Chromosomal locations of the maize (Zea mays L.) HtP and rt genes that confer resistance to Exserohilum turcicum

Juliana Bernardi Ogliari¹, Marco Antônio Guimarães² and Luis Eduardo Aranha Camargo³

¹Departamento de Fitotecnia, Centro de Ciências Agrárias, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil.
²Monsanto do Brasil Ltda, Uberlândia, MG, Brazil.
³Setor de Fitopatologia, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, SP, Brazil.

Abstract

We used 125 microsatellite markers to genotype the maize (Zea mays L.) near isogenic lines (NIL) L30HtPHtPRtRt and L30htphtpRtRt and the L40htphtprtrt line which contrast regarding the presence of the recently described dominant HtP and the recessive rt genes that confer resistance to Exserohilum turcicum. Five microsatellite markers revealed polymorphisms between the NIL and were considered candidate linked markers for the HtP resistance gene. Linkage was confirmed by bulked segregant sample (BSS) analysis of 32 susceptible and 34 resistant plants from a BC1F1 population derived from the cross (L30HtPHtPRtRt x L40htphtprtrt) x L40htphtprtrt. The bnlg198 and dupssr25 markers, both located on maize chromosome 2L (bin 2.08), were polymorphic between bulks. Linkage distances were estimated based on co-segregation data of the 32 susceptible plants and indicated distances of 28.7 centimorgans (cM) between HtP and bnlg198 and 23.5 cM between HtP and dupssr25. The same set of susceptible plants was also genotyped with markers polymorphic between L30HtPHtPRtRt and L40htphtprtrt in order to find markers linked to the rt gene. Marker bnlg197, from chromosome 3L (bin 3.06), was found linked to rt at a distance of 9.7 cM. This is the first report on the chromosomal locations of these newly described genes.

Key words: bulked segregant sample analysis, microsatellite markers, maize, near-isogenic lines, northern leaf blight.

Received: July 13, 2006; Accepted: November 6, 2006.
appears to be located on the short arm of chromosome 1 near the centromere (Carson, 1995). However, until now the chromosomal locations of the \( HtP \) and \( rt \) resistance genes mentioned above have not been determined.

Analyses of near-isogenic lines (NIL) and of bulked segregant samples (BSS) constitute two approaches commonly used to identify molecular markers linked to genes (Muchhal et al., 1988; Michelmore et al., 1991). In breeding programs, near-isogenic lines are easily obtained by backcrossing when a specific gene is introgressed from a donor line into a recurrent line. Thus, near-isogenic lines should be genetically identical over large portions of the genome, except for the segment containing the introgressed gene. Putative evidence of linkage between a marker and the target gene arises when the marker detects polymorphisms between near-isogenic lines. On the other hand, bulked segregant sample analysis involves comparisons between two-pooled DNA samples consisting of individual plants with extreme phenotypes identified in a segregating population. Evidence of linkage between a marker and the target gene arises when the marker is polymorphic between bulks. Thus, both strategies can be used concomitantly to identify putative markers linked to resistance genes. However, co-segregation analyses based on data from a segregating population is always required in order to confirm linkage and to estimate genetic distances.

The objective of the work described in this paper was to use near-isogenic lines and bulked segregant sample analyses to identify microsatellite (also called simple sequence repeats or SSRs) loci of known chromosomal locations linked to the \( HtP \) and \( rt \) genes. Linkage was confirmed by co-segregation analyses between these genes and candidate markers using susceptible individuals of a BC1F1 population. For this, the introgressed line L30\(^{HtPHtPR}\) was generated after six backcrossing cycles between the recurrent line and the donor line L10\(^{htphtpr}\) followed by two self-pollinations (BC 6S2) that brought the \( HtP \) locus to the homozygous state. The recurrent line was developed in central Brazil through self-pollinations and selections out of a synthetic composed of maize chromosomes. Markers that were monomorphic between these two and the recurrent line (L30\(^{htphtpr}\)) were considered potentially linked to the \( HtP \) locus. Such markers were further analyzed by bulked segregant sample analysis using individuals from a segregating BC1F1 population of 138 plants from the backcross [(L30\(^{HtPHtPR}\) x L40\(^{hphpRrt}\)) x L40\(^{hphpRrt}\)] previously evaluated for resistance to race 123x of \( E. \) turcicum (Ogliari et al., 2005). Two DNA bulks from 34 resistant and 32 susceptible plants were genotyped with the candidate markers identified in the near-isogenic lines analysis. Linkage between \( HtP \) and markers that revealed polymorphisms between bulks was tested in a co-segregation analysis as described below. Co-segregation between the \( rt \) gene and polymorphic microsatellite markers between L30\(^{HtPHtPR}\) and L40\(^{hphpRrt}\) was also tested in the same BC1F1 population.

