An EST-SSR Marker, \textit{bu099658}, and its Potential Use in Breeding for Yellow Rust Resistance in Wheat

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Abstract

EST-SSR markers, derived from the A and B genomes of wheat were used to identify molecular markers associated with yellow rust resistance. For this purpose, bulk segregant analysis was performed using 114 EST-SSR primer pairs. They were screened on the parent genotypes and resistant/susceptible DNA pools from the cross between Izgi2001 (resistant male parent) × ES14 (susceptible female parent) at the seedling and adult plant stage. An EST-SSR marker, \textit{bu099658}, generated the 206 bp DNA fragment that was present in the resistant parent and resistant bulk, but it was not present in the susceptible parent and the susceptible bulk. To investigate its association with \textit{Yr} genes, 20 individuals of NILs were also amplified with \textit{BU099658} and the 206 bp marker fragment was obtained only in \textit{Yr1/6} × Avocet S. Additionally, \textit{bu099658} was screened on 65 genotypes which possessed different \textit{Yr} genes/gene combination(s) and \textit{Yr1}. The results indicate a close linkage of \textit{bu099658} with the \textit{Yr1} gene.

Keywords: Bulk Segregant Analysis (BSA); Marker-Assisted Selection (MAS); \textit{Puccinia striiformis} f.sp. \textit{tritici}; \textit{Triticum aestivum} L.; \textit{Yr1}

Wheat (\textit{Triticum aestivum} L.) is the most important and strategic cereal crop for the majority of the world countries because of its basic nutrition supply. The largest proportion of yield losses in wheat production worldwide each year is due to rust diseases. Yellow rust (stripe rust), caused by \textit{Puccinia striiformis} f.sp. \textit{tritici}, is one of the major devastating factors worldwide in common wheat (\textit{Triticum aestivum} L.). Turkey is the 10\textsuperscript{th} largest wheat-producing country in the world with an average of 20 million tonnes per year and the economic damage caused by yellow rust as yield losses is quite serious. Growing resistant cultivars is considered the most effective, low-cost, and environmentally safe approach to controlling yellow rust (Line & Chen 1995). Several rust resistance genes have been identified and used in breeding for resistance but new variants of the pathogen overcome the resistance over a period of time. Molecular markers are becoming available for many genes and their use in marker-assisted selection will certainly have a considerable impact on practical breeding (Priyamvada & Tiwari 2011).
Molecular markers tightly linked to yellow rust resistance (Yr) genes are very useful for the introduction of resistance genes into wheat breeding programmes by Marker-Assisted Selection (MAS) (Todorovska et al. 2009). Moreover, they are very valuable tools to select the suitable genotypes for gene pyramiding to improve the new resistant genotypes containing two or more Yr genes (Chen 2007). This being the purpose, many molecular markers, such as Restriction Fragment Length Polymorphisms (RFLPs) (Helentjaris et al. 1986), Random Amplified Polymorphic DNAs (RAPDs) (Williams et al. 1990), Amplified Fragment Length Polymorphisms ( AFLPs) (Vos et al. 1995), Resistance Gene Analogs (RGAs) (Kanazin et al. 1996) and Simple Sequence Repeats (SSRs) (Akkaya et al. 1992) have been widely used in plants. The successful utility of DNA markers has been shown in different wheat breeding programmes, namely SCAR marker (SC-Y15) for Yr17 (Sharp et al. 2001), SSR markers (Xgwm413 and Xgwm273) for YrH52 (Peng et al. 2000) and SSR marker (Xgwm498) for Yr26 (Yildirim et al. 2004), for developing resistant wheat cultivars. Worldwide, yellow rust resistance genes Yr1–Yr48 and many provisionally designated genes have been identified in wheat and its relatives (Huang et al. 2011). However, more markers are needed for identifying and mapping the genes.

