Identification of newly developed wheat/winter barley addition lines using fluorescence in situ hybridization and SSR markers

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Summary

New disomic (2H, 3H, 4H, 1HS) and monosomic (2H, 3H, 6H, 7H) wheat/barley (*Triticum aestivum*/*Hordeum vulgare*) addition lines have been developed from hybrids produced in Martonvásár with the German two-rowed winter barley cultivar 'Igri' and the Ukrainan six-rowed winter barley cultivar 'Manas'. The addition lines were identified using genomic in situ hybridization (GISH) and two-colour fluorescent in situ hybridization (FISH) with the probes GAA, pAs1, HvT01, Afa family and pTa71 and with SSR markers.

Key words:

Triticum aestivum, *Hordeum vulgare*, wheat/barley addition lines, in situ hybridization, SSR markers

Introduction

The introgressive hybridization of barley to wheat makes it possible to transfer useful characters such as earliness, drought and soil salinity tolerance, and various traits for specific nutrition quality. The first wheat/barley hybrid was produced by KRUSE (1973) and the production of the first set of Chinese Spring/Betzes spring wheat/spring barley addition lines was described by ISLAM et al. (1978). Since then only KOBA et al. (1997) have published two new 5H and 6H addition lines from a hybrid between the wheat cultivar Shinchunaga and the barley cultivar Nyugoruden. It will be necessary to develop addition lines using different barley genotypes with great genetic variability for agronomic traits (two or six-row, winter or spring habit, biotic and abiotic resistance, etc.) if favourable characters from this cereal are to be mapped and transferred.

The main objective of the present study was to develop and identify wheat/barley addition lines derived from the 'Martonvásári 9 kr1' × 'Igri' and the 'Asakaze komugi' × 'Manas' hybrids using in situ hybridization and SSR markers.

Material and Methods

Plant materials: Wheat × barley hybrids were produced using two wheat (*T. aestivum* L.) genotypes as the maternal plants: the Hungarian winter wheat line 'Martonvásári 9 kr1' (Mv9 kr1), and the Japanese wheat variety 'Asakaze komugi'. The winter barley (*H. vulgare* L.) cultivars used as the male parent were the following: 'Igri', (two-rowed, German) and 'Manas' (six-rowed, Ukrainian). The 'Mv9 kr1'×'Igri' and the 'Asakaze komugi'×'Manas' hybrids were multiplied in tissue culture and the backcross pollination was carried out using the wheat genotypes 'Mv9 kr1', 'Asakaze komugi' and 'Chinese Spring' as described earlier (MOLNÁR-LÁNG et al. 2000).

Sequential fluorescence and genomic in situ hybridization: Mitotic metaphase chromosome preparations of the addition lines were used for in situ hybridization. FISH was carried out according to LINC et al. (1999) with the following DNA probes: GAA satellite sequences (PEDERSEN et al. 1996), pAs1 (RAYBURN and GILL 1986), HvT01 subtelomeric tandem repeat (SCHUBERT et al. 1998), Afa family (NAGAKI et al. 1995) and pTa71 (GERLACH and BEDBROOK 1979). The pAs1 and pTa71 DNA probes were labelled by nick translation in the presence of Fluorored (Roche) and Fluorogreen (Roche), respectively.

HvT01, GAA satellite sequences and the Afa family were amplified from the genomic DNA of barley and labelled by PCR with Fluorored and Fluorogreen, respectively, according to VRÁNA et al. (2000).

GISH was carried out on the same slides following the method of READER et al. (1994) with minor modifications (MOLNÁR-LÁNG et al. 2000). Total barley genomic DNA was labelled with Fluorored by nick translation. Unlabelled wheat genomic DNA was used at 20 times the quantity of the probe. The slides were counterstained with 1µg/ml DAPI (Amersham). A Zeiss Axioskop-2 epifluorescence microscope fitted with a Spot CCD camera (Diagnostic Instruments, USA) was used to document the hybridization signals.

SSR marker analysis: Genomic DNA was isolated from the BC_2 progenies, the addition lines and the parental lines. SSR markers were selected from a highly saturated genetic map of barley (RAMSAY et al. 2000). Besides the twenty SSR markers used in previous experiments (MOLNÁR-LÁNG et al. 2005) the following SSR markers were studied: HVHVA1 (1HL), HVM60 (3HL), HVM62 (3HL) and HVM4(7HS). The PCRs were performed as described by RAMSAY et al. (2000).

Results

Identification of barley chromosomes in the 'Mv9kr1'/'Igri' addition lines using FISH and SSR markers: The 4H disomic addition line was identified with sequential GISH and FISH using the GAA sequence and the pAs1 and HvT01 probes. SSR markers HvM40 and HvM67 confirmed the presence of 4H in this line. The spikes of the 4H disomic addition line bore the greatest resemblance to those of 'Mv9kr1'. They were compact with small awn stubs at

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the top, and their fertility was equal to that of the 'Mv9kr1' parent.

The 2H disomic addition line was identified with a combination of the probes GAA, pAs1 and HvT01. The presence of 2H was confirmed with GISH. The SSR marker HvM36 also gave the expected PCR product size. The 2H disomic addition line had a long, loose spike, with significantly poorer seed set (grains/spike) than 'Mv9kr1'. The plants were much taller than those of 'Mv9kr1' and the other addition lines.

The 3H disomic addition line was identified using FISH with the following DNA probes GAA, pAs1 and HvT01 *(Figure 1A*). The 3H-specific SSR markers HvM60 and HvM62 confirmed the presence of the 3H chromosome in this line. The spikes of the 3H disomic addition line were short, very compact with awn stubs, and broader towards the top (*Figure 1B*). The plants were short, but had relatively good fertility.

