INCORPORATION OF A WINTER BARLEY CHROMOSOME SEGMENT INTO CULTIVATED WHEAT AND ITS CHARACTERIZATION WITH GISH, FISH AND SSR MARKERS

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Abstract: Spontaneous wheat-barley translocation chromosomes were detected by genomic in situ hybridisation (GISH) in the progenies of wheat/barley addition lines produced from the wheat cultivar Asakaze komugi and the Ukrainan six-rowed barley cultivar Manas. The homozygous translocation line was selected from a heterozygous translocation line with the help of molecular markers and fluorescence in situ hybridisation (FISH) using repetitive DNA probes. The wheat chromosome arm involved in the translocation was identified by FISH as 4BS. The barley chromosome segment could not be unequivocally determined with the hybridisation of the repetitive DNA sequences used in the present work (Afa family, pSc119.2, pTa71). The microsatellite marker analysis revealed the presence of an almost complete 7HL chromosome arm, but the centromeric region of 7HL was missing from this translocation line. The rearranged chromosome, identified as 4BS.7HL with a centromeric deletion of 7HL, represents a unique genetic material which can be used for the physical mapping of genes or genetic markers within 7HL. As the barley chromosome 7H is considered to be the most important chromosome for drought tolerance, the translocation line will make it possible to reveal the effect of the abiotic stress-related genes situated on the incorporated 7HL segment in the genetic background of wheat.

Keywords: wheat, barley, 7H chromosome, translocation, microsatellite markers

Introduction

The wide hybridisation of common wheat (*Triticum aestivum* L.) opens up the possibility to transfer agronomically useful genes from related species into bread wheat. Barley (*Hordeum vulgare* L.), which is tolerant of various abiotic stresses and has good nutritional parameters, represents a potential gene source for wheat improvement. In order to transfer agronomically useful traits from barley into wheat a new wheat-barley hybrid was developed in Martonvásár (Hungary) by crossing the Ukrainan six-rowed barley cultivar Manas with the Japanese wheat cultivar Asakaze komugi (Molnár-Láng et al. 2000). After several backcrosses, wheat/barley addition lines were selected from the progenies. Wheat/alien addition lines are excellent genetic materials for studying the effect of alien chromosomes in the genetic background of wheat and they can be used as a starting point for gene transfer from an alien chromosome. Translocation lines can be selected from the progenies of addition lines. A spontaneous translocation was detected in progenies of the 7H wheat/barley addition line using GISH. The translocation line carries 42 chromosomes. The importance of the translocation line is that the long arm of the 7H chromosome is considered to be the most important genomic region for drought tolerance (Cattivelli et al. 2002). The objective of the present work was to identify the chromosomal regions involved in the translocation, and to detect which chromosome segments have been deleted from the Asakaze komugi/Manas translocation line using GISH, FISH and SSR markers.

Materials and methods

Plant material: A wheat×barley hybrid was developed previously in Martonvásár (Hungary) by crossing the Japanese wheat cultivar Asakaze komugi (*Triticum aestivum* L.) with the Ukrainian six-rowed winter barley cultivar Manas (*Hordeum vulgare* L.)

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(Molnár-Láng et al. 2000). The hybrids were backcrossed twice with Chinese Spring wheat, and the translocation line was selected from the BC_2 progenies (Molnár-Láng et al. 2005).

GISH (Genomic *in situ* hybridisation): Total genomic DNA from the barley cultivar Manas and the wheat cultivar Asakaze komugi was isolated using the phenol-chloroform method described by Sharp et al. (1988). Barley DNA was labelled with Fluorored (rhodamine-4-dUTP, Amersham) using nick translation. Wheat DNA was used as blocking DNA at a ratio of 30:1. The *in situ* hybridisation was carried out according to Molnár-Láng et al. (2000).

FISH (Fluorescence *in situ* hybridisation): The GISH hybridisation signals were washed off the slides in 4×SSC Tween at 25 ºC overnight. The Afa family, pSc119.2 and pTa71 repetitive DNA sequences were used for three-colour FISH. The probe labelling was carried out as described by Sepsi et al. (2008) and the *in situ* hybridisation experiment was performed according to Szakács and Molnár (2008).

SSR marker analysis: Genomic DNA was isolated from three BC_2 plants, from Chinese Spring and from Manas according to Anderson et al. (1992). PCR reactions were performed in a final volume of 25µl. The reaction mixture contained 25ng of template DNA, 2× GoTaq Green Master Mix (Promega, USA) and 0.2 µM of both primers. Amplification was carried out in an Eppendorf Mastercycler (Eppendorf, Germany), with the corresponding profile described previously for each primer pair. The following microsatellites were tested: 4BS-specific markers: Xgwm368, Xgwm113 (Roder et al. 1998) and Xbarc1045 (Sourdille et al. 2004); 4BL-specific markers: Xgwm149, Xgwm165 and Xgwm251 (Röder et al. 1998); 7HS-specific markers: HvM4 (Liu et al. 1996) and Bmag0021 (Ramsay et al. 2000); and 7HL-specific markers: HvID, Bmag0120, Bmac 0156 (Ramsay et al. 2000), EBmac0827, EBmac0785 (Rostoks et al. 2005) and GBM1102 (Varshney et al. 2006, Hearnden et al. 2007). The PCR products were separated using 2.5% SeaKem agarose (Cambrex, USA) gels and the fragments were stained with ethidium bromide. A 50bp DNA ladder (Fermentas, Lithuania) was used to estimate molecular weights. The patterns were documented and analysed using a GeneGenius gel documentation system (Syngene, UK).