Express Sequence Tags derived from SSRs (EST-SSRs), as genetic markers, have been evaluated in several studies and these tend to be considerably less polymorphic than those from genomic DNA for wheat (Eujayl et al. 2001). However, EST-SSRs have received a lot of attention because they are derived from the transcribed region of genes responsible for the traits of interest. This feature can provide opportunities for gene discovery and enhance the role of markers by assaying variation in the transcribed and known gene function (Andersen & Lubberstedt 2003). Recently, an increasing number of ESTs being deposited in databases for wheat (1.361.764; http://www.ncbi.nlm.nih.gov/dbEST) and EST-SSRs can be rapidly developed from the in silico analysis of these databases at low cost. EST-SSRs are already available for wheat (www.wheat.pw.usda.gov) and are transferred to adapted varieties. To date, few EST-SSR markers linked to yellow rust resistance have been reported (Ercan et al. 2010; Jia et al. 2011).

The objective of the present study was to identify associated EST-SSR markers for Yr genes that can be used for MAS in wheat breeding programmes. Here we report on the identification of bu099658 marker and its close association with Yr1 gene.

**MATERIAL AND METHODS**

**Plant material.** A cross between the yellow rust resistant and susceptible Turkish bread wheat cultivar, Izgi2001 and ES14, respectively, was made in the wheat breeding programme of the Anatolian Agricultural Research Institute (AARI). Izgi2001 has Yr1 resistance gene according to the result of the gene postulation study conducted by Colin Wellings and Zafer Mert at the Plant Breeding Institute, Sydney University (Personal communication). The F1 individuals derived from Izgi2001 × ES14 were evaluated for yellow rust resistance at the seedling stage in the greenhouse and adult stage in the field. Randomly selected 100 F1 individuals were used for an inheritance study of the marker locus. Additionally, 20 near isogenic lines (NIL) (Table 1) and a total of 65 wheat genotypes carrying Yr1 or different Yr gene/gene combination(s) (Table 2) that were supplied from Australia, Syria and Denmark were used for the identification of linkage between bu099658 marker locus and Yr1 gene.

**Inoculation and disease assessment.** Five hundred F1 individuals derived from the cross were evaluated for yellow rust resistance both at the seedling stage in the greenhouse and at the adult stage in the field. For the inoculations, urediospores of a Pst gene according to the result of the gene postulation study conducted by Colin Wellings and Zafer Mert at the Plant Breeding Institute, Sydney University (Personal communication). The F1 individuals derived from Izgi2001 × ES14 were evaluated for yellow rust resistance at the seedling stage in the greenhouse and adult stage in the field. Randomly selected 100 F1 individuals were used for an inheritance study of the marker locus. Additionally, 20 near isogenic lines (NIL) (Table 1) and a total of 65 wheat genotypes carrying Yr1 or different Yr gene/gene combination(s) (Table 2) that were supplied from Australia, Syria and Denmark were used for the identification of linkage between bu099658 marker locus and Yr1 gene.

