

SZENT ISTVÁN UNIVERSITY

Characterization of winter wheat ×
Triticum timopheevii hybrid progenies

Main points of the PhD thesis

Andrea Horváthné Uhrin

Martonvásár

2013

Postgraduate school

Name: Postgraduate School for Plant Science

Branch of science: Crop Production and Horticultural Sciences

Head: Prof. Lajos Helyes, DSc
Department of Horticulture and Technology
Szent István University

Supervisors: László Láng, DSc
Scientific Consultant
Head of Cereal Breeding Department

Márta Molnár-Láng, DSc
Scientific Consultant
Head of Plant Genetics Resources and Organic
Breeding Department

.....
Dr. Lajos Helyes
Head of Postgraduate School

.....
Dr. László Láng
Supervisor

.....
Dr. Márta Molnár-Láng
Supervisor

BACKGROUND AND AIMS

In Hungary the outstanding role played by winter wheat (*Triticum aestivum* ssp. *aestivum* L., $2n=6x=42$, AABBDD) in the human diet, the magnitude of the area on which it is grown and the quantities exported all give a clear reflection of the importance of this species in crop production. The quantity and quality of wheat production is greatly influenced by unfavourable environmental effects. The agronomic properties of wheat, particularly its resistance to various diseases, can be improved by using plant species with broader genetic diversity in breeding. One efficient way of increasing genetic variability is crossing with species related to wheat, in the course of which chromosomes from alien species are transferred into the recipient plant. These species-alien chromosomes may carry genes for useful agronomic traits, such as resistance to various biotic and abiotic stress factors. In addition to these favourable properties, however, they may also introduce negative traits into wheat, so the final aim of gene transfer is for the recipient cultivar to contain not the whole alien chromosome, but only the fragment containing the genes coding for the properties desired. It is important for the incorporated chromosome or chromosome segment to be able to compensate for the genes on the missing wheat chromosome or chromosome segment. This compensation can best be achieved in the case of homoeologous chromosomes. The pairing of homoeologous chromosomes, and thus the induction of intergenomic translocations, is inhibited by the *Ph1* locus on the 5B chromosome, which controls chromosome pairing. The breakage of the alien chromosome and the occurrence of translocations is only possible if the *Ph1* locus is inhibited or deleted. Chromosome-specific microsatellite markers can be used to localise the alien chromosome segment, and if the marker density is sufficiently great, the breakpoints can also be identified. Chromosome fragments originating from individual genomes can be detected using cytogenetic methods, with which chromosome rearrangements within the genome and the location of the breakpoints can also be analysed. The identification of the chromosomes belonging to the genome in question can also be performed using cytogenetic methods. In addition to molecular markers, biochemical markers such as the storage proteins characteristic of the given species can also be used to indicate the presence or absence of alien chromosomes. After the breakage of the alien chromosome, progeny plants carrying translocations of varying size and location can be analysed (e.g. by artificial inoculation with pathogens) to demonstrate that chromosome segments carrying genes coding for useful traits are present in the genome. In the next stages of breeding the aim is to achieve cytogenetic stability in translocation lines carrying the desired trait. Due to developments in

biotechnological methods, it is now possible to cross wheat with more distantly related species and to raise plants from these crosses. By means of crossing, followed by the development of amphiploids, useful genes can be transferred to wheat from any species having at least one genome homoeologous with wheat. *Triticum timopheevii* (Zhuk.) is known for its excellent disease resistance. Although it has been the subject of a relatively large volume of research, which has detected a number of resistance genes, it may well carry numerous other genes that could be exploited to improve the agronomic traits of cultivated wheat.

In the course of the work the aim was to develop wheat lines carrying the 6B.6G translocation and possessing leaf rust resistance derived from *T. timopheevii* without any of the undesirable agronomic and quality traits that may be introduced with the alien chromosome. For this purpose the following experiments were performed:

