Euphytica (2008) 162:457–466
DOI 10.1007/s10681-007-9615-z

# Mapping of the leaf rust resistance gene *Lr38* on wheat chromosome arm 6DL using SSR markers

**S. A. Mebrate · E. C. Oerke · H. W. Dehne · K. Pillen**

Received: 16 July 2007 / Accepted: 2 November 2007 / Published online: 27 November 2007


**Abstract** Leaf rust caused by the fungus *Puccinia triticina* is one of the most important diseases of wheat (*Triticum aestivum*) worldwide. The use of resistant wheat cultivars is considered the most economical and environment-friendly approach in controlling the disease. The *Lr38* gene, introgressed from *Agropyron intermedium*, confers a stable seedling and adult plant resistance against multiple isolates tested in Europe. In the present study, 94 $F_2$ plants resulting from a cross made between the resistant Thatcher-derived near-isogenic line (NIL) RL6097, and the susceptible Ethiopian wheat cultivar Kubsa were used to map the Thatcher *Lr38* locus in wheat using simple sequence repeat (SSR) markers. Out of 54 markers tested, 15 SSRs were polymorphic between the two parents and subsequently genotyped in the population. The *P. triticina* isolate DZ7-24 (race FGJTJ), discriminating *Lr38* resistant and susceptible plants, was used to inoculate seedlings of the two parents and the segregating population. The SSR markers *Xwmc773* and *Xbarc273* flanked the *Lr38* locus at a distance of 6.1 and 7.9 cM, respectively, to the proximal end of wheat chromosome arm 6DL. The SSR markers *Xcfd5* and *Xcfd60* both flanked the locus at a distance of 22.1 cM to the distal end of 6DL. In future, these SSR markers can be used by wheat breeders and pathologists for marker assisted selection (MAS) of *Lr38*-mediated leaf rust resistance in wheat.

**Keywords** *Agropyron intermedium* · *Triticum aestivum* · *Puccinia triticina* · Leaf rust resistance · SSR markers

S. A. Mebrate · E. C. Oerke · H. W. Dehne
Institute of Crop Science and Resource Conservation, Phytomedicine, University of Bonn, Nussallee 9, 53115 Bonn, Germany

*Present Address:*
S. A. Mebrate
Debre Zeit Agricultural Research Center, P.O. Box 32, Debre Zeit, Ethiopia

K. Pillen
Institute of Crop Science and Resource Conservation, Crop Genetics and Biotechnology, University of Bonn, Katzenburgweg 5, 53115 Bonn, Germany

*Present Address:*
K. Pillen (✉)
Max-Planck-Institute for Plant Breeding Research, Barley Genetics Research Group, Carl-von-Linné-Weg 10, 50829 Cologne, Germany
e-mail: pillen@mpiz-koeln.mpg.de

## Introduction

Leaf rust caused by the fungus *Puccinia triticina* Eriks. is considered as one of the most important foliar diseases of wheat (*Triticum aestivum* L.) worldwide. It occurs almost everywhere where wheat is grown (Dehne and Oerke 1998) and severe wheat yield losses due to damage by leaf rust can range from

30 to 50% (McIntosh et al. 1995). The use of resistant wheat varieties is considered an economical and eco-friendly method of controlling the disease. So far, more than 50 leaf rust resistance genes have been described (McIntosh et al. 1995, 2005). However, only two leaf rust resistance genes, *Lr10* and *Lr21*, have been cloned so far (Feuillet et al. 2003; Huang et al. 2003). Both genes are typical NBS-LRR type resistance genes. Host genetic resistance to leaf rust can be utilized effectively through proper knowledge of the identity of resistance genes in commonly used wheat cultivars (Kolmer 1996). A number of leaf rust resistance genes have been transferred from wild relatives to the cultivated forms of wheat with the objective of improving the resistance of existing wheat cultivars. For instance, genes derived from *Aegilops* species and transferred to common wheat include *Lr9* (Sears 1956), *Lr28* (Riley et al. 1968; McIntosh et al. 1982), *Lr35* (Kerber and Dyck 1990), and *Lr36* (Dvorak 1977; Dvorak and Knott 1980, 1990). Furthermore, leaf rust resistance genes *Lr25* (Driscoll and Sears 1965), *Lr26* (Kattermann 1937, 1938), and *Lr45* (Mukade et al. 1970) were derived from cultivated rye (*Secale cereale*). On the other hand, leaf rust resistance genes transferred from *Agropyron* species include *Lr19* (Sharma and Knott 1966; Knott 1968; Dvorak and Knott 1977; Gupta et al. 2006), *Lr24* (Smith et al. 1968; McIntosh et al. 1977), *Lr29* (Sears 1973; 1977), and *Lr38* (Wienhues 1966; 1973; Friebe et al. 1992, 1993).