**Table 1.** NIL 06 sets used to study the association of bu099658 with Yr1 gene

<table>
<thead>
<tr>
<th>No.</th>
<th>Pedigree</th>
<th>Gene</th>
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<tbody>
<tr>
<td>1</td>
<td>Yr1/6 × Avocet S</td>
<td>Yr1</td>
</tr>
<tr>
<td>2</td>
<td>Yr5/6 × Avocet S</td>
<td>Yr5</td>
</tr>
<tr>
<td>3</td>
<td>Yr6/6 × Avocet S</td>
<td>Yr6</td>
</tr>
<tr>
<td>4</td>
<td>Yr7/6 × Avocet S</td>
<td>Yr7</td>
</tr>
<tr>
<td>5</td>
<td>Yr8/6 × Avocet S</td>
<td>Yr8</td>
</tr>
<tr>
<td>6</td>
<td>Yr9/6 × Avocet S</td>
<td>Yr9</td>
</tr>
<tr>
<td>7</td>
<td>Yr10/6 × Avocet S</td>
<td>Yr10</td>
</tr>
<tr>
<td>8</td>
<td>Yr11/3 × Avocet S</td>
<td>Yr11</td>
</tr>
<tr>
<td>9</td>
<td>Yr12/3 × Avocet S</td>
<td>Yr12</td>
</tr>
<tr>
<td>10</td>
<td>Yr15/6 × Avocet S</td>
<td>Yr15</td>
</tr>
<tr>
<td>11</td>
<td>Yr17/6 × Avocet S</td>
<td>Yr17</td>
</tr>
<tr>
<td>12</td>
<td>Yr18/3 × Avocet S</td>
<td>Yr18</td>
</tr>
<tr>
<td>13</td>
<td>Yr24/3 × Avocet S</td>
<td>Yr24</td>
</tr>
<tr>
<td>14</td>
<td>Yr26/3 × Avocet S</td>
<td>Yr26</td>
</tr>
<tr>
<td>15</td>
<td>YrSP/6 × Avocet S</td>
<td>YrSP</td>
</tr>
<tr>
<td>16</td>
<td>YrSK/3 × Avocet S</td>
<td>Yr27</td>
</tr>
<tr>
<td>17</td>
<td>Jupateco R (S)</td>
<td>Yr18+</td>
</tr>
<tr>
<td>18</td>
<td>Jupateco S</td>
<td>YrA</td>
</tr>
<tr>
<td>19</td>
<td>Avocet R</td>
<td>YrA</td>
</tr>
<tr>
<td>20</td>
<td>Avocet S</td>
<td>YrA</td>
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</table>
Table 2. Wheat genotypes screened for validation of the association between *bu099658* and the *Yr1* gene