Co-segregation analyses between polymorphic markers and the \( HtP \) and \( rt \) genes were carried out using the 32 plants from the susceptible bulk. Because \( HtP \) is dominant and \( rt \) is recessive (Ogliari et al., 2005), the genotype of all susceptible plants of the backcross [(L30\(^{HtPHtPR}\) x L40\(^{hphpRrt}\)) x L40\(^{hphpRrt}\)] should be \( hptphpRrt \). Thus, for markers linked to \( HtP \), the ratio between homozygotes and heterozygotes should differ by the chi-squared \( (\chi^2) \) test from the 1:1 ratio expected for non-linkage, that is, an excess of homozygotes for marker genotypes should be detected in relation to heterozygous recombinants. The same reasoning applies to \( rt \), except that in this case the heterozygotes should be in excess.

Assuming that all susceptible plants were homozygous recessive for \( HtP \) (\( hptphp \)) (Ogliari et al., 2005), the recombination frequency \( (c_1) \) between the linked marker locus and \( HtP \) relative to the susceptible plants can be estimated by \( c_1 = \frac{N_1}{N_s} \), where \( N_1 \) corresponds to the number of heterozygous recombinant plants for the marker locus and \( N_s \) to the total number of susceptible plants genotyped.
Similarly, the recombination frequency (c1) between the linked marker locus and rt can be estimated by c1 = N2/Ns, where N2 corresponds to the number of homozygous recombinant plants for the marker locus and Ns to the total number of susceptible plants genotyped. For both genes, standard error estimates of c1 were given by Vc1/2 = \(\sqrt{\frac{(1-c1)/N_s}{N_s/2}}\) (Liu, 1998) and the genetic distances were estimated by Haldane’s mapping function (Haldane, 1919).

Sixty-one microsatellite markers were considered informative since they revealed polymorphism between the donor L10\(^{HtPHtP}\) and the recurrent L30\(^{HtPHtPRtRt}\) lines. Of these, however, 56 were monomorphic between L30\(^{HtPHPRtRt}\) and L30\(^{HtphPRtRt}\), whereas the remaining five marker loci (the bnlg198 and dupssr25 from chromosome 6L (bin 2.08), ole2 (bin 5.02) and dupssr15 from chromosome 6L (bin 6.06)) were monomorphic between L30\(^{HtPHPRtRt}\) and L10\(^{HtPHPH}\), but polymorphic between these two lines and L30\(^{HtphPRtRt}\) and thus were considered potentially linked to the HtP locus. However, only two of these (bnlg198 and dupssr25) were confirmed by bulked segregant sample analysis as being linked to HtP since they were polymorphic between the bulks, that is, the susceptible bulk amplified the same marker allele of the resistant line L40\(^{HtphPRtRt}\), whereas the resistant bulk amplified the alleles from both lines (L40\(^{HtphPRtRt}\) and L30\(^{HtPHPRtRt}\)). These marker genotypes were expected, considering that HtP confers dominant resistance and rt confers recessive resistance (Ogliari et al., 2005). In this case, the genotype of all susceptible plants of the backcross \([\text{L}30^{HtPHPRtRt} \times \text{L}40^{HtphPRtRt}]\) should be HtphPRtRt and the genotype for marker loci linked to HtP should be homozygous in the susceptible bulk for marker alleles of the resistant line L40\(^{HtphPRtRt}\).