From a group of wheat—*Agropyron intermedium* translocation lines developed by Wienhues (1966, 1973), the *A. intermedium* chromosome segment 7Ai#2L was found to be present in five different translocations all containing *Lr38* (Friebe et al. 1993, 1996). The 7Ai#2L segments in the five translocation lines T4, T7, T24, T25, and T33 were independently transferred to wheat chromosomes 3DS, 6DL, 5AS, 1DL, and 2AL, respectively (Dyck and Friebe 1993). The sizes of the 7Ai#2L segments in mitotic metaphases of these translocations were 2.78, 4.19, 4.20, 2.55, and 2.42 µm, respectively (Friebe et al. 1993). Unfortunately, all five translocation lines were reported to exhibit reduced yield (Dyck and Friebe 1993). From line T7, *Lr38* was introgressed to the long arm of chromosome 6D (6DL) of the wheat cultivar Thatcher by a series of backcrosses and the near-isogenic line RL6097 (Tc*6/T7), containing the gene *Lr38*, was developed (Dyck and Friebe 1993). Due to the low yield associated with the 7Ai#2L segment, *Lr38* has so far not been used for the development of leaf rust resistant wheat cultivars. Nonetheless, *Lr38*, present in RL6097, was found to be resistant to all isolates of *P. triticina* tested by Dyck and Friebe (1993). Similar results were obtained in a leaf rust virulence survey made by Mesterházy et al. (2000) covering 12 European countries. In their survey, *Lr38* was found to be among the most effective genes for leaf rust resistance. Other studies confirmed this finding also for Italy, Germany and Russia, indicating the immense potential of the gene for leaf rust resistance breeding in wheat (Pasquini et al. 1998; Kovalenko et al. 2002; Lind and Gultyaeva 2007). For this reason, the goal of the outlined study was to identify and map DNA markers closely linked to the leaf rust resistance gene *Lr38*. These markers would ultimately pave the route to reduce the size of the *A. intermedium* introgression containing *Lr38* by identification of recombinations in the vicinity of *Lr38*.

## Materials and methods

### Generation of $F_2$ and $F_{2:3}$ generations, segregating for *Lr38*

A cross was made between the resistant, *Lr38*-bearing, Thatcher-derived nearly isogenic line RL6097 and the susceptible cultivar Kubsa from Ethiopia. The resulting $F_2$ population, in the following denoted TK*Lr38*, comprised 94 segregating $F_2$ individuals and was used to map *Lr38* on wheat chromosome arm 6DL using simple sequence repeat (SSR) markers. The harvested seeds of each $F_2$ plant were bulked to give rise to 94 $F_{2:3}$ families used for re-evaluation of leaf rust phenotypes.

### Inoculum production

For inoculum production, seedlings of the susceptible wheat cultivar Monopol were grown in a cellophane chamber on a greenhouse bench. When seedlings were 10–15 days old, leaves were harvested and cut into 5–7 cm segments. Leaf segments were placed on 5% water agar (Mebrate and Cooke 2001) containing benzimidazole (35 ppm) and streptomycin sulphate (50 ppm). Leaf segments, placed on water agar, were sparsely inoculated using a moistened cotton swab with spores of the *P. triticina* isolate DZ7-24 for

further multiplication. The inoculated leaf segments were incubated in a growth chamber adjusted to 20°C with continuous white light until usage.

Inoculation of plants and disease scoring

For disease scoring, 75–100 mg spores diluted in 150 ml water of the *P. triticina* isolate DZ7-24 produced on cultivar Monopol were used to inoculate 2-leaf stage seedlings of the parents RL6097 and Kubsa, the $F_2$ plants of population TK*Lr38* and the bulked $F_{2:3}$ families. The two parents and the $F_2$ plants were grown under greenhouse conditions whereas a minimum of 10 $F_{2:3}$ plants were grown in a growth chamber. The latter were used to re-confirm the $F_2$ disease scores and to distinguish homozygous and heterozygous resistant $F_2$ plants. The inoculated plants were kept in a moist chamber under dark conditions at ambient temperature for 24 h. The seedlings were then transferred to a growth chamber with 16/8 h light/dark regime and a temperature of 20–22°C. Scoring of leaf rust symptoms (infection types) was carried out 10–12 days post inoculation. Infection types were quantified based on a standard 0–4 scale (Long and Kolmer 1989). The scale values can be interpreted as follows '0': immune, ';': hypersensitive fleck without uredinia, '1': small uredinia surrounded by necrosis, '2': small uredinia surrounded by chlorosis, '3': moderate size uredinia that may be associated with chlorosis, '4': large uredinia without chlorosis. Mixtures of two infection types were represented by two values with the most common value listed first. Designations of '+' and '−' were used in conjunction with the 0–4 scale to indicate larger and smaller uredinia than normal, respectively. Generally, scores 0–2+ were considered resistant while scores 3–4 were considered susceptible reactions.

DNA isolation

A leaf sample of 100 g, taken when the plantlets started to tiller, was ground with liquid nitrogen. Then the DNeasy Plant Mini Prep Kit (Qiagen, Hilden, Germany) was used to extract DNA.

SSR analysis

Two protocols were applied for SSR analysis. SSRs from set I in Table 1 were labeled with IRD700 and, thus, run on the Li-COR automated fragment analysis system. SSRs from set II in Table 1 were subjected to a silver staining technique because only primer aliquots (not labeled with IRD700) were received from Sourdille et al. (2004).

SSR analysis on Li-COR system

PCR was conducted in a final volume of 20 µl containing 5 µl of genomic DNA (15 ng/µl), 0.75 µl of 2 mM dNTP, 2.0 µl 10× buffer, 0.2 µl of 10 µM tailed forward primer, 0.2 µl of 10 µM reverse primer, 0.5 µl of 25 mM $MgCl_2$, 0.5 µl of 1 µM M13 forward primer labeled with IRD700, 0.5 µl of diluted *Taq* polymerase (0.5 U/µl, Promega, Mannheim, Germany) in 10.35 µl $ddH_2O$. The wheat specific forward primer was tailed at the 5′ end with the M13 forward sequence to serve as a template for the IRD700-labeled M13 primer. The reactions were PCR amplified in a GeneAmp PCR system 9600 (Perkin Elmer Corp., Norwalk, CT, USA), programmed for the following steps: a 'hot-start' at 94°C for 3 min, 10 cycles of 1 min at 94°C, 0.5 min starting at 64°C and decreasing 0.8°C per cycle, extension for 1 min at 72°C, followed by 30 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 55°C, and final extension for 1 min at 72°C. Subsequently, the IRD700-labeled PCR products were size-separated on 6% polyacrylamide gels with the Li-COR 4200 system (Li-COR Biosciences, Bad Homburg, Germany).