<table>
<thead>
<tr>
<th>No.</th>
<th>Genotype</th>
<th>Yr gene(s)</th>
<th><em>bu099658</em> fragment (206 bp)</th>
<th>Seed source</th>
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<tbody>
<tr>
<td>1</td>
<td>Buster-1</td>
<td><em>Yr1</em></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Buster-2</td>
<td><em>Yr1</em></td>
<td>+</td>
<td></td>
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<tr>
<td>3</td>
<td>Galahad</td>
<td><em>Yr1</em></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Hobbit</td>
<td><em>Yr1</em></td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Ritmo</td>
<td><em>Yr1</em></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Chinese 166</td>
<td><em>Yr1</em></td>
<td>+</td>
<td>Sydney University, Australia</td>
</tr>
<tr>
<td>7</td>
<td>Forno</td>
<td><em>Yr1</em></td>
<td>+</td>
<td></td>
</tr>
<tr>
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<td>ISR678.1</td>
<td><em>Yr1</em></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>ISR678.39</td>
<td><em>Yr1</em></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>ISR 678.40</td>
<td><em>Yr1</em></td>
<td>+</td>
<td></td>
</tr>
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<td>ISR.679.19</td>
<td><em>Yr1</em></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>ISR 679.20</td>
<td><em>Yr1</em></td>
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<td>13</td>
<td>Chinese 166</td>
<td><em>Yr1</em></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Suwon 92 × Omar</td>
<td><em>Yr1</em></td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>AVS/ 6 × Yr 1</td>
<td><em>Yr1</em></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Chinese 166</td>
<td><em>Yr1</em></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Avocet S</td>
<td><em>Yr1</em></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>IBIS</td>
<td><em>Yr1, Yr2</em></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Tadorna</td>
<td><em>Yr1, Yr2</em></td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Fenman</td>
<td><em>Yr1, Yr2</em></td>
<td>–</td>
<td>ICARDA, Syria</td>
</tr>
<tr>
<td>21</td>
<td>Stetson</td>
<td><em>Yr1, Yr9</em></td>
<td>–</td>
<td></td>
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<tr>
<td>22</td>
<td>Bounty</td>
<td><em>Yr1, Yr13</em></td>
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<tr>
<td>23</td>
<td>Galahad</td>
<td><em>Yr1, Yr2, Yr14</em></td>
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<tr>
<td>24</td>
<td>Maris Ranger</td>
<td><em>Yr1, Yr3a, Yr4a, Yr6</em></td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Virtue</td>
<td><em>Yr1, Yr3a, Yr4a, Yr13</em></td>
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<td></td>
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<tr>
<td>26</td>
<td>Mardler</td>
<td><em>Yr1, Yr2, Yr3a, Yr4a, Yr13</em></td>
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<td></td>
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<tr>
<td>27</td>
<td>Hustler</td>
<td><em>Yr1, Yr2, Yr3a, Yr4a, Yr13</em></td>
<td>–</td>
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<tr>
<td>28</td>
<td>Avocet</td>
<td><em>Yr32</em></td>
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<tr>
<td>29</td>
<td>Carstens V</td>
<td><em>Yr32, Yr25</em></td>
<td>+</td>
<td></td>
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<tr>
<td>30</td>
<td>VPM 1</td>
<td><em>Yr17, +</em></td>
<td>–</td>
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<tr>
<td>31</td>
<td>Avocet</td>
<td><em>Yr10</em></td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>Moro</td>
<td><em>Yr10</em></td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>Avocet</td>
<td><em>Yr9</em></td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>Sleipner</td>
<td><em>Yr9, +</em></td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Heines Kolben</td>
<td><em>Yr6, Yr2</em></td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>Suwon 92 /Omar</td>
<td>So/Yr4</td>
<td>–</td>
<td>Aarhus University, Denmark</td>
</tr>
<tr>
<td>37</td>
<td>Hybrid 46</td>
<td><em>Yr4, +</em></td>
<td>–</td>
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<tr>
<td>38</td>
<td>Vilmorin23</td>
<td><em>Yr3, +</em></td>
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<tr>
<td>39</td>
<td>Heines VII</td>
<td><em>Yr2, Yr25+</em></td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>Kalyansona</td>
<td><em>Yr2, +</em></td>
<td>–</td>
<td></td>
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<tr>
<td>41</td>
<td>Spaldings Prolific</td>
<td><em>Sp, Yr25</em></td>
<td>–</td>
<td></td>
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<tr>
<td>42</td>
<td>Strubes Dickkopf</td>
<td><em>Sd, Yr25</em></td>
<td>–</td>
<td></td>
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<tr>
<td>43</td>
<td>Avocet Yr8</td>
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<td></td>
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<tr>
<td>44</td>
<td>Chinese 166</td>
<td><em>Yr1</em></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>Compair</td>
<td><em>Yr8, +</em></td>
<td>–</td>
<td></td>
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</table>
which was virulent for \( Yr_2, Yr_6, Yr_7, Yr_8, Yr_9, Yr_{11}, Yr_{12}, Yr_{17}, Yr_{18}, Yr_{27}, Yr_{A+} \) and avirulent for \( Yr_1, Yr_5, Yr_{10}, Yr_{15}, Yr_{24}, Yr_{SP}, Yr_{CV} \) genes were used. The most resistant and susceptible \( F_2 \) individuals at the seedling and adult stage were selected for Bulk Segregant Analysis (BSA) \((\text{Michelmore et al. 1991})\) after 15–20 days following the inoculation. The infection type was recorded, using the 0–9 scale of \( \text{McNeal et al.} (1971) \) at the seedling stage and the 0-100 scale of the modified Cobb scale (CI) at the adult stage \((\text{Roelfs et al. 1992})\).

Based on the global virulence mapper (http://wheatrust.org/international-services/yellow-rust/global-virulence-mapper/), since 2010 yellow rust isolates have been avirulent for \( Yr_1 \) in the Middle East including Turkey, Azerbaijan and Syria whereas the effectiveness of \( Yr_1 \) gene has been decreasing in Northern Europe (Figure 1).