1. A search for microsatellite markers that are polymorphic for the 'AMP12' line carrying the 6G(6B) substitution, for the donor of the 6G chromosome, *T. timopheevii* and for the 6B chromosome of wheat.
2. The testing of polymorphic and non-polymorphic markers on 6B-nullisomic genotypes in order to determine their specificity.
3. The use of polymorphic microsatellite markers to identify possible rearrangements between chromosomes 6B and 6G in three consecutive generations of progeny from a cross performed between 'AMP12', found to carry the 6G(6B) substitution, and the Chinese Spring line CO4 in order to induce recombination between chromosomes 6B and 6G.
4. Identification of *T. timopheevii* chromosomes and the determination of their karyotypes using genomic and fluorescent *in situ* hybridisation.
5. Identification of the 6G chromosome of the 'AMP12' line with the help of FISH using repetitive DNA probes.
6. Analysis of progeny carrying the 6B.6G translocation using *in situ* hybridisation, and their comparison with progeny containing the 6G(6B) substitution or the 6B chromosome.
7. Analysis of progeny carrying the 6G(6B) substitution or translocation and of gene bank accessions of *T. timopheevii* by means of acidic polyacrylamide gel electrophoresis in order to identify the alien chromosome or chromosome segment.

8. Use of polymorphic microsatellite markers to analyse progeny plants originating from crosses between the ‘AMP 12’ line bearing the 6G(6B) substitution and a number of Martonvásár wheat cultivars.
9. Tests on the leaf rust resistance of ‘AMP12’, the control genotypes and the progeny of crosses between ‘AMP12’ (with or without the 6B.6G translocation) and Chinese Spring CO4 and between ‘AMP12’ and various Martonvásár wheat cultivars after artificial inoculation in the greenhouse or under field conditions.

MATERIALS AND METHODS

Plant material

1. The *T. timopheevii*/*T. aestivum* 6G(6B) substitution line (‘AMP12’): a 42-chromosome progeny resistant to powdery mildew and leaf rust, obtained by backcrossing the Fleischmann-481/*Triticum timopheevii* amphiploid with the wheat cultivars Mironovskaya-808 and Mv14.
2. *T. timopheevii* genotypes: a total of 12 *T. timopheevii* genotypes were used in the various experiments.
3. Progeny of 6G(6B) substitution line (‘AMP12’) × CO4 crosses: progeny plants in the F₃ to F₆ generations of crosses between the ‘AMP12’ line and a wheat line based on Chinese Spring but carrying the *Ph1* suppressor gene derived from *Ae. speltoides* (Chinese Spring CO4).
4. Progeny of crosses between ‘AMP12’ and various Martonvásár wheat cultivars.
5. 6B nulli-tetrasomic lines of Chinese Spring CO4.
6. Control plants: the parental lines of the ‘AMP12’ line (Mv14, Fleischmann-481, Mironovskaya-808, *T. timopheevii* accession TRI677, *T. timopheevii* accession MvGB573) and the cultivars Chinese Spring CO4, Mv Magdaléna, Mv Palotás, Mv27-2000, Mv Emese, Mv Csárdás and Mv Mezőföld were used in the various experiments

Testing of molecular markers, PCR reactions

A total of 42 microsatellite markers mapped by various authors to the wheat 6B chromosome were tested on *T. timopheevii*, ‘AMP12’ and the control wheat genotypes (Fig. 1).

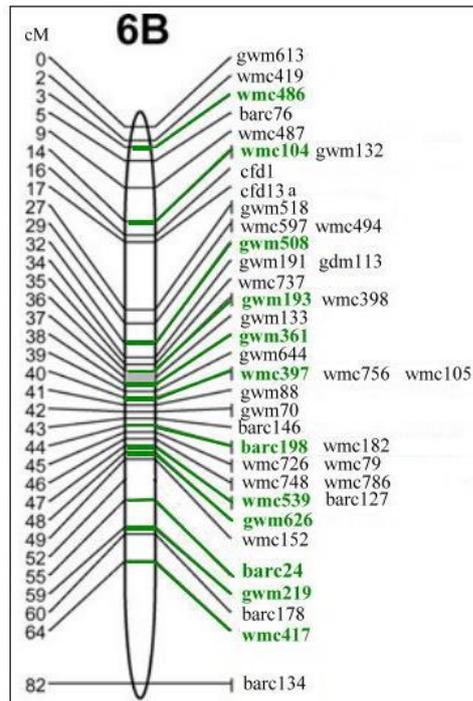


Fig. 1. Location of the microsatellite markers used in the analysis on the 6B chromosome

Cytogenetic methods

Slides were prepared from the root tips of germinating seeds for the cytological analysis. These were examined under a microscope and those with a satisfactory mitotic index were stored at -20°C until required for analysis.