SSR analysis with silver staining technique

The PCR was run in a final volume of 20 µl containing 5 µl genomic DNA (15 ng/µl), 2.0 µl of 2 mM dNTP, 2.0 µl of 25 mM $MgCl_2$, 2.0 µl of 10× buffer, 0.15 µl of the primer pair provided by Dr. Sourdille, 0.5 µl of *Taq* polymerase (5 U/µl, Promega, Mannheim, Germany), and 8.35 µl of $ddH_2O$. The PCR was basically conducted as described by Pillen et al. (2000) using a GeneAmp PCR system 9600 (Perkin Elmer Corp., Norwalk, CT, USA) programmed with a 'hot-start' at 94°C for 3 min followed by 35 cycles of 1 min at 94°C denaturing at 94°C for 30 s, annealing at 60°C for 0.5 min and extension at 72°C for 1 min. Subsequently, the PCR products were size-separated on 6% polyacrylamide gels and silver stained as explained in Pillen et al. (2000).

**Table 1** List of 54 SSR markers from wheat chromosome 6D screened for polymorphisms between the parents RL6097 and Kubsa

| Set I | | | | | | Set II | | |
|---|---|---|---|---|---|---|---|---|
| No. | SSR | Source[a] | No. | SSR | Source[a] | No. | SSR | Source[a] |
| 1 | *Xbarc5* | G | 22 | *Xcfd95* | G | 1 | *Xgpw312* | Su |
| 2 | *Xbarc21* | Sg | 23 | *Xcfd132* | So | 2 | *Xgpw362* | Su |
| 3 | *Xbarc54* | So | 24 | *Xcfd135* | So | 3 | *Xgpw1019* | Su |
| 4 | *Xbarc96* | So | 25 | *Xcfd188* | So | 4 | *Xgpw1034* | Su |
| 5 | *Xbarc123* | So | 26 | *Xcfd219* | So | 5 | *Xgpw1101* | Su |
| 6 | *Xbarc146* | Sg | 27 | *Xcfd287* | So | 6 | *Xgpw2232* | Su |
| 7 | *Xbarc183* | So | 28 | *Xgwm325* | R | 7 | *Xgpw3087* | Su |
| 8 | *Xbarc196* | So | 29 | *Xgwm469* | R | 8 | *Xgpw3214* | Su |
| 9 | *Xbarc202* | Sg | 30 | *Xgwm582a* | R | 9 | *Xgpw4440* | Su |
| 10 | *Xbarc273* | Sg | 31 | *Xgwm732* | K | 10 | *Xgpw5125* | Su |
| 11 | *Xbarc1121* | Sg | 32 | *Xwmc469* | So | 11 | *Xgpw5130* | Su |
| 12 | *Xcfd5* | G | 33 | *Xwmc773* | So | 12 | *Xgpw5170* | Su |
| 13 | *Xcfd37* | So | | | | 13 | *Xgpw5179* | Su |
| 14 | *Xcfd42* | So | | | | 14 | *Xgpw5182* | Su |
| 15 | *Xcfd45* | G | | | | 15 | *Xgpw5205* | Su |
| 16 | *Xcfd47* | P | | | | 16 | *Xgpw5207* | Su |
| 17 | *Xcfd49* | So | | | | 17 | *Xgpw5210* | Su |
| 18 | *Xcfd60* | G | | | | 18 | *Xgpw7292* | Su |
| 19 | *Xcfd75* | So | | | | 19 | *Xgpw7303* | Su |
| 20 | *Xcfd76* | So | | | | 20 | *Xgpw7433* | Su |
| 21 | *Xcfd80* | So | | | | 21 | *Xgpw8068* | Su |

[a] Sources of SSRs: G: Guyomarc'h et al. (2002); K: Khlestkina et al. (2004); P: Paillard et al. (2003); R: Röder et al. (1998); Sg: Song et al. (2005); So: Somers et al. (2004); Su: Sourdille et al. (2004)

### Linkage analysis

Linkage between the *Lr38* locus and the SSR markers was calculated using the MAPMAKER software version 3.0 (Lander et al. 1987). The Kosambi function (Kosambi 1944) was applied to calculate centiMorgan (cM) distances between the SSR markers and the *Lr38* locus. The linkage map was plotted using the MAPCHART software version 2.2 (Voorrips 2002).

## Results

In this study, the 94 $F_2$ plants of the cross RL6097 × Kubsa and 54 SSR markers were used for mapping the *A. intermedium*-derived leaf rust resistance gene *Lr38* in wheat.