### DNA isolation and EST-SSR screening

Genomic DNA of leaves was extracted as described by \( \text{Weining and Langridge 1991} \). Aliquots of DNA from 28 resistant and 28 susceptible plants from \( F_2 \) segregating population were mixed, respectively, to produce resistant and susceptible bulks of both growth stages to be used for BSA. 114 EST-SSR markers derived from A and B genomes of wheat \((\text{Gadaleta et al. 2009})\) were employed to determine the markers associated with yellow rust resistance.

#### Fragment analysis by capillary electrophoresis

A fluorescently labelled BU099658 forward primer was used for the determination of the polymorphic DNA fragment. PCR mixtures were prepared according to the GenomeLab GeXP System manufacturer’s (Beckman Coulter, Brea, USA) instructions. The electrophoretic separation was performed using the GenomeLab GeXP Genetic Analysis System and the data was analysed by a fragment analysis module of the system. Each experiment was replicated at least three times to verify the reproducibility of the marker analysis.

### RESULTS AND DISCUSSION

Infection type and CI values of selected resistant \( F_2 \) individuals were between 0 and 1 at the seedling stage and about 20 at the adult plant stage, while susceptible \( F_2 \) individuals were 8–9 at the seedling stage and 60–90 at the adult plant stage. The isolates used in this study are avirulent for \( Yr_1 \) based on the gene postulation study (supplementary info). Based on disease scoring data, the 28 most resistant and 28 most susceptible \( F_2 \) seedlings were taken into consideration for BSA.

<table>
<thead>
<tr>
<th>No.</th>
<th>Genotype</th>
<th>( Yr ) gene(s)</th>
<th>\text{bu099658} fragment (206 bp)</th>
<th>Seed source</th>
</tr>
</thead>
<tbody>
<tr>
<td>46</td>
<td>Avocet ( Yr_7 )</td>
<td>( Yr_7 )</td>
<td>–</td>
<td>Aarhus University, Denmark</td>
</tr>
<tr>
<td>47</td>
<td>Lee</td>
<td>( Yr_7, + )</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>Avocet ( Yr_6 )</td>
<td>( Yr_6 )</td>
<td>–</td>
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</tr>
<tr>
<td>49</td>
<td>Heines Peko</td>
<td>( Yr_6, Yr_2, Yr_{25+} )</td>
<td>–</td>
<td></td>
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<tr>
<td>50</td>
<td>TP 981</td>
<td>( Yr_{25?} )</td>
<td>–</td>
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<tr>
<td>51</td>
<td>Ambition</td>
<td>several</td>
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<td>53</td>
<td>Avocet S</td>
<td>( U2 )</td>
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<tr>
<td>54</td>
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<tr>
<td>55</td>
<td>Avocet ( Yr_{24} )</td>
<td>( Yr_{24} )</td>
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<tr>
<td>56</td>
<td>Brigadier</td>
<td>( Yr_9, Yr_{17+} )</td>
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<td>57</td>
<td>Anja</td>
<td>( U1 )</td>
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<tr>
<td>58</td>
<td>Opata</td>
<td>( Yr_{27}, Yr_{18} )</td>
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<td>59</td>
<td>Cortez</td>
<td>( Yr_{15} )</td>
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<td>Cartago</td>
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<tr>
<td>61</td>
<td>Chinese 166 (winter type)</td>
<td>( Yr_1 )</td>
<td>+</td>
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<tr>
<td>62</td>
<td>Moro (winter type)</td>
<td>( Yr_{10} )</td>
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<tr>
<td>63</td>
<td>( Yr_{1/6} \times \text{Avocet S} )</td>
<td>( Yr_1 )</td>
<td>+</td>
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</tr>
<tr>
<td>64</td>
<td>( Yr_{10/6} \times \text{Avocet S} )</td>
<td>( Yr_{10} )</td>
<td>+</td>
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<tr>
<td>65</td>
<td>Chinese 166 (winter type)</td>
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</tbody>
</table>
Among the 114 EST-SSR markers (Table 3) employed in this study, 99 of them (87%) revealed a monomorphic band profile between the two parents. The remaining 15 markers (13%) amplified polymorphic DNA fragments except for *bu099658*, while others did not produce such polymorphic band profiles between bulks. *BU099658* amplified a 206 bp DNA fragment that was present in the resistant parent and in the resistant bulks, but not in the susceptible ones both for seedling and adult plant stages (Figure 2). A linkage between the *bu099658* locus mapped on chromosome group 2A and 2B and yellow rust resistance was confirmed in 28 resistant F$_2$ individuals at both growth stages. The 206 bp fragment, indicated by an arrow, was present in all 28 individuals in the resistant bulks, but not in the susceptible ones at both stages (Figure 2).