Probe labelling

Total genomic DNA was isolated for *in situ* hybridisation from the leaves of young plants using phenol-chloroform extraction. For GISH, total genomic DNA was isolated from rye, *Ae. speltoides* and *T. urartu*. The DNA of rye ($2n=14$, RR) and *T. urartu* ($2n=14$, A^uA^u) was labelled with digoxigenin-11-dUTP using the random priming method, while that of *Ae. speltoides* Tausch. ($2n=14$, SS) was labelled with biotin-16-dUTP, again using the random priming method. The repetitive probes pSc119.2, Afa family, (GAA)₇ and pTa71 were applied for FISH. Depending on the combination, the probes were labelled with biotin-16-dUTP or digoxigenin-11-dUTP.

Fluorescent in situ hybridisation

Prior to hybridisation the selected slides were treated with RN-ase, digested in freshly prepared pepsin solution and then rinsed. The chromosomes were then post-fixed, after

which the slices were dehydrated in an ice-cold ethanol series with increasing concentrations. After denaturing, the hybridisation mixture containing the labelled probe and blocking DNA was hybridised overnight at 37°C. Following incubation with streptavidin-FITC and anti-digoxigenin-rhodamine, the slides were rinsed and counterstained with an anti-fade mixture containing DAPI.

Genomic in situ hybridisation

GISH was carried out on the *T. timopheevii* chromosome preparations after washing off the FISH hybridisation signals. FISH and GISH were also carried out on the ‘AMP12’ line. In the case of *T. timopheevii* the mixture contained 70 ng labelled A genome-specific probe (*T. urartu*) and 200× this concentration of blocking DNA (*Ae. speltoides*). For the ‘AMP12’ line whole rye (*Secale cereale* L.) genomic DNA was labelled with digoxigenin-11-dUTP. Genomic wheat (Mv9 kr1) DNA was used as blocking DNA at 35× the concentration of the labelled probe.

Analysis of storage protein (gliadin) using a one-dimensional A-PAGE method

During protein isolation the germ part of the wheat grains was excised, after which the part containing the endosperm was ground to a powder in a mortar. After adding the buffer the samples were loaded onto acrylamide gel and the gel was run in a vertical electrophoresis unit at 10°C for 10 min at 220 V, 30 mA and 10 W resistance, followed by 2 h 30 min at 550 V, 70 mA and 38 W resistance.

Artificial rust inoculation in the greenhouse

In the greenhouse experiments, artificial rust inoculation was performed with a uredospore suspension on selected genotypes from the F₆ and F₅ generations of the ‘AMP12’ × CO4 cross and on plants from the F₄ generation of the ‘AMP12’ × Mv27-2000, ‘AMP12’ × Mv Csárdás, ‘AMP12’ × Mv Magdaléna, ‘AMP12’ × Mv Emese, ‘AMP12’ × Mv Pálma and ‘AMP12’ × Mv9 crosses, proved by preliminary molecular marker analysis to contain chromosome 6G. The genotypes Chinese Spring CO4, ‘AMP12’, Mv14, Mv Emese, Mv27-2000, Mv Csárdás, Mv Pálma and Mv Magdaléna were used as controls. The inoculants required for the artificial inoculation was developed on susceptible plants of the winter wheat cultivar Alcedo. The analysis of leaf rust susceptibility was performed using a mixture of pathotypes. The population used for inoculation was avirulent to differentiating lines carrying the *Lr9* and *Lr19* genes, caused traces of infection on the *Lr24*, *Lr28* and

Lr29 near-isogenic lines of Thatcher and was virulent to 31 *Lr* genes or alleles (*Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3*, *Lr3bg*, *Lr3ka*, *Lr10*, *Lr11*, *Lr12*, *Lr13*, *Lr14a*, *Lr14b*, *Lr15*, *Lr16*, *Lr17*, *Lr18*, *Lr20*, *Lr21*, *Lr22*, *Lr23*, *Lr25*, *Lr26*, *Lr30*, *Lr32*, *Lr33*, *Lr34*, *Lr35*, *Lr37*, *Lr38*, *Lr44*).

Inoculation was carried out in two phenophases in two replications. In the first experiment young plants in the 2-leaf stage were inoculated, while in the second experiment artificial inoculation was performed on vernalised wheat plants in the Zadoks 13 stage of development. The lowest leaves were inoculated with the uredospore suspension, after which optimum conditions for fungal development were maintained for 48 h. The degree of infection was scored on the 12th and 20th days.