Results and discussion

A wheat/barley translocation was detected by GISH using barley DNA as a probe (Fig. 1). Using three repetitive DNA probes, FISH identified the wheat chromosome segment involved in the translocation as 4BS. The presence of 4BS was confirmed with the 4BS specific markers (Xgwm368, Xgwm113 and Xbarc1045) and the lack of signals for markers mapped to 4BL (Xgwm149, Xgwm165, Xgwm251) proved the absence of 4BL.

The barley chromosome segment could not be clearly identified using FISH, but the presence of an almost complete 7HL was proved by the signals given by four 7HL-specific microsatellites. The elimination of the centromeric region of 7HL was suggested by the absence of the Afa family signal characteristic of the 7HL centromere (Fig. 1). The 7HS-specific markers (HvM4, Bmag0021) gave no signals, indicating the elimination of 7HS.

As a physical map of the 7H-specific SSR markers was not available, the position of the translocation breakpoint could not be compared to the breakpoint of known deletion

lines. The 7HL-specific SSR markers were selected from three genetic maps (Rostoks et al. 2005, Hearnden et al. 2007, Ramsay et al. 2000). Some of the markers were included in more then one genetic map, making it possible to compare their positions within different mapping populations. In the centromeric regions several markers were mapped genetically within a small distance of each other. Greater distances were found between markers in the middle of the chromosome arm, while in the telomeric region the markers were situated closer to each other. The FISH pattern of the translocation chromosome suggested the absence of the centromeric region of 7HL. Six 7HL-specific microsatellites were tested in order to identify the eliminated 7HL segment. Two of these markers (EBmac0785, Ebmac0827) were mapped to the centromeric region (Rostoks et al. 2005, Hearnden et al. 2007), a third marker (GBM1102) was positioned near the centromeric region (Varshney et al. 2006, Hearnden et al. 2007), two markers (Bmag0120, Bmac0156) were mapped near the middle of the chromosome arm (Ramsay et al. 2000, Hearnden et al. 2007, Karsai et al. 2007), and the sixth marker (HvID) was the most distal marker on 7HL (Ramsay 2000, Rostoks 2005).
The SSR marker analysis confirmed the results obtained using FISH and proved the presence of an almost complete 7HL. The absence of two 7HL-specific markers from the translocation line (EBmac0785 and Ebmac0827), which were previously mapped genetically close to the centromere, revealed the absence of the 7HL centromeric region. The translocation breakpoint could be placed between markers Ebmac0827 and GBM1102 (Fig. 1).

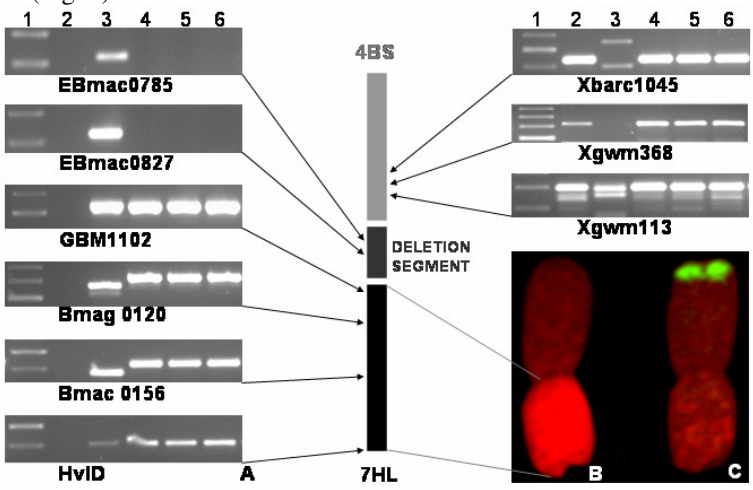

Figure 1: Physical map of SSR markers within the 4BS.7HL translocation, including the centromeric deletion of 7HL (A) The electrophoretic patterns of the 7HL-specific markers are indicated on the left of the schematic chromosome while the electrophoretic patterns of the 4BS-specific markers are indicated on the right (1: size marker 50bp, 2: Chinese Spring wheat DNA, 3: Manas barley DNA, 4, 5, 6: DNAs from the translocation line). The 4BS.7HL translocation with the centromeric deletion of 7HL was identified by GISH(B) and FISH (C). The Afa family, pSc119.2 and pTa71 repetitive DNA sequences were used for three-colour FISH.

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Conclusions

The 4BS/7HL translocation line described in the present study makes it possible to study the effects of genes situated on 7HL. Moreover, this is an excellent genetic material for the physical mapping of genes or molecular markers in the centromeric region of 7HL, which plays an important role in drought tolerance. It is planned to test the drought tolerance of the 4BS/7HL translocation line in order to study the effect of QTLs involved in drought tolerance in the genetic background of wheat.

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