

PHYSICAL MAPPING OF THE 7D CHROMOSOME USING A WHEAT/BARLEY TRANSLOCATION LINE (5HS.7DL) PRODUCED IN A MARTONVÁSÁRI WHEAT BACKGROUND USING MICROSATELLITE MARKERS

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Abstract: A wheat/barley translocation line was previously developed in Martonvásár from the Mv9kr1 × Igrí hybrid and was identified as a 5HS.7D translocation using fluorescence *in situ* hybridization (FISH) and 5H-specific barley SSR markers. The aim of the present study was to characterize the rearranged wheat chromosome and to identify the 7D chromosome segment eliminated by the translocated 5HS segment using 7D-specific SSR markers. The elimination of the 7DS terminal region was proved by three of the twenty-four tested markers. The breakpoint of the 5HS.7DS translocation was considered to be closer to the telomere than the breakpoint of known deletion lines, which provides a new physical landmark for future deletion mapping studies. The fine mapping of 7D makes it possible to localize agronomically useful genes to the precise chromosomal region of the eliminated 7DS segment, opening up the possibility of marker-assisted breeding and map-based cloning.

Keywords: wheat-barley translocation line, *in situ* hybridisation, SSR markers, physical mapping

Introduction

Barley (*Hordeum vulgare* L.), one of the most important crops, carries several genes involved in biotic and abiotic stress tolerance, which can be transferred to wheat via wide hybridisation. The production of wheat-barley translocation lines is an important intermediate step in transferring genes of interest into wheat. Besides their importance in wheat breeding programmes, translocation lines are excellent genetic materials for the physical mapping of genes or molecular markers to specific breakpoint intervals (bins). Using the translocation breakpoint as a physical landmark, genes or molecular markers can be located to a precise chromosomal region (Nagy et al. 2002). When comparing genetic and physical maps, discrepancies were found in marker order and in the distances between markers. Due to the recombination hotspots, which are more frequent in the gene-rich regions close to the telomeres, genetic maps provide only an estimate of marker positions (Gustafson et al. 1990; Lukaszewski and Curtis 1993). It is particularly difficult to assess the marker order using genetic mapping in regions of high marker density, where genetic distances are very short (Sourdille et al. 2004). Microsatellite markers are highly polymorphic genetic markers making it possible to discriminate between closely related species. Translocations and their breakpoint positions can be visualised using genomic *in situ* hybridisation (GISH) (Schwarzacher et al. 1989, Le et al. 1989, Molnár-Láng et al. 2000b) and the rearranged chromosomes can be identified with fluorescence *in situ* hybridisation (FISH) using repetitive DNA sequences (Molnár et al. 2009).

The 5HS.7D translocation line analysed in the present study was developed in Martonvásár from the 'Mv9kr1' × 'Igrí' hybrid (Molnár-Láng et al. 2000a) and was identified using fluorescence *in situ* hybridization (FISH). This genetic material was previously used for the physical mapping of the 5H barley chromosome (Nagy et al 2002, Molnár-Láng et al. 2005) but it was not completely clarified which region of the

7D chromosome was substituted by barley chromosome arm 5HS. The aim of the present study was to describe the precise composition of the translocated chromosome and to identify the substituted wheat chromosome segment.

Materials and methods

Plant material:

The wheat/barley translocation line was developed from the Mv9kr1 × Igri hybrid produced in Martonvásár, Hungary, as described in detail by Molnár-Láng et al. (2000a). The 5HS.7D translocation line was selected from the backcrossed progenies of the hybrid using GISH.

Genomic *in situ* hybridization: Total genomic DNA from the barley cultivar Igri was labelled with fluorored (rhodamine-4-dUTP, Amersham) using nick-translation, while DNA from Mv9kr1 wheat was used as blocking DNA. The *in situ* hybridization experiment was carried out as described previously by Szakács and Molnár-Láng (2008), except that the hybridisation temperature was 65°C.

SSR marker analysis: DNA was isolated from the wheat cultivar Mv9kr1, the barley cultivar Igri and from the Mv9kr1/Igri translocation line. The DNA extraction was performed as described by Anderson et al. (1992).

Twenty-four SSRs distributed over the 7D chromosome (7DS-specific SSRs: Xwmc506, Xgdm130, Xbarc184, Xgwm44, Xgwm295, Xgdm86, Xbarc126, Xgwm111, Xbarc214 and Xgwm350; 7DL-specific SSRs: Xwmc437, Xwmc94, Xbarc1046, Xbarc172, Xbarc111, Xgdm46, Xgdm67, Xbarc53, Xgwm428, Xgwm37, Xcfd69, Xgdm142, Xgdm150 and Xgdm84) were selected from the GrainGenes 2.0 database (<http://wheat.pw.usda.gov/GG2/index.shtml>). The PCR reactions were performed in an Eppendorf Mastercycler (Eppendorf-Netheler-Hinc Inc., Hamburg, Germany) using the PCR mixture described by Schneider and Molnár-Láng. (2008). The 45 cycles were performed with 30 sec at 94 °C, 30 sec at either 50°C, 55°C or 60°C (depending on the annealing temperature of the microsatellite markers), 30 sec at 72 °C, and a final extension step of 10 min at 72 °C. The PCR products were separated on 2% agarose gels. The bands were stained using ethidium bromide, and DNA was visualised using a SynGene GelDoc system (SynGene, Cambridge, England).

