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Production and Molecular Cytogenetic Identification of Wheat-Barley Hybrids and Translocations

Márta Molnár-Láng*, Gabriella Linc, József Sutka

Department of Genetics, Agricultural Research Institute of the Hungarian Academy of Sciences, 2462, Martonvásár, P.O. Box, 19, Hungary(author for correspondence)*

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Abstract

New winter wheat winter barley hybrids were produced (Mv9 kr1 Igri, Mv9 kr1 Osnova, Asakazekomugi Manas). The wheat-barley hybrids showed entire male sterility and were multiplied in tissue culture. Chromosome configurations were studied with GISH in meiosis in the Mv9 kr1 x Igri hybrid and in its progenies multiplied *in vitro*. Chromosome pairing between wheat and barley has been observed in some cells in the hybrids multiplied *in vitro*. Backcross plants with 43 and 44 chromosomes were selected with the aim of developing new wheat-barley addition lines.

Wheat-barley translocations were demonstrated with GISH in backcross progenies originating from *in vitro* regenerated wheat (*Triticum aestivum* L. cv. Chinese Spring) x barley (*Hordeum vulgare* L. cv. Betzes) hybrids. Five different translocations were observed. Sequential N-banding and GISH analyses were performed to further identify the translocations. The N-banding pattern of the Robertsonian translocation suggests that this chromosome consists of the short arm of barley chromosome 4H translocated to the long arm of 2B of wheat. Plants with four different homozygous translocations were selected from the following BC2F3 generation.

Introduction

Bread wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) are two of the most important cereal crops worldwide. Hybridization between these species makes it possible to transfer desirable traits (e.g. earliness) from barley into wheat. The first successful hybridization was reported by Kruse [9] between wheat and barley and not much later a set of wheat-barley addition lines was produced [3]. However, very few new hybrid combinations have since been reported from wheat barley crosses [5, 16]. Homoeologous pairing between the chromosomes of these species is very rare, so only a small number of recombinants have been produced between wheat and barley [4]. Recently Koba et al. [8] reported wheat-barley translocations.

Genomic *in situ* hybridization (GISH), which utilizes total genomic DNA from one of the parental species as a probe, allows chromosomes of different parental origins to be "painted" in different colours in the nuclei of interspecific hybrids. GISH is the most efficient and most accurate technique for identifying the breakpoints and estimating the amount of alien chromatin in the translocation chromosomes [15, 7].

Our aim was to produce new winter wheat winter barley hybrids and to induce wheat-barley translocations lines via tissue culture with the aim of gene transfer from barley into wheat. The chromosome pairing in new winter wheat winter barley hybrids and mitotic chromosomes in backcross seeds originating from *in vitro* regenerated wheat barley hybrid plants were analysed with GISH to detect barley chromosomes and chromosome segments in a wheat background.

Table 1. Production of wheat-barley hybrids. Number of plants developed when wheat is pollinated with different barley varieties

Maternal partner, wheat variety	Pollinator, barley variety	No. of pollinated flowers	No. of embryos	No. of plants
Chinese Spring	Betzes	3232	14	6
Mv9 kr1	Igri	3012	3	1
Mv9 kr1	Osnova	254	1	1
Askazekomugi	Manas	676	2	1

Production of winter wheat-winter barley hybrids

Wheat barley crosses were carried out between several genotypes. Three wheat (*Triticum aestivum* L.) genotypes were used as the maternal plants: Chinese Spring, the Hungarian winter wheat line Martonvásári 9 kr1 (Mv9 kr1), which carries recessive kr1 and kr2 alleles [12] and the Japanese winter wheat variety Asakazekomugi. The barley (*Hordeum vulgare* L.) varieties tested for hybrid production were the following: Betzes, Martonvásári 50, Martonvásári 37, Martonvásári 38, Osnova, Manas, Igri, Kompolti korai, Attila, Botond, Kaliforniai Mariont, Duet, P-284-90, Intro, Swift, NRPB and Trasco. Pollination was carried out as described earlier [13].

Wheat-barley hybrids were produced with four barley (*Hordeum vulgare*) varities: Betzes (German spring barley variety), Igri (German winter barley variety), Osnova and Manas (Ukrainian winter barley varieties). Mv9 kr1 Igri, Mv9 kr1 Osnova and Asakazekomugi Manas hybrids were produced with the help of embryo culture (Table 1).

In situ hybridization using total barley DNA labelled with fluorescein-11-dUTP as the probe and wheat DNA as the blocking DNA was performed as described by Reader et al. [14]. Young anthers from the wheat barley hybrid Mv9 kr1 Igri and its progenies multiplied *in vitro* were used for making meiotic chromosome preparations for genomic *in situ* hybridization.