Natural rust infection in the field

The ‘AMP12’ line and numerous ‘AMP12’ × Chinese Spring CO4 progeny (in the F₄ and F₅ generations) were sown in the field so that their leaf rust resistance could be tested under natural conditions. A susceptible wheat genotype was sown immediately next to ‘AMP12’ and around the whole experimental block. The degree of infection was scored on a 0–4 scale (0 = resistant, 4 = completely susceptible)

RESULTS

Phenotypic characterisation

The spike of ‘AMP12’ has a characteristically spindle shape, is moderately dense and has short awn stubs on the upper spikelets. Among the parental wheat lines, Fleischmann-481 and the *T. timopheevii* genotype used in the cross had awned spikes. The progeny of the ‘AMP12’ × Chinese Spring CO4 had very variable spike types. Although the majority were awnless, like those of ‘AMP12’ or Chinese Spring CO4, some had awn stubs, awns, short awns or, in two cases, elongated husks.

Microsatellite marker analysis of polymorphism on chromosomes 6B and 6G

In order to detect polymorphism between chromosomes 6G and 6B, the 42 microsatellite markers were first tested on the Mv14, CO4, ‘AMP12’ and *T. timopheevii* genotypes. After optimising the PCR reactions, 12 of the 42 microsatellite markers (wmc486, wmc104, gwm508, gwm193, gwm361, wmc397, barc198, wmc539, gwm626, barc24, gwm219, wmc417) were found to be polymorphic for the wheat 6B and the *T. timopheevii* 6G chromosomes. Three of these markers (wmc486, wmc104, gwm508) were mapped to the short arm of the 6B chromosome, three (gwm193, gwm361, wmc397) to the

centromere region and six (barc198, wmc539, gwm626, barc24, gwm219, wmc417) to the long arm of the chromosome.

Testing of microsatellite markers on 6B nullisomic lines

The markers mapped to the 6B chromosome were then tested on 6B nullisomic lines to determine whether they were specific to the 6B chromosome. Nulli 6B–tetra 6D and nulli6B–tetra 6A Chinese spring lines in which the nullisomy was preliminary checked by means of *in situ* hybridisation were used in the study. The microsatellite markers which had proved to be polymorphic for the 6G and 6B chromosomes gave no products, or only non-specific products, on samples from the nullisomic lines, proving that they were specific for the 6B chromosome.

Microsatellite marker analysis of the progeny generations of the 6G(6B) substitution line ('AMP12') × CO4 cross

Crossing the 'AMP12' line with the Chinese Spring CO4 line carrying the *Ph1* suppressor gene facilitated recombination between the 6G chromosome of 'AMP12' and the 6B chromosome of Chinese Spring, and thus the induction of 6B.6G translocations. The experiments were begun on 51 progeny in the F₃ generation, which were sown and raised in the greenhouse. In order to map any possible translocations, the plants were then tested with microsatellite markers proved by preliminary analysis to be polymorphic. The F₄ progeny arising from these plants were also sown in the greenhouse and tested with polymorphic microsatellite markers to identify plants carrying translocations. Nine of the 165 wheat seeds sown were found to carry translocations, shown by analysis with SSR markers to be of three types (Table 1). In one type a segment from the 6B chromosome was translocated to the end of the long arm of chromosome 6G (samples A and B) and the markers showed bands characteristic of the 6G chromosome of *T. timopheevii*, while the wmc417 marker gave a signal characteristic of wheat. In samples C, D, I and E part of the short arm of 6G was observed to replace a similar segment on the 6B chromosome. For this type, markers wmc486, wmc104 and gwm508 showed a pattern typical of wheat 6B, while the other markers gave patterns characteristic of the *T. timopheevii* 6G chromosome. In the third type (samples F, G and H) a large segment from the long arm of 6B was translocated to the 6G chromosome (Table 1). For these samples, markers gwm219 and wmc417 amplified products characteristic of wheat chromosome 6B, while the others gave products specific to the 6G chromosome of *T. timopheevii*.