### Segregation of resistance to leaf rust

Out of 94 TK*Lr38* plants, 76 segregated for resistance and the other 18 plants for susceptibility to infection by the isolate DZ7-24 (race FGJTJ) of *P. triticina* (Table 2). The observed segregation supported the existence of a monogenic resistance in population TK*Lr38*, segregating 3:1 (resistant to susceptible) with a chi-square *P* value of 0.190. To ensure the genetic resistance status of $F_2$ plants and to differentiate between homozygous and heterozygous resistant plants, a minimum of 10 $F_{2:3}$ plants derived from each $F_2$ plant was tested again for resistance. In the $F_3$ families, all plants resistant, a mixture of resistant and susceptible plants, and all plants susceptible indicated a homozygous resistant (RR), a heterozygous resistant (Rr), and a homozygous susceptible (rr) $F_2$ plant, respectively. The $F_3$ families segregated 25:51:18 for homozygous resistant, heterozygous

**Table 2** Genotypic segregation for leaf rust resistance in $F_2$ population TK*Lr38*, assessed by inoculating $F_3$ families with the *P. triticina* isolate DZ-724

| $F_2$ plant | $F_3$ seg[a] | LRR[b] | $F_2$ plant | $F_3$ seg[a] | LRR[b] | $F_2$ plant | $F_3$ seg[a] | LRR[b] |
|---|---|---|---|---|---|---|---|---|
| K38-1 | 6:10 | Rr | K38-60 | 15:1 | Rr | K38-99 | 1:15 | Rr |
| K38-2 | 0:16 | rr | K38-61 | 16:0 | RR | K38-100 | 16:0 | RR |
| K38-5 | 14:2 | Rr | K38-62 | 19:12 | Rr | K38-101 | 13:3 | Rr |
| K38-14 | 16:0 | RR | K38-64 | 11:3 | Rr | K38-103 | 12:10 | Rr |
| K38-15 | 10:6 | Rr | K38-65 | 14:2 | Rr | K38-104 | 0:15 | rr |
| K38-16 | 16:0 | RR | K38-67 | 11:4 | Rr | K38-105 | 10:6 | Rr |
| K38-17 | 15:0 | RR | K38-68 | 17:13 | Rr | K38-106 | 0:16 | rr |
| K38-18 | 0:16 | rr | K38-69 | 16:9 | Rr | K38-108 | 0:16 | rr |
| K38-19 | 12:3 | Rr | K38-70 | 0:14 | rr | K38-109 | 16:0 | RR |
| K38-20 | 16:0 | RR | K38-71 | 0:16 | rr | K38-110 | 0:16 | rr |
| K38-22 | 15:13 | Rr | K38-72 | 12:4 | Rr | K38-118 | 12:4 | Rr |
| K38-24 | 16:0 | RR | K38-73 | 10:3 | Rr | K38-120 | 10:6 | Rr |
| K38-26 | 13:3 | Rr | K38-75 | 16:0 | RR | K38-125 | 0:16 | rr |
| K38-28 | 0:16 | rr | K38-76 | 20:10 | Rr | K38-130 | 16:0 | RR |
| K38-29 | 12:16 | Rr | K38-78 | 15:0 | RR | K38-131 | 16:0 | RR |
| K38-30 | 18:13 | Rr | K38-80 | 14:1 | Rr | K38-133 | 0:16 | rr |
| K38-31 | 12:4 | Rr | K38-81 | 0:16 | rr | K38-136 | 14:2 | Rr |
| K38-35 | 16:0 | RR | K38-82 | 18:17 | Rr | K38-137 | 0:16 | rr |
| K38-36 | 0:16 | Rr | K38-83 | 16:0 | RR | K38-138 | 11:5 | Rr |
| K38-37 | 14:0 | RR | K38-85 | 11:5 | Rr | K38-139 | 14:2 | Rr |
| K38-39 | 0:16 | rr | K38-86 | 14:2 | Rr | K38-140 | 0:16 | rr |
| K38-40 | 16:0 | RR | K38-87 | 10:6 | Rr | K38-141 | 15:0 | RR |
| K38-43 | 0:16 | rr | K38-88 | 15:1 | Rr | K38-142 | 17:14 | Rr |
| K38-47 | 16:0 | RR | K38-89 | 15:1 | Rr | K38-143 | 18:13 | Rr |
| K38-48 | 15:1 | Rr | K38-90 | 16:0 | RR | K38-144 | 15:0 | RR |
| K38-49 | 15:1 | Rr | K38-91 | 16:0 | RR | K38-145 | 16:0 | RR |
| K38-50 | 16:0 | RR | K38-92 | 15:1 | Rr | K38-146 | 13:3 | Rr |
| K38-51 | 16:0 | RR | K38-93 | 11:3 | Rr | K38-147 | 13:3 | Rr |
| K38-52 | 13:3 | Rr | K38-94 | 12:4 | Rr | K38-148 | 10:6 | Rr |
| K38-53 | 17:14 | Rr | K38-95 | 16:0 | RR | K38-79 | 0:10 | rr |
| K38-54 | 11:5 | Rr | K38-96 | 19:13 | Rr | RL6097 | 30:0 | RR |
| K38-57 | 13:2 | Rr | K38-97 | 0:16 | rr | Kubsa | 0:32 | rr |

[a] Ratio of resistant to susceptible plants in $F_3$ family

[b] Leaf rust resistance genotype of $F_2$ individual, inferred from segregation in $F_3$ family with RR: homozygous resistant; Rr: heterozygous resistant; rr: homozygous susceptible

resistant, and homozygous susceptible. This ratio fits to the expected 1:2:1 segregation for a monogenic trait with a chi-square *P* value 0.422 (Table 3).