Slides were analysed by epifluorescence microscopy using a Zeiss Axioskop microscope. Images were captured by means of a Spot CCD camera (Diagnostic Instruments, Inc. USA) and processed using Image Pro Plus software.

The morphological and cytological analyses demonstrated the hybrid nature of the new combinations. Chromosome configurations were analysed in the pollen mother cells of the Mv 9 kr1 x Igri and the Asakazekomugi x Manas hybrids. In 120 cells an avarage of 25. 15 univalents, 1.00 bivalents (0.52 rods and 0.48 rings), 0. 025 trivalents and 0.008 quadrivalents and 1.51 telocentrics were observed in the hybrid Mv9 kr1 x Igri. The number of chiasmata per cell was 1.53, the average chromosome number being 28. In 75 cells an average of 25.21 univalents, 1.14 bivalents (0.69 rods and 0.45 rings) and 0.85 telocentrics were observed in the hybrid Asakazekomugi x Manas. The number of chiasmata per cell was 1.47, the average chromosome number being 28.

When the chromosome configurations in meiosis in the Mv9 kr1 x Igri hybrid were studied with GISH it was found that the barley chromosomes could be clearly distinguished from wheat. Mostly univalents were observed. The few bivalents were mostly formed between wheat chromosomes and only rarely between barley chromosomes. Chromosome pairing between wheat and barley has been observed in some cells in the hybrids multiplied *in vitro* (Figure 1). Misdivision was more typical of the barley chromosomes.

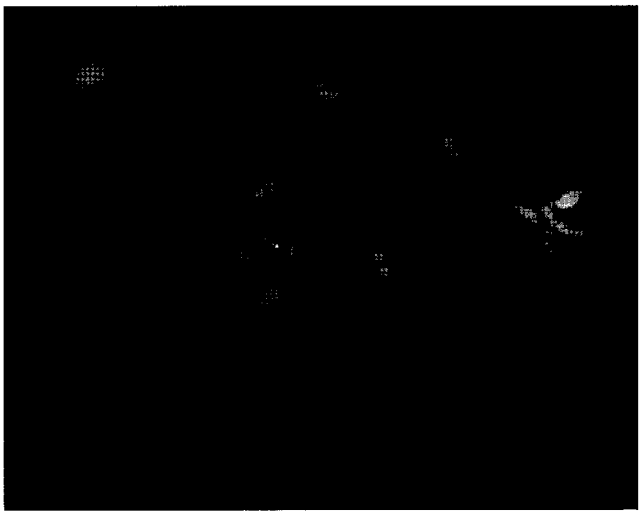

Figure 1. Genomic in situ hybridization pattern of meiotic metaphase chromosomes of the wheat (Triticum aestivum L. cv Mv9 k1) x barley (Hordeum vulgare L. cv Igri) hybrids multiplied in vitro. Bright yellowish-green colour marks the barley chromosomes, counterstaining was carried out with DAPI. A rod bivalent is formed between a wheat and a barley chromosome, marked with an arrow.

Table 2. Pollination of wheat-barley hybrids multiplied in vitro by wheat

Hybrid	Pollinator wheat variety	No. of pollinated flowers	No. of embryos	No. of plants
Mv9 kr1 × IgriR	Mv9 kr1	4606	9	6
Mv9 kr1 × IgriR	CO – 4	340	2	1
Asakazekomugi × ManaszR	Mv9 kr1	1280	3	1
Asakazekomugi × ManaszR	Asakazekomugi	3150	–	–
(Mv9 kr1 × Igri)R Mv9 kr1	Mv9 kr1	656	–	24
(Mv9 kr1 × Igri)R CO – 4	Mv9 kr1	354	–	1

The winter wheat- winter barley hybrids showed entire male sterility and were pollinated with wheat. No backcross seeds were developed, so the hybrids were multiplied in tissue culture. More than 100 regenerants were developed from the Mv9 kr1 Igri hybrid. The regenerated hybrids were backcrossed with the wheat line Mv9 kr1 and seven BC1 plants were developed. These were backcrossed again with wheat and plants with 43 and 44 chromosomes were selected among the BC2F1 plants with the aim of developing new wheat-barley addition lines (Table 2).

Production of wheat-barley translocations

Wheat (*Triticum aestivum* L. cv. Chinese Spring) barley (*Hordeum vulgare* L. cv. Betzes) hybrids previously produced in Martonvásár were multiplied in tissue culture according to the method described earlier [11, 13]. Altogether 384 Emasculated flowers on 12 regenerated hybrids were pollinated with the wheat line Mv 9 kr1. Two backcross plants (CS Betzes)RMv9 kr1 were developed. The BC1 plants were pollinated with the wheat line Mv9 kr1 and 47 BC2F1 plants were grown. Seeds developed after self-pollination on the (CS x Betzes)RMv9 kr12 BC 2F1 plants were used for genomic *in situ* hybridization.