Table 1. Chromosome rearrangements mapped using polymorphic microsatellite markers on genotypes in the F₄ generation found to carry the 6B.6G translocation

G and B indicate the presence (or absence) of products of a size characteristic of the 6G chromosome of *T. timopheevii* and the 6B chromosome of wheat (Chinese Spring CO4), respectively.

| Genotípus | Rövid kar | | | Centroméra | | | Hosszú kar | | | | | |
|-----------|-----------|--------|--------|------------|--------|--------|------------|--------|--------|--------|--------|--------|
| | wmc486 | wmc104 | gwm508 | gwm193 | gwm361 | wmc397 | barc198 | wmc539 | gwm626 | barc24 | gwm219 | wmc417 |
| A | G | G | G | G | G | G | G | G | G | G | G | B |
| B | G | G | G | G | G | G | G | G | G | G | G | B |
| C | B | B | B | G | G | G | G | G | G | G | G | G |
| D | B | B | B | G | G | G | G | G | G | G | G | G |
| E | B | B | B | G | G | G | G | G | G | G | G | G |
| F | G | G | G | G | G | G | G | G | G | G | B | B |
| G | G | G | G | G | G | G | G | G | G | G | B | B |
| H | G | G | G | G | G | G | G | G | G | G | B | B |
| I | B | B | B | G | G | G | G | G | G | G | G | G |

Spikes were isolated from progeny of the F₄ generation found to carry translocations, after which each of the spike progeny was sown in the greenhouse, while the seeds of plants without translocations were sown in the field.

In the following generation, testing with microsatellite markers demonstrated that 40% of the progeny carried a translocation of some kind. In samples G and H the size of the translocated segment varied.

In the course of the analysis a total of 150 F₃ seeds from the crosses ‘AMP12’ × Mv Magdaléna, ‘AMP12’ × Mv Palotás, ‘AMP12’ × Mv27-2000, ‘AMP12’ × Mv Emese, ‘AMP12’ × Mv Csárdás and ‘AMP12’ × Mv Mezőföld were sown in the greenhouse. Microsatellite markers polymorphic for the 6B and 6G chromosomes were used to test the plants for the presence of the 6G(6B) substitution, which was detected in the ratios listed in Table 2.

Table 2. Appearance of the 6G(6B) substitution in the F₃ generation of crosses between the 6G(6B) substitution line ‘AMP12’ and a number of wheat cultivars

| Combination | Progenies carrying 6G(6B) substitution | Number of tested plants | Combination | Progenies carrying 6G(6B) substitution | Number of tested plants |
|---------------------|--|-------------------------|-----------------------|--|-------------------------|
| „AMP12”× Mv Csárdás | 5 | 30 | „AMP12”× Mv Pálma | 3 | 33 |
| „AMP12”× Mv27-2000 | 4 | 16 | „AMP12”× Mv Magdaléna | 7 | 28 |
| „AMP12”× Mv Emese | 3 | 15 | „AMP12”× Mv Mezőföld | 8 | 28 |

Cytogenetic characterisation of the 6G(6B) substitution line ‘AMP12’, the wheat cultivars Mv14 and Fleischmann-481, and T. timopheevii

The FISH karyotypes of the Fleischmann-481 and Mv14 cultivars were determined using the repetitive probes pSc119.2, Afa family and pTa71. Mv14 carries the 1BL.1RS translocation originating from rye. The *T. timopheevii* chromosomes were identified by performing FISH and GISH analysis on the same slides. It proved possible to identify the *T. timopheevii* chromosomes using the pSc119.2, Afa family and pTa71 probes. The 6G chromosome can be easily distinguished from the others on the basis of the strong pTa71 and pSc119.2 hybridisation signals. The pSc119.2 probe hybridised to the terminal part of the short and long arms of the 6G chromosome. The Afa family signals appeared on the subterminal part of the 6G chromosome satellite. The (GAA)₇ repetitive probe was also used for the precise determination of the *T. timopheevii* karyotype.

The ‘AMP12’ line carries the 6G chromosome of *T. timopheevii* in place of the 6B chromosome of wheat. The hybridisation pattern observed on the 6G chromosome was in agreement with the hybridisation signals appearing on the 6G chromosome of *T. timopheevii*. The pattern obtained on the 6G chromosome using the (GAA)₇ repetitive probe was very similar to the (GAA)₇ hybridisation signals recorded for the 6G chromosome of *T. timopheevii*.

In addition to the 6G(6B) substitution the ‘AMP12’ line also carries the 1BL.1RS translocation, which was identified with GISH.

Cytogenetic analysis of the progeny generations of the 6G(6B) substitution line ‘AMP12’ × CO4 cross

After testing the F₅ progeny of the ‘AMP12’ × CO4 crosses with molecular markers, the F₆ generation of plants carrying the translocation and of those without the translocation

(containing a complete 6G or 6B chromosome) were examined using FISH. Chinese Spring CO4, the *T. timopheevii* TRI677 genotype and the Mv14 cultivar were chosen as control genotypes. The FISH analysis was performed using the repetitive probes pSc119.2, Afa family, pTa71 and (GAA)₇.

