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

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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).

A spontaneous translocation was detected in progenies of the 7H wheat/barley addition line using GISH. 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.

Summary of abiotic stress tolerance QTLs mapped on the homoeologous Triticeae chromosome seven. On the left side of the chromosomes small characters indicate RFLP and RAPD (underlined) marker loci. Capitals indicate QTL peaks and most significant marker loci associated with QTLs: DT, drought tolerance and vernalization-responsive (Vrn) are reported. (Cattivelli et al. 2002)

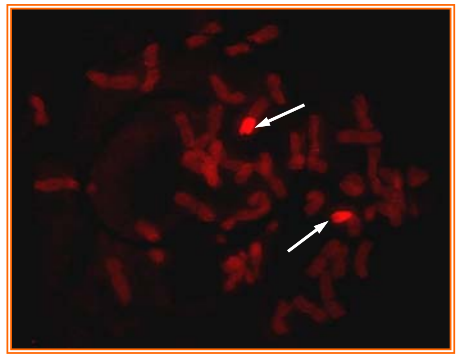

Figure 1: A spontaneous wheat/barley (Asakaze komugi×Manas) translocation line detected using GISH. Total barley genomic DNA was labelled with Fluorored, thus barley chromosome arms are red (arrows).

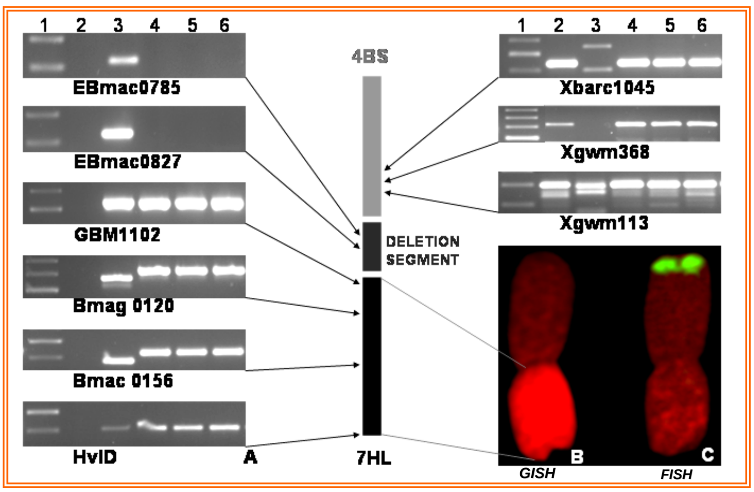

Figure 3: Physical map of SSR markers within the 4BS.7HL translocation, including the centromeric deletion of 7HL (A) The electrophoretic patterns of the 7HL-specific markers are indicated on the left of the schematic chromosome while the electrophoretic patterns of the 4BS-specific markers are indicated on the right (1: size marker 50bp, 2: Chinese Spring wheat DNA, 3: Manas barley DNA, 4, 5, 6: DNAs from the translocation lines).

Materials and methods

Plant material:

A wheat/barley translocation line selected from the progenies of the Asakaze komugi (*Triticum aestivum* L. , Japanese wheat cultivar) × Manas (*Hordeum vulgare* L. Ukrainian six-rowed winter barley cultivar) hybrid (Molnár-Láng et al. 2000). Chromosome preparations were made of root tips.

GISH (Genomic in situ hybridisation):

Probe: Total genomic DNA from the barley cultivar Manas. Labelling: Nick translation.

FISH (Fluorescence in situ hybridisation):

Probes: **Afa family**, **pSc119.2**, pTa71 repetitive DNA sequences. The probe labelling was carried out as described by Sepsi et al. (2008) with Fluorored (rhodamine-4-dUTP), biotin-streptavidin-FITC, digoxigenin-antidig-rhodamine and the in situ hybridisation experiment was performed according to Szakács and Molnár (2008).

SSR marker analysis:

Genomic DNA was isolated from three BC2 plants, and from the parental lines. The following microsatellites were tested: 4BS-specific markers: Xgwm368, Xgwm113 and Xbarc1045; 4BL-specific markers: Xgwm149, Xgwm165 and Xgwm251; 7HS-specific markers: HvM4 and Bmag0021; and 7HL-specific markers: HvID, Bmag0120, Bmac 0156, EBmac0827, EBmac0785 and GBM1102.

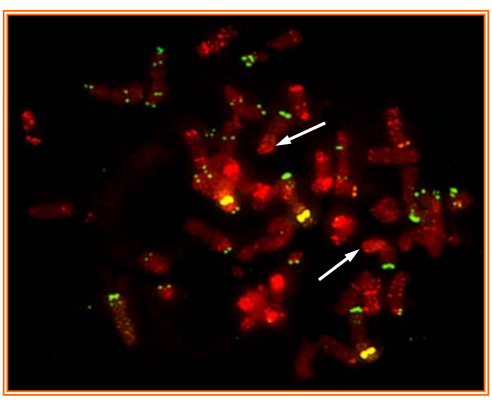

*Figure 2: Chromosome identification on the somatic chromosomes of the wheat/barley translocation line using FISH with the help of three repetitive DNA probes: **Afa family**, **pSc119.2**, **pTa71**.*

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 (Fig. 2) involved in the translocation as 4BS. The presence of 4BS was confirmed with the 4BS specific markers (Xgwm368, Xgwm113 and Xbarc1045) (Fig. 3) 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. 3/C). The 7HS-specific markers (HvM4, Bmag0021) gave no signals, indicating the elimination of 7HS.

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.

References

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Acknowledgements

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