A new generation fluorescence-based capillary electrophoresis system was also used for the verification of the sizes of fragments generated by the *BU099658*. Figure 3 shows the fragment profile of the Izgi2001 with six peaks labelled as 168.20; 206.20; 222.43; 225.61; 227.70 bp and 230.75 bp, and also shows the fragment profile of the ES14 with five peaks 168.80; 222.54; 225.58; 227.57 bp and 230.84 bp. The 206 bp fragment was amplified in the resistant parent and resistant bulks at both growth stages but not in the susceptible ones.

Table 3. Screened EST-SSR markers (polymorphic markers used in this study are shown in bold); primer sequences are reported on the website http://wheat.pw.usda.gov

| Marker     | Primer 1 | Primer 2 | Band Size (bp) | Allele Width (bp) | Parent | Bulk | Other
|------------|----------|----------|----------------|------------------|--------|------|-------
| TC91851    | TC74823  | TC65966  | TC88833        | CA681959         | BJ262177 | BQ170801
| TC69046    | TC91851  | TC84481  | TC70788a       | CA716906         | BJ239878 | BQ805704
| TC87195a   | TC71236  | TC85303  | TC70788b       | CA594434         | BJ306922 | BQ246417
| TC87195b   | TC77302  | TC84464  | TC67645        | CA668788         | BJ213673 | BEA19757
| TC85294    | TC88560  | TC85125a | TC77994        | CA724675         | BJ267382 | BE427655
| TC95235    | TC80528  | TC85125b | TC77993        | CA594434         | BJ318987 | BF483631
| TC84551    | TC89014  | TC92445  | TC101037       | CA681959         | BJ227727 | BU999658
| TC91645    | TC87011  | TC86653  | TC70722        | CA662535         | BJ253815 | BP234852a
| TC69046    | TC85050  | TC69177  | CA741577       | CA677684         | BJ237020a | NP234852b
| TC85294    | TC77481  | TC95791  | CA703897       | CA623872         | BJ370202b | AL825137ab
| TC81688a   | TC80528  | TC84481a | CA594434       | CA499463b        | BJ261777 | AL825137b
| TC81688b   | TC80528  | TC84481b | CA597228       | CA499463a        | BJ261821 | AL825137b
| TC88378    | TC69937  | TC85035a | CA679329       | CA499463b        | BJ236800a | AL825137b
| TC90641    | TC72953  | TC85035b | CA651264       | CA694714         | BJ236800b | AL825137b
| TC90640    | TC86610  | TC85037a | CA677684       | CA663888         | BJ213673 | BQ607256
| TC82001    | TC89014  | TC85037b | CA658758       | CA707573         | BQ838884 | AL825137b
| TC81096    | TC82742  | TC67416  | CA695634       | CA668775         | BQ838884 | AL825137b