## Linkage analysis

Out of the 54 SSR primers screened, 15 were found to be polymorphic between the two parents RL6097 and Kubsa (Table 3). Eleven out of 15 polymorphic SSR markers were codominant while the remaining four were dominant markers. Two codominant markers, *Xgpw5179* and *Xgpw5210*, showed distorted segregation ratios with chi-square *P* values of 0.003 and 0.001, respectively. At both loci, an excess of heterozygotes was observed (Table 3). An example of marker segregation in population

**Table 3** Chi-square test for segregation of $F_2$ plants in population TK*Lr38* for the *Lr38* resistance gene and 15 polymorphic SSR markers

| Marker/Gene | Segregation ratio[a] | | | | Total | Class | Df[b] | *P* value* |
|---|---|---|---|---|---|---|---|---|
| | a | h | b | c | | | | |
| *Lr38* | 25 | 51 | 18 | – | 94 | [a:h:b] | 2 | 0.422 |
| *Xbarc5* | 21 | 50 | 22 | – | 93 | [a:h:b] | 2 | 0.760 |
| *Xbarc96* | 30 | – | – | 64 | 94 | [a:c] | 1 | 0.122 |
| *Xbarc146* | 17 | 52 | 25 | – | 94 | [a:h:b] | 2 | 0.297 |
| *Xbarc196* | 27 | 42 | 24 | – | 93 | [a:h:b] | 2 | 0.589 |
| *Xbarc273* | 22 | 46 | 18 | – | 86 | [a:h:b] | 2 | 0.673 |
| *Xcfd5* | 17 | – | – | 76 | 93 | [a:c] | 1 | 0.134 |
| *Xcfd37* | 26 | 46 | 21 | – | 93 | [a:h:b] | 2 | 0.760 |
| *Xcfd42* | 29 | 41 | 24 | – | 94 | [a:h:b] | 2 | 0.356 |
| *Xcfd60* | 17 | – | – | 75 | 92 | [a:c] | 1 | 0.149 |
| *Xcfd76* | 25 | 53 | 16 | – | 94 | [a:h:b] | 2 | 0.067 |
| *Xcfd188* | 24 | 46 | 20 | – | 90 | [a:h:b] | 2 | 0.819 |
| *Xgwm469* | 25 | 47 | 20 | – | 92 | [a:h:b] | 2 | 0.746 |
| *Xgpw5179* | 14 | 63 | 16 | – | 93 | [a:h:b] | 2 | 0.003 |
| *Xgpw5210* | 8 | 67 | 19 | – | 94 | [a:h:b] | 2 | 0.001 |
| *Xwmc773* | 18 | – | – | 75 | 93 | [a:c] | 1 | 0.209 |

[a] a: homozygous RL6097 genotype; h: heterozygous genotype; b: homozygous Kubsa genotype; c: homozygous Kubsa or heterozygous genotype for dominant/recessive marker

[b] Df: degrees of freedom for chi-square test

**P* value of chi-square test for distorted segregation. Significant deviations ($P < 0.05$) from the expected 1:2:1 segregation for class [a:h:b] or 1:3 segregation for class [a:c] are highlighted in bold

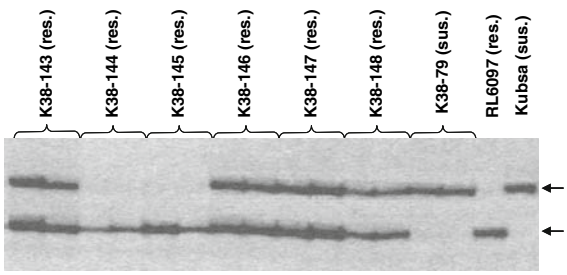


**Fig. 1** Segregation of SSR marker *Xbarc273* in $F_2$ population TK*Lr38*. The $F_2$ individuals were double loaded in adjacent lanes. The reaction to leaf rust is indicated as resistant (res.) or susceptible (sus.) The SSR fragments inherited from parent RL6097 (upper arrowhead) and parent Kubsa (lower arrowhead) are indicated

TK*Lr38* is given for the codominant marker *Xbarc273* in Fig. 1.

The linkage analysis indicated that 12 SSRs could be placed in a map with the resistance gene *Lr38* (Fig. 2). The SSR marker *Xwmc773* was found to be the closest proximal marker to *Lr38* with 6.1 cM distance followed by *Xbarc273*, which was 7.9 cM distant from the gene *Lr38*. Markers *Xcfd5* and *Xcfd60* were located 22.1 cM distant from *Lr38* towards the distal region of chromosome arm 6DL. The order of these markers was in agreement with the chromosome 6D wheat consensus map (Somers et al. 2004) and the physical map established by Sourdille et al. (2004).

## Discussion

Our segregation analyses in population TK*Lr38* confirmed that the *Lr38* leaf rust resistance conferred by the Thatcher-derived NIL, containing an introgression from *A. intermedium*, is of monogenic dominant inheritance. *Lr38* is effective both at seedling and adult plant stages (Mesterházy et al. 2000) indicating its potential use in wheat breeding programs. The gene *Lr38* proved to give effective protection of wheat against a wide array of virulence in many

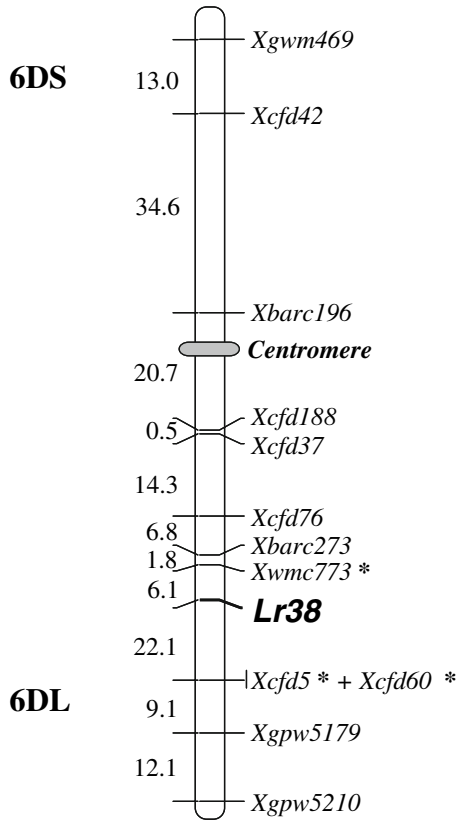


**Fig. 2** Genetic map of markers linked with *Lr38* on wheat chromosome 6D deduced from $F_2$ population TK*Lr38*. Genetic distances in cM and SSR markers are indicated on the left and right sides of the map, respectively. Asterisks specify three SSR markers which are inherited in a dominant/recessive manner in TK*Lr38*

