection. To achieve that, it has first to attribute molecular markers to useful genes by a genetic analysis implying analysis of co-segregation of the markers with genes (or desired trait controlled by genes), Bulked Segregant Analysis (BSA) or utilization of Near Isogenic Lines (NIL), (Michelemore et al., 1991; Mohan et al., 1997; Chagué et al., 1999; Adhikari et al., 2004).

The RAPD markers, and the markers derived from it, like ISSR (Inter Simple Sequence Repeats), rely on random amplification of small DNA fragments from genomal DNA using PCR technique with a single oligonucleotid primer (usually 10-mer). Polymorphism in nucleotide sequence is sometimes sufficient for it to function as a molecular marker, revealed by presence of an amplification product in one individual and absent in the other. This type of marker is dominant. Unfortunately the polymorphism in bread wheat is quite reduced and to find a marker that marks a desired gene, like a gene for resistance to STB (*Septoria tritici* bloch),(*Stb* genes) or resistance to *Tilletia* sp. (*Bt* genes) is not very probable.

SSR markers rely on amplification of a DNA microsatellite sequence (Simple Sequence Repeats) using a pair of specific primers (left and right) flanking a microsatellite sequence. If the amplification products of the two alleles are of different size, this type of marker is co-dominant, being possible to discriminate the homozygous from heterozygous. If already there
exit primers, which mark, for instance, the alleles for resistance to *Septoria* or *Tilletia*, the selection of resistant forms it is easy to do.

In this paper we present our attempts to find RAPD markers linked with resistance genes to *Septoria* and *Tilletia* relying on co-segregation and our results from testing the PCR markers, identified in literature, in order to do selection of bread wheat for resistance to *Septoria* and *Tilletia*.

**MATERIAL AND METHOD**

The biological material used is represented by 14 lines of bread wheat with field resistance to *Septoria* (1-14), 8 varieties susceptible to *Septoria* (15-22) and 5 lines with field resistance to *Tilletia* (23-27). Lines 1-6 were obtained by hybridization with resistance sources derived from D genome, lines 7-9 were obtained by hybridization with resistance sources derived from A genome and lines 11-14 were obtained by hybridization with resistance sources derived from R genome (Rye). DNA extraction was made on individual plants, using CTAB protocol (Rogers and Benedich, 1994).

RAPD amplification was performed using twenty decamer primers, nine primers being polymorphic (table 1).

<table>
<thead>
<tr>
<th>Primers</th>
<th>Nucleotidic sequence of primers</th>
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</thead>
<tbody>
<tr>
<td>OPA-11</td>
<td>5’-CAATCGCCGT</td>
</tr>
<tr>
<td>OPC-4</td>
<td>5’-CCGCATCTAC</td>
</tr>
<tr>
<td>OPC-13</td>
<td>5’-AAGCCTCGTC</td>
</tr>
<tr>
<td>OPC-20</td>
<td>5’-ACTTCGCCAC</td>
</tr>
<tr>
<td>70.08</td>
<td>5’-CTGTACCCCC</td>
</tr>
<tr>
<td>Mic-14</td>
<td>5’-TGAGTGAGGTG</td>
</tr>
<tr>
<td>Set 5</td>
<td>5’-AAGAGCCCGT</td>
</tr>
<tr>
<td>UBC-196</td>
<td>5’-CTTCCTCCCC</td>
</tr>
<tr>
<td>OPH-20</td>
<td>5’-GGGAGACATC</td>
</tr>
</tbody>
</table>

ISSR amplification was performed using four primers: ISSR-1 (CA)$_8$RG; ISSR-4 (GA)$_8$YC; ISSR-6 (AC)$_8$YA and ISSR-7 (GT)$_8$YG, R meaning a purine and Y meaning a pyrimidine (Fang and Roose, 1997).

As SSR marker, for *Septoria* resistance genes, we used five markers, each marker being defined by a specific pair of primers, left and right, (table 2) and one pair of primers for *Tilletia* resistance genes, FSD (5’-GTTTTATCTTTTATTTTTC) and RSA (5’-CTCCTCCCCCCC), (Laroche et al.,2000).

Because introgressions of the rye genome into the wheat genome could include disease resistance genes we tried one primer which is known to give rye specific RAPD products (OPH-20) and a pair of specific primers derived from rye specific RAPD clone pSC20H, namely F3 (5’-GATCGCCTCTTTTGGCAAGA) and R3 (5’-TCACTGATCACAAGAGCTTG), (Katto et al.,2004).

<table>
<thead>
<tr>
<th>Marker</th>
<th>Resistance gene</th>
<th>Positioned on chromosome</th>
<th>Primers</th>
<th>Structure of the primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xgwm 389</td>
<td>Stb 2</td>
<td>3BS</td>
<td>WMS389-L R</td>
<td>5’-ATCATGTGCATCCTCTTTGACG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5’-TGCCATGCACATTTAGCAGAT</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSIONS

RAPD markers linked with *Septoria* resistance genes could be revealed by analysis of molecular polymorphism obtained with different primers among different resistant wheat lines and susceptible wheat parental forms. For each primer, we can consider as candidate that marks a resistance gene, the amplification products present in resistant lines and absent in the susceptible parent. The OPC4 primer gives two candidates, one of approximately 510 bp and another of approximately 1500 bp revealed in almost all resistant lines (figure 1).