The fourth line had a disomic addition of the isochromosome 1HS. First this addition line was analysed using FISH with a combination of the DNA probes GAA and HvT01. Two minor, perfectly symmetrical interstitial pTa71 signals identified this chromosome pair as 1HS isochromosomes. The presence of 1HS and the absence of the 1HL chromosome arm in this line was also confirmed by the SSR markers Bmac0213 (1HS) and HVHVA1 (1HL). In a climatic chamber the heading of this line was about one week earlier than that of the other disomic addition lines. The spikes were as long as those of the 2H addition line (11 cm) and loose, with awn stubs at the top. This plant produced enough seeds for further multiplication.

Monosomic additions of 7H were identified among the selfed seeds of a BC_2 progeny of the 'Mv9kr1' × 'Igri' hybrid. The 7H chromosome was identified with the probes HvT01 and Afa family. Seeds (506) were developed on the three 7H monosomic additions, so it will be possible to select the disomic addition in the next generation. The spikes were similar to the wheat parent, with small awn stubs.

Identification of barley chromosomes in the 'Asakaze komugi'/'Manas' monosomic and disomic additions using FISH and SSR markers: The aim of this study was to produce backcross progenies on the 'Asakaze komugi' × 'Manas' hybrid. The chromosome constitution of the sixteen BC_2 plants was determined with a combination of GISH and SSR markers.

A disomic 4H addition in a wheat background was detected by GISH from selfed BC_2 seeds originating from a 4H monosomic addition (*Figure 1C*). The spike structure of this line was similar to the spikes of the 4H 'Mv9kr1'/'Igri' disomic addition; but the 4H 'Asakaze komugi'/'Manas' addition had long awns at the top of the head (*Figure 1D*). This line had very good fertility (96, 113 and 261 seeds/plant).

Three other BC_2 plants contained monosomic additions of the 3H, 6H and 7H chromosomes. Disomic additions have not yet been found as the number of selfed seeds on these plants was very limited (10, 3 and 13). However, further plants with monosomic additions have been selected again using GISH in the next generation. A large number of seeds are already available on the 3H, 6H and 7H monosomic progenies, which will make it possible to select for disomic additions in the near future.

A 2H monosomic addition was found among the progenies of a BC_2 plant which carried three barley chromosomes (2H, 3H and 6H). The identification of these chromosomes was carried out with the help of the HvT01 and pTa71 probes in the next generation. The 2H monosomic addition had good fertility, so it should be possible to select the disomic 2H addition in the future.

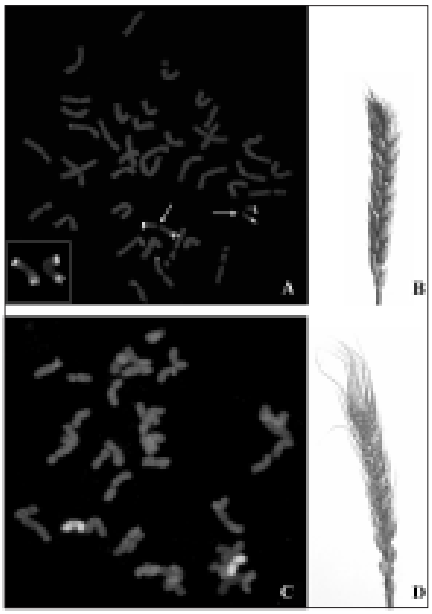

Figure 1A: **Identification of 'Mv9kr1'/'Igri' addition line 3H with FISH using HvT01 as a probe. The HvT01 probe hybridizes mainly to the barley chromosomes (arrows); no hybridization signals can be seen on the wheat chromosomes except on the long arm of wheat chromosome 4B. The pair of 3H chromosomes can identified by the three hybridization signals on the long arms.**
B: **Spike of the 'Mv9kr1'/'Igri' disomic addition line 3H.**
C: **Demonstration of the presence of a barley chromosome pair in the 'Asakaze komugi'/'Manas' disomic addition line 4H using GISH. The barley chromosomes show up brightly.**
D: **Spike of the 'Asakaze komugi'/'Manas' disomic addition line 4H.**

Conclusions

Combinations of four probes (HvT01-GAA, HvT01-pTa71, HvT01-Afa family) in FISH made it possible to identify all the barley chromosomes. One very advantageous feature of the repetitive DNA probe HvT01 is that it gives few, if any, weak hybridization signals on wheat chromosomes.

Monosomic and disomic alien addition lines could be helpful both for breeding and for basic research (GALE and MILLER 1987). The 'Mv9kr1'/'Igri' and 'Asakaze komugi'/'Manas' disomic addition lines and the translocation lines originating from these additions may provide important additional information on barley genetics, as the barley genotypes are of the winter type and carry different genes (e.g. vernalization, frost resistance) compared to the spring type Chinese Spring/Betzes addition lines reported previously. From the point of view of wheat breeding these addition lines are potential sources of abiotic stress resistance, particularly for drought and salt tolerance.

Acknowledgements

The technical assistance of Mrs. I. BUCSI and Mrs. J. HAVASI is gratefully acknowledged. Thanks are due to Dr. A. SCHNEIDER for her help in the microsatellite marker examinations and to Mrs. B. HARASZTOS for revising the manuscript linguistically. This work was

financed by the Hungarian National Scientific Research Fund (No. T 043502).

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