wheat growing regions of the world (Dyck and Friebe 1993; Pasquini et al. 1998; Mesterházy et al. 2000; Kovalenko et al. 2002; Lind and Gultyaeva 2007). Despite its immense potential in leaf rust control, *Lr38* is, to our knowledge, currently not used in breeding programs. This is because *Lr38* is associated with low yield, most likely due to the large size of the original chromosomal segment transferred to wheat. This limitation demands considerable efforts in backcross breeding to improve yield of lines possessing *Lr38* if the gene is to be used in wheat breeding (Dyck and Friebe 1993).

Many molecular marker systems have been used to tag resistance genes in wheat, of which SSR markers are PCR-based robust markers that are ready-to-use, user-friendly and amenable for high throughput assays (Khan et al. 2005). Molecular markers, other than SSRs, need to be sequenced and converted into PCR-based markers for more rapid and reliable use. For instance, the cosegregating RFLP marker *Lr*k10 was converted into a PCR-based sequence-tagged-site (STS) marker for the gene *Lr10* (Schachermayr et al. 1997) and Naik et al. (1998) converted the random amplified polymorphic DNA (RAPD) marker OPJ-$01_{378}$ into an STS marker for the gene *Lr28*. Seyfarth et al. (1999) also developed an STS marker for the gene *Lr*35 through cloning and sequencing of a cosegregating RFLP probe and, currently, Urbanovich et al. (2006) developed and applied DNA marker assays to select leaf rust resistance at nine loci in wheat.

In this study, we mapped the SSR markers *Xwmc773* and *Xbarc273* proximal and *Xcfd5* and *Xcfd60* distal to *Lr38* on wheat chromosome arm 6DL. The SSR markers *Xwmc773*, *Xcfd5* and *Xcfd60* were dominantly inherited in population TK*Lr38* and linked in repulsion phase with *Lr38*, i.e. the amplified fragments were indicative for the Kubsa allele whereas the *A. intermedium*-derived marker allele could not be amplified by PCR. Thus, heterozygous and homozygous Kubsa genotypes could not be distinguished at these loci. The dominant inheritance of three *Lr38*-linked SSRs in population TK*Lr38* suggests that, in these cases, the sequence of the *Lr38*-bearing introgression from *A. intermedium* do not match with the *T. aestivum*-derived SSR primer sequences prohibiting a PCR amplification. On the other hand, the SSR marker *Xbarc273* was found to be codominant, allowing the amplification of both parental alleles. *Xbarc273* might, thus, be more useful for breeding purposes than the other markers because of its codominant nature and fairly good linkage with the *Lr38* locus. If needed, the *A. intermedium* introgression can be selected with *Xbarc273* plus the second flanking codominant marker *Xgpw5179* (Fig. 2). In future, the use of the flanking SSR markers will assist in predicting the presence or absence of *Lr38* in wheat cultivars and hence can be utilized in marker assisted selection and gene pyramiding efforts (Ashikari and Matsuoka 2006). However, no close linkage between the tested SSR markers and the *Lr38* locus was found so far. We, thus, propose to increase the number of markers mapped in close proximity to *Lr38* by a massive screen for polymorphic DNA markers. For this purpose, amplified fragment length polymorphism (AFLP) markers (Vos et al. 1995; Faris and Gill 2002; Haen et al. 2004) or the recently

developed wheat diversity array technology (DArT) markers (Semagn et al. 2006; Akbari et al. 2006) appear very attractive due to the large number of parallel studies which are possible with these techniques.

The use of PCR-based markers for the identification of leaf rust resistance genes can assist to reduce time, efforts and expenses for backcross breeding and pyramiding of major resistance genes into wheat cultivars (Naik et al. 1998; Gupta et al. 1999; Seyfarth et al. 1999). For this reason, we believe that the SSR markers linked to *Lr38* can facilitate the effort to reduce the introgression size in future backcrosses. Thus, the yield disadvantages associated with *Lr38* might be overcome by means of selecting recombinant plants possessing *Lr38* but missing most of the originally introgressed DNA segment from *A. intermedium*.

**Acknowledgements** The authors thank Dr. Pierre Sourdille (INRA, Clermont Ferrand, France) for providing primer aliquots of SSR markers from set II, Dr. Volker Lind (Federal Center for Breeding Research on Cultivated Plants, BAZ, Quedlinburg, Germany) for provision of seeds of the near-isogenic line RL6097 and Dr. Bedada Girma (Kulumsa Agricultural Research Center, Ethiopia) for providing seeds of cultivar Kubsa. We are also indebted to Ms Hedda von Quistorp for her excellent technical assistance on the SSR analysis.