Results and discussion

Twenty-four microsatellites distributed over the 7D chromosome were used to determine the 7D chromosome segment eliminated by the barley chromosome arm 5HS. Polymorphism was detected for all the markers between PCR products amplified by wheat and barley DNA, so all the markers could be used to characterise the rearranged chromosome. All the 7DL-specific markers produced PCR products of the expected size on wheat and on the translocation DNA, revealing the presence of the complete 7DL. In the case of three 7DS-specific markers (Xbarc184, Xwmc506 and Xgdm130) the Mv9kr1/Igri translocation line failed to amplify the 7DS-specific fragments given by wheat DNA, signaling the elimination of a short chromosome segment carrying these markers. None of these three markers were previously mapped physically within 7DS but genetic mapping studies located them close to the telomere of 7DS. The other seven 7DS-specific markers were present on the translocation chromosome. Four of these markers (Xbarc126, Xgwm44, Xgwm295, Xbarc214) were mapped previously to the

terminal bin of 7DS, between fraction lengths (FL) 0.61-1.00 (Sourdille et al. 2004). As the FISH experiment detected the absence of a strong Afa family signal characteristic of the terminal 7DS region, it was concluded that this region had been deleted. The 7DS-specific markers absent from this genetic material were thus located on the deleted, most distal region of 7DS. Obviously, the three missing markers (Xbarc184, Xwmc506, Xgdm130) were situated distally to the four markers present on the translocation chromosome (Xbarc126, Xgwm44, Xgwm295, Xbarc214) and mapped previously to the terminal bin of 7DS. The present study physically mapped Xbarc184, Xwmc506 and Xgdm130 to FL 0.61-1.00, but distally to markers Xbarc126, Xgwm44, Xgwm295 and Xbarc214.

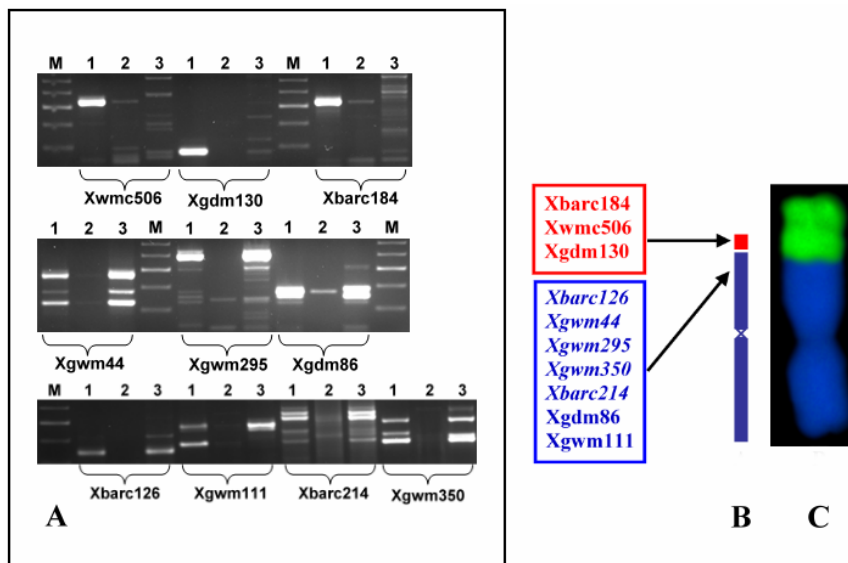


Figure 1 A: Agarose gel electrophoresis pattern of the 7DS-specific markers on wheat line Mv9kr1 (1), barley cultivar Igri (2) and on the Mv9kr1/Igri 5HS.7DS.7DL translocation line (3). The translocation line lacked fragments amplified by markers Xwmc506, Xgdm130 and Xbarc184.

B: Physical mapping of the tested 7DS-specific markers on the 5HS.7DS.7DL translocation chromosome. The positions of the markers are indicated by arrows. The markers previously mapped physically to the 7DS terminal region are in italics, while markers mapped physically in the present study to the deleted 7DS chromosome segment are visualised in red.

C: GISH pattern of the 5HS.7DS.7DL translocation chromosome using barley DNA as probe. The barley chromosome segment is visualised in green and the 7D chromosome in blue.

The marker order revealed in the present study showed good accordance with the wheat microsatellite consensus map established by Somers et al. (2004), except for the marker Xgwm350, which was mapped genetically as the most distal marker within 7DS. The analysis of the 5HS.7DS.7DL translocation clarified the position of this marker and placed it proximally to Xbarc184, Xwmc506 and Xgdm130.

The 5HS.7DS.7DL translocation provides a new breakpoint interval within FL 0.61-1.00 characterised by three microsatellites, making it possible to construct more detailed physical maps involving the terminal 7DS region.

Conclusions

A new breakpoint interval within 7DS was detected by means of the fine mapping of 7DS, involving the *in situ* hybridisation of the 5HS.7DS.7DL translocation line, combined with microsatellite marker analysis. More detailed physical mapping analysis of the terminal regions of 7DS is thus possible, opening up the way for map-based cloning and marker-assisted selection.

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