The OPC 13 primer gives one candidate of 350 bp and the Mic 14 primer gives one candidate of 550 bp (figure 2). The UBC 534 primer gives three candidates (figure 3), the OPA 11 primer gives four candidates (figure 4), and the Set 5 primer gives one candidate (figure 5). The OPH20 primer that usually gives one 1400 bp product of amplification for rye introgression in the wheat genome (Ko at all. 2002), has given such a product in almost all resistant lines obtained by hybridization with *Triticale* (figure 6), but also in resistant lines obtained by hybridization with resistance sources derived from D and A genomes (figure 7).

The ISSR markers used, could not usually discriminate the resistant from susceptible forms, the electrophoresis profiles being monomorphic.

![Figure 1. RAPD amplification products from OPC4 primer in the *Septoria* resistant lines (1-14) and in the susceptible form (22). M-100 bp molecular](image)
Figure 2. RAPD amplification products from OPC13 (a) and Mic 14 (b) primers in the *Septoria* resistant lines (1-14) and the susceptible form (22). M-100 bp molecular marker.

Figure 3. RAPD amplification products from UBC534 primer in the *Septoria* resistant lines (1-14) and the susceptible form (22). M-100 bp molecular marker.

Figure 4. RAPD amplification products from OPA11 primer in the *Septoria* resistant lines (1-14) and the susceptible form (22). M-100 bp molecular marker.

Figure 5. RAPD amplification products from Set5 primer in the *Septoria* resistant lines (1-14) and the susceptible form (22). M-100 bp molecular marker.
In our experiment, two markers (Xgwm111 and Xgwm44) from the five SSR markers could not discriminate the *Septoria* resistant from the susceptible form. The Xgwm389 marker revealed a not very obvious segregation for the 190 bp product of amplification associated with Stb2 resistance gene. The Xgwm577 also produced a 180 bp product of amplification (figure 8) associated with Stb8 resistance gene and the Xgwm313 gave a 197 bp product of amplification (figure 9) present in genotypes with Stb7 resistance gene. Unfortunately in the last case this band was also obtained for some susceptible forms.

It is important to mention that the marker F3/R3, specific to rye genome, appeared in the individual plants from 11, 13 and 14 resistant lines to *Septoria* (figure 10). This suggests that the resistance genes come from rye genome. In the next step we are going to look for which gene to *Septoria* is associated with this marker.

Figure 6. RAPD amplification products from OPH20 primer in individual plants of *Septoria* resistant lines (11-14) and the susceptible forms (16-22). M-100 bp molecular marker.

Figure 7: RAPD amplification products from OPH20 primer in individual plants of *Septoria* resistant lines (1,7) and the susceptible form (17). M-100 bp molecular marker.
RAPD markers possibly linked with *Tilletia* resistance genes were fewer than for *Septoria* and also the polymorphic bands were obtained only in some resistant lines. Knowing that there are 15 Bt resistance genes to *Tilletia*, we have to emphasize that dwarf bunt and common bunt are controlled by the same resistance genes (Blair, 2004). In case of OPA 16 primer we obtained a polymorphic band (400 bp) only in 24 line (figure 11) and in case of Mic 14 primer a polymorphic band (280 bp) was obtained only in 23 line (figure 12).

Figure 8. PCR (SSR) amplification products from WMS577 primers in the individual plants of resistant lines to *Septoria* (8-12) and the sensible form (16-17). M-100 bp molecular marker.

Figure 9. PCR (SSR) amplification products from WMS313 primers in the individual plants from a) resistant lines to *Septoria* (1-3) and the sensible forms (15-19) and b) resistant lines to *Septoria* (3-5) and sensible forms (15-20). M-100 bp molecular marker.
The pair of primers FSD/RSA, known to be a specific PCR primer (Laroche, 2000), behaved as a RAPD primer generating many products of amplification. Among these products we can notice polymorphic band (420 bp) that segregates only in 25 and 27 lines (figure 13).
CONCLUSIONS

- We have obtained several candidate RAPD markers possibly linked with _Septoria_ resistance genes and fewer linked with _Tilletia_ resistance genes.

- Some RAPD and SSR markers were only detected in some resistant lines. These markers, probably marked different resistance genes in different lines.

- It seems that some _Septoria_ resistance genes were introgressed from the rye genome because these genes were marked with rye genome specific primers (OPH20 and F3/R3)

- We found segregation for these markers in some lines, showing that the analyzed lines are not completely stabilized.

- The ISSR markers used by us could not discriminate the _Septoria_ and _Tilletia_ resistant from susceptible forms.
REFERENCES