## References

Akbari M, Wenzl P, Caig V, Carling J, Xia L, Yang SY, Uszynski G, Mohler V, Lehmensiek A, Kuchel H, Hayden MJ, Howes N, Sharp P, Vaughan P, Rathmell B, Huttner E, Kilian A (2006) Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. Theor Appl Genet 113:1409–1420

Ashikari M, Matsuoka M (2006) Identification, isolation and pyramiding of quantitative trait loci for rice breeding. Trends Plant Sci 11:344–350

Dehne HW, Oerke E-C (1998) Impact of diseases and disease control on crop production. In: Hutson DH, Miyamoto J (eds) Fungicidal activity: chemical and biological approaches to plant protection. Wiley and Sons, Chichester, New York, pp 1–21

Driscoll CJ, Sears ER (1965) Mapping of a wheat-rye translocation. Genetics 51:439–443

Dvorak J (1977) Transfer of leaf rust resistance from *Aegilops speltoides* to *Triticum aestivum*. Can J Genet Cytol 19:133–141

Dvorak J, Knott DR (1977) Homoeologous chromatin exchange in radiation-induced gene transfer. Can J Genet Cytol 19:125–131

Dvorak J, Knott DR (1980) Chromosome location of two leaf rust resistance genes transferred from *Triticum speltoides* to *T. aestivum*. Can J Genet Cytol 22:281–289

Dvorak J, Knott DR (1990) Location of a *Triticum speltoides* chromosome segment conferring resistance to leaf rust in *Triticum aestivum*. Genome 33:892–897

Dyck PL, Friebe B (1993) Evaluation of leaf rust resistance from wheat chromosomal translocation lines. Crop Sci 33:687–690

Faris JD, Gill BS (2002) Genomic targeting and high-resolution mapping of the domestication gene Q in wheat. Genome 45:706–718

Feuillet C, Travella S, Stein N, Albar L, Nublat A, Keller B (2003) Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome. Proc Natl Acad Sci USA 100:15253–15258

Friebe B, Zeller FJ, Mukai Y, Forster BP, Bartos P, McIntosh RA (1992) Characterization of rust-resistant wheat-*Agropyron intermedium* derivatives by C-banding, in situ hybridization and isozyme analysis. Theor Appl Genet 83:775–782

Friebe B, Jiang J, Gill BS, Dyck PL (1993) Radiation-induced nonhomoelogous wheat-*Agropyron intermedium* chromosomal translocations conferring resistance to leaf rust. Theor Appl Genet 86:141–149

Friebe B, Jiang J, Raupp WJ, McIntosh RA, Gill BS (1996) Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. Euphytica 91:59–87

Gupta PK, Varshney RK, Sharma PC, Ramesh B (1999) Molecular markers and their applications in wheat breeding. Plant Breed 118:369–390

Gupta SK, Charpe A, Prabhu KV, Haque QMR (2006) Identification and validation of molecular markers linked to the leaf rust resistance gene *Lr19* in wheat. Theor Appl Genet 113:1027–1036

Guyomarc'h H, Sourdille P, Charmet G, Edwards KJ, Bernard M (2002) Characteristics of polymorphic microsatellite markers from *T. tauschii* and transferability to the D-genome of bread wheat. Theor Appl Genet 104:1164–1172

Haen KM, Lu HJ, Friesen TL, Faris JD (2004) Genomic targeting and high-resolution mapping of the *Tsn1* gene in wheat. Crop Sci 44:951–962

Huang L, Brooks SA, Li WL, Fellers JP, Trick HN, Gill BS (2003) Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat. Genetics 164:655–664

Kattermann G (1937) Zur Zytologie halmbehaarter Stämme aus Weizenroggenbastardierung. Der Züchter 9:196–199

Kattermann G (1938) Über konstante halmbehaarte Stämme aus Weizenroggenbastardierung mit 2n = 42 Chromosomen. Z Induk Abst und Vererbungsl 74:354–375

Kerber ER, Dyck PL (1990) Transfer to hexaploid wheat of linked genes for adult-plant leaf rust and seedling stem rust resistance from an amphiploid of *Aegilops speltoides* × *Triticum monococcum*. Genome 33:530–537

Khan RR, Bariana HS, Dholakia BB, Naik SV, Lagu MD, Rathjen AJ, Bhavani S, Gupta VS (2005) Molecular mapping of stem and leaf rust resistance in wheat. Theor Appl Genet 111:846–850

Khlestkina KE, Than MHM, Pestsova EG, Röder MS, Malyshev SV, Korzun V, Boerner A (2004) Mapping of 99 new microsatellite-derived loci in rye (*Secale cereale* L.)