Figure 1. Map of *Yr1* virulence in Northern Europe and Middle East during 2010–2012; data provided by: Institut National de la Recherche Agronomique (France), Julius Kühn-Institut, Federal Research Centre for Cultivated Plants (Germany and Austria), National Institute of Agricultural Botany (United Kingdom) and Aarhus University (Denmark and Sweden); N – No. of virulent *Pst* isolates/No. of analysed *Pst* isolates in a country
NILs were screened by the BU099658 primer pair to validate associations between the marker locus and Yr gene. As expected, while only Yr1 including Yr1/6 × Avocet S produced a 206 bp marker fragment, others did not. In addition, 21 genotypes carrying Yr1, 10 genotypes carrying Yr1 in combination with other Yr gene/genes and 34 genotypes lacking Yr1 (Table 2) were used for validation. Genotypes carrying the Yr1 gene including Chinese 166, as the reference sources for this gene, were used by Bansal et al. (2009) for validation of the linkage between Yr1 and stm673acag. The marker fragment (206 bp) belonging to the bu099658 locus was amplified in all of the 19 genotypes carrying Yr1 and another 4 individuals carrying Yr1 gene in combination with other Yr gene/genes, which demonstrated the same pattern with the resistant parent, Izgi2001. The remaining genotypes gave exactly the same amplification profile with the susceptible parent, ES14, and did not amplify the marker fragment (Table 2).

In order to determine the inheritance of the bu099658 locus, PCR amplification was performed in 100 F2 individuals of Izgi2001 × ES14. In this analysis, 71 plants produced the polymorphic 206 bp marker fragment, while it was not produced by 29 plants, which fits a 3:1 ratio (χ2 test, \( P = 0.25–0.50 \)). This chi-squared analysis supported monogenic inheritance of Izgi2001 resistance to yellow rust. In this study, we reported on the detection of the bu099658 EST-SSR marker, linked to the seedling and adult plant resistance to yellow rust. An F2 population from a cross between Izgi2001 and ES14 was visually assessed for seedling infection type in the greenhouse and adult plant infection in the field. In repeated amplifications, the presence of the 206 bp EST-SSR marker may significantly enhance the selection of wheat genotypes for yellow rust resistance. Screening of NILs and 65 wheat genotypes which have Yr1 or other Yr genes by bu099658 showed that only Yr1/6 × Avocet S from NILs and all of the 19 genotypes carrying Yr1 from validation sets amplified the 206 bp EST-SSR marker fragment. These results supported our suggestion that this marker fragment is closely linked to the Yr1 gene. Bariana and McIntosh (1993) predicted a distal location of Yr1 on chromosome 2AL based on recombination studies of rust resistance loci. Bansal et al. (2009) reported the genetic relationship between the genes Yr1 and Sr48 on chromosome 2AL. The close linkage was identified between Yr1 and the PCR-based molecular marker stm673acag. Genotyping with stm673acag amplified a 120-bp fragment in 8 of 9 wheat genotypes carrying Yr1, also used in this study for validation. However, the line ISR679.20 amplified a 124-bp allele present in Australian cultivars lacking Yr1. This marker failed to differentiate Avocet S × 6 Chinese 166 (Yr1) and Avocet S by amplifying a 120-bp product in both genotypes. Thus, the line ISR679.20 and Avocet S were genotyped as false negative and false positive by the author. In contrast, BU099658 did not produce any false positive or false negative results for Yr1 in our work. Therefore, we estimated that this marker could be useful for
MAS in breeding programmes aimed at the large scale for the screening of segregating populations for Yr1. Bansal et al. (2009) mapped markers Xgwm311 and Xgwm382 with a 5 and 5.6 cM proximal distance to Yr1 in an Arina/Forno RIL population. Previously, we also detected the presence of Xgwm382 (Akfirat-Senturk et al. 2010) and Xgwm311 (Akfirat-Senturk et al. 2013) markers in resistant germplasm of the Izgi2001 × ES14 cross. According to the genetic map presented in Somers et al. (2004), the marker Xgwm311 was the most distal marker on chromosome 2AL, followed by the marker Xgwm382. Identification of a close or loose genetic association between Yr1 and bu099658 will be confirmed by linkage mapping in the Izgi2001 × ES14 population in our forthcoming studies.

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References


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