Altogether fifty-one BC2F2 seeds were analysed by genomic *in situ* hybridization and wheat-barley translocations were detected in twelve progenies. Five different translocations were identified, which originated from different BC2 plants, except in two cases, where centric fusions were demonstrated in plants derived from two of the BC2 (Table 3). GISH analysis mapped the translocation breakpoints at fraction lengths of 0.5 and 0.8 with the distal segment being derived from barley in three terminal translocations. In one terminal translocation, with the translocation breakpoint at a fraction length of 0.5, the barley chromosome segment was derived from a SAT chromosome, either 5H or 6H (Figure 2). In one submetacentric chromosome the translocation breakpoint was in the short arm at a fraction length of about 0.2, the distal segment being derived from wheat and the rest of the chromosome from barley. The other arm of this chromosome was derived from a SAT barley chromosome.

Sequential N-banding and GISH analysis followed the protocol of Jiang and Gill [6] to further identify the translocation chromosomes. Three of the translocation

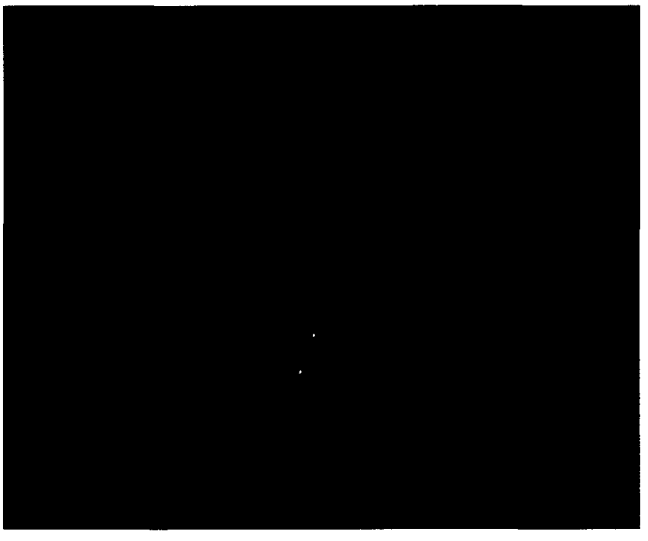

Figure 2. Genomic in situ hybridization pattern of mitotic metaphase chromosomes of backcross progenies originating from in vitro regenerated wheat x barley hybrids (CS x Betzes)RMv9 kr 12F3. Bright yellowish-green colour marks the barley chromosome segments, counterstaining was carried out with DAPI. Homozygous wheat-barley translocation and two barley chromosomes are present in a BC2F3 plant. About 50% of the long arm of an acrocentric chromosome was derived from a

SAT

BC2F1 plant No.[1]	No. of BC2F2 plants examined	No. of BC2F2 plants with alien translocations	Type of translocation (No. of plants)[2]
1/33	2	–	–
1/25	4	4	TT(4); TS(2)
1/31	1	–	–
1/5	2	–	–
1/8	5	–	–
1/28	1	–	–
2/2	4	–	–
2/4	5	3	TT*(3)
2/6	2	–	–
2/10	2	–	–
2/13	3	–	–
2/17	4	3	CF(3)
2/19	2	–	–
2/21	4	–	–
2/23	3	1	CF(1)
2/26	4	–	–
2/27	3	1	TT**(1)

Table 3. Number of plants carrying wheat-barley translocations among the [(Chinese Spring Betzes) Mv9 kr12] BC2F2 backcross progenies of an in vitro- multiplied wheat-barley hybrid

[1]BC2 plants Nos 1/5-33 originated from the 1st BC1 plant, and Nos 2/2-27 from the 2nd BC1 plants.

chromosomes lacked diagnostic N-bands and thus could not be identified. The N-banding pattern of the Robertsonian translocation suggests that this chromosome consists of the short arm of barley chromosome 4H translocated to the long Sequential N-banding and GISH analyses were performed to further identify the translocation chromosomes. Three of the translocation chromosomes lacked diagnostic N-bands and thus could not be identified. The N-banding pattern of the Robertsonian translocation suggests that this chromosome consists of the short arm of barley chromosome 4H translocated to the long arm of 2B of wheat.

Plants with four different homozygous wheat-barley translocations were selected from the following BC2F2 generation which set seeds after selfing.

It is assumed that the relatively high frequency of wheat-barley translocated chromosomes among the progenies of wheat-barley hybrids multiplied *in vitro* could be the result of the *in vitro* regeneration process, as it is a well known fact that tissue culture may result in chromosome breakages [1, 2, 10]. The Robertsonian translocations may be the result of the misdivision of univalent chromosomes in the hybrids and in the backcross progenies. It is planned to determine which wheat and barley chromosomes are involved in these translocations.

Acknowledgements

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