including 39 expressed sequence tags. Theor Appl Genet 109:725–732
Knott DR (1968) Translocations involving *Triticum* chromosomes and *Agropyron* chromosomes carrying rust resistance. Can J Genet Cytol 10:695–696
Kolmer JA (1996) Genetics of resistance to wheat leaf rust. Annu Rev Phytopathol 34:435–455
Kosambi DD (1944) The estimation of map distances from recombination values. Annu Eugen 12:172–175
Kovalenko ED, Zhemchuzina AI, Kryazheva NN (2002) Virulence of *Puccinia triticina* in the Russian Federation in 2000. Annu Wheat Newsl 48:120–122
Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic maps of experimental and natural populations. Genomics 1:174–181
Lind V, Gultyaeva E (2007) Virulence frequences of *Puccinia triticina* in Germany and the European regions of the Russian Federation. J Phytopathol 155:13–21
Long DL, Kolmer JA (1989) A North American system of nomenclature for *Puccinia recondita* f. sp. *tritici*. Phytopathology 79:525–529
McIntosh RA, Dyck PL, Green GJ (1977) Inheritance of leaf rust and stem rust resistance in wheat cultivars Agent and Agatha. Aust J Agric Res 28:37–45
McIntosh RA, Miller TE, Chapman V (1982) Cytological studies in wheat XII. *Lr*28 for resistance to *Puccinia recondita* and *Sr*34 for resistance to *P. graminis tritici*. Z Pflanzenzüchtg 89:295–306
McIntosh RA, Wellings CR, Park RF (1995) Wheat rusts: an atlas of resistance genes. CSIRO Publications, Victoria, Australia
McIntosh RA, Devos KM, Dubcovsky J, Rogers WJ, Morris CF, Appels R, Anderson OA (2005) Catalogue of gene symbols for wheat. Annu Wheat Newsl 51:250–285
Mebrate SA, Cooke BM (2001) Response of wheat cultivars to infection by *Stagonospora nodorum* isolates/mixture on detached and intact seedling leaves. Euphytica 122:263–268
Mesterházy A, Batos P, Goyeau H, Niks RE, Csösz M, Andersen O, Casulli F, Ittu M, Jones E, Manisterski J, Manninger K, Pasquini M, Rubiales D, Schachermayr G, Strzembicka A, Szunics L, Todorova M, Unger O, Vanco B, Vida G, Walther U (2000) European virulence survey for leaf rust in wheat. Agronomie 20:793–804
Mukade K, Kamio M, Hosoda K (1970) The transfer of leaf rust resistance from rye to wheat by intergeneric addition and translocation. Gamma Field Symp. No. 9. 'Mutagenesis in Relation to Ploidy Level', pp 69–87
Naik S, Gill KS, Prakasa Rao VS, Gupta VS, Tamhankar SA, Pujar S, Gill BS, Ranjekar PK (1998) Identification of a STS marker linked to the *Aegilops speltoides*-derived leaf rust resistance gene *Lr*28 in wheat. Theor Appl Genet 97:535–540
Paillard S, Schnurbusch T, Winzeler M, Messmer M, Sourdille P, Abderhalden O, Keller B, Schachermayr G (2003) An integrative genetic linkage map of winter wheat (*Triticum aestivum* L.). Theor Appl Genet 107:1235–1242
Pasquini M, Casulli F, Sereni L, Casini F (1998) Pathogenicity of *Puccinia recondita* f.sp. *tritici* in Italy during 1997. Annual wheat newsletter 44: items from Italy. http://grain.jouy.inra.fr/ggpages/awn/44/Textfiles/ITALY.html. Accessed 21 Nov 2007
Pillen K, Binder A, Kreuzkam B, Ramsay L, Waugh R, Foerster J, Léon J (2000) Mapping new EMBL-derived barley microsatellites and their use in differentiating German barley cultivars. Theor Appl Genet 101:652–660
Riley R, Chapman V, Johnson R (1968) The incorporation of alien disease resistance in wheat by genetic interference with the regulation of meiotic chromosome synapsis. Genet Res Camb 12:198–219
Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023
Schachermayr G, Feuillet C, Keller B (1997) Molecular markers for the detection of the wheat leaf rust resistance gene *Lr*10 in diverse genetic backgrounds. Mol Breed 3:65–74
Sears ER (1956) The transfer of leaf rust resistance from *Aegilops umbellulata* to wheat. Brookhaven Symp Biol 9:1–22
Sears ER (1973) *Agropyron*-wheat transfers induced by homoeologous pairing. In: Sears ER, Sears LMS (eds) Proceedings of the fourth international wheat genetics symposium, Agricultural Research Station, University of Missouri, Columbia, MO, USA, pp 191–199
Sears ER (1977) Analysis of wheat-*Agropyron* recombinant chromosomes. *In*: Proceedings of the 8th Eucarpia Congress, Madrid, Spain, pp 63–72
Semagn K, Bjornstad A, Skinnes H, Maroy AG, Tarkegne Y, William M (2006) Distribution of DArT, AFLP, and SSR markers in a genetic linkage map of a doubled-haploid hexaploid wheat population. Genome 49:545–555
Seyfarth R, Feuillet C, Schachermayr G, Winzeler M, Keller B (1999) Development of a molecular marker for the adult plant leaf rust resistance gene *Lr*35 in wheat. Theor Appl Genet 99:554–560
Sharma D, Knott DR (1966) The transfer of leaf-rust resistance from *Agropyron* to *Triticum* by irradiation. Can J Genet Cytol 8:137–143
Smith EL, Schlehuber AM, Young HC, Edwards LH (1968) Registration of Agent wheat. Crop Sci 8:511–512
Somers DJ, Isaac P, Edwards K (2004) A high-density wheat microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114
Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theor Appl Genet 110:550–560
Sourdille P, Singh S, Cadalen T, Brown-Guedira GL, Gay G, Qi L, Gill BS, Dufour P, Murigneux A, Bernard M (2004) Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). Funct Integr Genomics 4:12–25
Urbanovich OY, Malyshev SV, Dolmatovich TV, Kartel NA (2006) Identification of leaf rust resistance genes in wheat *(Triticum aestivum* L.) cultivars using molecular markers. Russ J Genet 42:546–554
Voorrips RE (2002) MapChart: Software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78
Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Wienhues A (1966) Transfer of rust resistance of *Agropyron* to wheat by addition, substitution and translocation. In: MacKey J (ed) Proceedings of the second International wheat genetic symposium, Lund, Sweden, Hereditas suppl Berlingska Boktryckeriet Lund, 19–24 August 1963, pp 328–341

Wienhues A (1973) Translocations between wheat chromosomes and an *Agropyron* chromosome conditioning rust resistance. In: Sears ER, Sears LMS (eds) Proceedings of the fourth International wheat genetic symposium, Columbia, MO, University of Missouri, Columbia, 6–11 August 1973, pp 201–207