Only seven bnlg198 heterozygotes and six dupssr25 heterozygotes were found among the 32 susceptible plants of the backcross \([\text{L}30^{HtPHPRtRt} \times \text{L}40^{HtphPRtRt}]\), which differ from the expected number (p = 0.01) of 16 plants in the case of non-linkage (Table 1). Thus, the recombination frequency (c1) between bnlg198 and HtP was estimated to be 0.22 ± 0.07 [7 \(\text{HtP}\)/\(\text{HtP}\) recombinants / 32 (\(\text{HtP}\)/\(\text{HtP}\) + \(\text{HtP}\)/\(\text{HtP}\))], whereas the recombination frequency between bnlg198 and dupssr25 was 0.03 ± 0.03. These data indicate that HtP is located on the long arm of chromosome 2 and is 28.7 cM from bnlg198 and 23.5 cM from dupssr25 (bin 2.08), the bnlg198 and dupssr25 markers being 3.2 cM from each other.

Twenty-four of the 125 microsatellite loci tested, were polymorphic between L30\(^{HtPHPRtRt}\) and L40\(^{HtphPRtRt}\), with bnlg197 being found to be linked to rt since only three homozygotes for this marker were found. This differs (p = 0.01) from the 1:1 ratio expected (16 heterozygotes: 16 homozygotes) when there is no linkage. Assuming that the 32 susceptible plants were heterozygous for rt, the c1 value between bnlg197 and rt was estimated to be 0.094 ± 0.05 [3 \(\text{RtZ6}/\text{rtZ6}\) recombinant/32 (\(\text{RtZ6}/\text{rtZ6} + \text{RtZ5}/\text{rtZ6}\))]. These data indicate that rt is located on the long arm of chromosome 3 (bin 3.06) distant 10.38 cM of bnlg197.

In conventional linkage analysis, all markers that detect polymorphisms between the parental lines should be

**Table 1 - Genetic linkage between HtP, bnlg198 (M), and dupssr25 (L) and between rt and bnlg197 in the BC\(_1\)F\(_1\) population from \([\text{L}30^{HtPHPRtRt} \times \text{L}40^{HtphPRtRt}]\).**

<table>
<thead>
<tr>
<th>Linked loci</th>
<th>Expected frequency</th>
<th>Observed frequency</th>
<th>Significant at p &lt; 0.01 by the (\chi^2) test</th>
<th>Recombination frequency estimate ((c_1, %})</th>
<th>Standard error associated with the estimated recombination frequency ((V_{c1/2}, %})</th>
</tr>
</thead>
<tbody>
<tr>
<td>HtP/bnlg198</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(hptM_1/hptM_2)</td>
<td>16</td>
<td>7</td>
<td>Yes</td>
<td>21.87</td>
<td>7.31</td>
</tr>
<tr>
<td>(hptM_2/hptM_2)</td>
<td>16</td>
<td>25</td>
<td>No</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HtP/dupssr25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(hptL_3/hptL_4)</td>
<td>16</td>
<td>6</td>
<td>Yes</td>
<td>18.75</td>
<td>6.90</td>
</tr>
<tr>
<td>(hptL_3/hptL_4)</td>
<td>16</td>
<td>26</td>
<td>No</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(M_L/M_L)</td>
<td>8</td>
<td>6</td>
<td>Yes</td>
<td>3.12</td>
<td>3.07</td>
</tr>
<tr>
<td>(M_L/M_L)</td>
<td>8</td>
<td>1</td>
<td>No</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(M_L/M_L)</td>
<td>8</td>
<td>0</td>
<td>No</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(M_L/M_L)</td>
<td>8</td>
<td>25</td>
<td>No</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>rt/bnlg197</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(RtZ6/rtZ6)</td>
<td>16</td>
<td>3</td>
<td>Yes</td>
<td>9.37</td>
<td>5.15</td>
</tr>
<tr>
<td>(RtZ6/rtZ6)</td>
<td>16</td>
<td>29</td>
<td>No</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
subsequently used for genotyping the entire segregating population. However, when near-isogenic lines or bulked segregant sample analyses are used, the number of genotyping reactions are greatly reduced, thus allowing a faster identification of linkage between markers at a reduced cost (Muehlbauer et al., 1988). The advantage of these techniques is improved if they are used together due to the low probability of false positives, i.e., informative markers that are polymorphic between near-isogenic lines or bulks but are not linked to the target gene (Michelmore et al., 1991).

In near-isogenic line analysis false positives can arise due to the retention of small segments of the genome of the donor parent in chromosomes of the converted line other than the chromosome carrying the target gene. False positives may also result from extensive retention of portions of donor parent-derived DNA in linkage blocks containing the gene of interest (linkage-drag). On the other hand, the probability of such false positives is smaller in bulked segregant sample analysis since only markers tightly linked to the target loci (< 30 cM) are detected, even if few (e.g. 10 F2 plants in each bulk) individual plants are used to construct the bulks (Michelmore et al., 1991). This probability is still smaller if a backcross population is used, because such crosses provide a good opportunity for crossing-over to occur between the desired gene(s) from the non-recurrent parent and any undesirable gene or genes linked to them (Wang and Peterson, 1994) thus resulting in a narrower “genetic window” around the target region compared to that occurring in F2 populations. In our study, for instance, 5 markers were polymorphic between the isogenic lines for HtP, but only two of these were selected for co-segregation analysis because they also detected polymorphisms between bulks. As it turned out, these two were linked to HtP.

Co-segregation analysis using only susceptible plants confirmed linkage of the HtP to the bnlg198 and dupssr25 markers, both located on chromosome 2L (bin 2.08). This shows that HtP is located on the same chromosome as Ht1, a gene that was described more than 40 years ago (Patterson et al., 1965). These genes are distinct since they confer resistance to distinct races of E. turcicum (Ogliari et al., 2005) but our data did not allow us to confirm whether or not the HtP and Ht1 are alleles or if they are located at different loci. However, it is interesting to note that Hooker and Tsung (1980) suggested the existence of a second allele at the Ht1 locus, although they did not report any information regarding its phenotypic effects.

Since the backcross population used to map HtP also segregated for rt and because these genes act independently and have opposite modes of gene action regarding resistance to E. turcicum, it was possible to find a marker linked to rt as well using the same population. However, bulked segregant sample analysis could not be used in this case because no polymorphisms between bulks for markers linked to rt would be expected, since the susceptible plants in the backcross population used in this study were heterozygous at this locus (i.e., htphtpRtrt) and the resistant plants could be either homo and/or heterozygous at this same locus (i.e., htphtpRrtt, HtPhpRrtt and/or HtPhpRtrt). Linkage between rt and bnlg197 allowed locating this gene in the long arm of chromosome 3 (bin 3.06), where previous reports have indicated the existence of a quantitative loci (QTL) controlling resistance to E. turcicum (Freymark et al., 1993). As in the case of HtP, the results indicate either that rt may be an allele of this locus with strong phenotypic effects or that it is linked to the QTL.

The identification of these two resistance genes (HtP and rt) is an important finding for defining genetic breeding strategies for maize to helminthosporiosis, because both the HtP and rt genes confer resistance to a wide spectrum of races of E. turcicum, even those that possess multiple virulence factors such as races 123x and 123N (Ogliari et al., 2005). In addition, qualitative resistance genes to E. turcicum have proved to be effective for long-term resistance to E. turcicum, with, for example, the Ht1 gene providing protection against E. turcicum which lasted for 15 years after the release of cultivars containing this gene (Leath et al., 1990).

In Brazil, E. turcicum populations seems to be more diverse in terms of race composition, even so some studies observed a predominance of race 0 and the presence of some races capable of overcoming resistance conferred by the Ht1 gene (Gianasi et al., 1996). In this way, breeding strategies aiming to incorporate two or more wide-spectrum resistance genes into a single genotype could be an interesting strategy to control E. turcicum. Thus, both L30HtPhtpRtRt and L40htphtpRt should be considered good sources of resistance to this pathogen in Brazil.

Even though our findings provided useful insights on the genetic basis of HtP and rt mediated resistance to E. turcicum, the linked markers found in our study are not adequate to be used in marker assisted selection programs due to their genetic distance. In order to do so, both allelism tests between Ht1 and HtP and saturation of the chromosomal regions around these loci with other markers should be accomplished.

Acknowledgments

The authors thank the Brazilian governmental agency CAPES for granting a scholarship to the first author, the São Paulo state agency FAPESP for financial support through grant 97/9531-4 and Sementes Agroceres S/A for providing the plant material.

References


*Associate Editor: Everaldo Gonçalves de Barros*