In many of the samples differences were observed in the hybridisation patterns of the pSc119.2 probe on chromosomes thought to be 6G. Two pSc119.2 hybridisation signals can be seen terminally on the satellite part of the 6G chromosome of *T. timopheevii*, but these could not be detected in some of the samples. This terminal pSc119.2 signal, present in the hybridisation patterns of the majority of wheat varieties, is missing from the short arm of the 6B chromosome in the control Chinese Spring CO4 genotype. In ‘AMP12’ × CO4 samples where the pSc119.2 probe did not hybridise to the terminal part of the satellite it can thus be assumed that part of the short arm of the 6G chromosome was replaced by the 6B arm of Chinese Spring CO4. In samples with a translocation on the long arm, rather than on the short arm, the same pSc119.2 hybridisation signal was detected on the terminal part of the satellite as in *T. timopheevii*. The 1BL.1RS translocation present in 6B.6G and in ‘AMP12’ was also present, absent, or present in heterozygous form in the progeny after crossing with Chinese Spring CO4.

Comparison of analysis with cytogenetic methods and molecular markers

Among the F₆ progeny of the ‘AMP12’ × CO4 cross tested with cytogenetic methods, the FISH results were used to select those carrying the 6B.6G translocation or only carrying the 6G(6B) substitution or the 6B chromosome. After excising the roots required for FISH from the germinated seeds of these plants, the seedlings were grown for 14 days, after which the DNA was isolated. The 6B.6G translocation could be detected with FISH in plants where the translocation involved part of the short arm. In these plants, analysis using molecular markers confirmed the conclusions drawn from the FISH patterns.

Analysis of the gliadin fraction

Acidic polyacrylamide gel electrophoresis was performed to compare the storage protein patterns of ‘AMP12’, Chinese Spring CO4 and 25 ‘AMP12’ × CO4 progeny in the F₅ generation. In addition, the cultivars used to develop the ‘AMP12’ line (MIR808, Fleischmann-481) were also analysed, together with progeny of the ‘AMP12’ × Mv Csárdás and ‘AMP12’ × Mv27-2000 crosses found to carry the 6G(6B) substitution (a total of 38 samples). As the aim of the analysis was to detect patterns indicating substitutions or

translocations involving the 6B chromosome, the alpha/beta gliadin region was examined. It proved possible to identify the 6G chromosome substitution on the basis of the alpha/beta gliadin bands of samples known to carry the 6G(6B) substitution, but the 6B.6G translocation could not be identified in a one-dimensional acid urea gel.

Analysis of the leaf rust resistance of progeny carrying the translocation

F₆ progeny of the ‘AMP12’ × CO4 cross found to carry the 6B.6G translocation were examined in two phases of development: in older plants transferred to pots after a 6-week vernalisation period, and in young plants in the 2-leaf stage.

Artificial leaf rust inoculation in the greenhouse

Both resistant and susceptible plants were observed in the non-vernalised group. Scoring was carried out between the 12th and 20th days after inoculation. In both the replications the ‘AMP12’ line proved to be very resistant to leaf rust, while the Mv14 and Chinese Spring CO4 plants used as controls were found to be susceptible or moderately susceptible.

Out of 41, the majority of the 29 ‘AMP12’ × CO4 progeny that carried 6 types of 6B.6G translocation were very resistant or resistant to infection at the second scoring date (with scores of no more than 2), while 6 plants were moderately susceptible or susceptible. Among the three progeny carrying the 6G(6B) substitution, one was found to be susceptible, as were all the progeny carrying only the 6B chromosome.

The progeny of crosses between ‘AMP12’ and various Martonvásár cultivars were more resistant to rust infection than the control cultivars (Mv Csárdás, Mv Magdaléna, Mv Emese).

Scoring for leaf rust infection was also carried out between the 12th and 20th days in the vernalised group. The ‘AMP12’ line was found to be very resistant, while the *T. timopheevii* controls were completely immune. Mv14 and Chinese Spring CO4 were susceptible and moderately susceptible. Out of the total 42, eight of the 29 genotypes carrying the 6B.6G translocation were moderately susceptible or susceptible, while 19 were resistant or very resistant. Even at the first scoring date, more severe symptoms were observed on the susceptible genotypes. One of the ‘AMP12’ × CO4 progeny carrying the 6G chromosome was very susceptible, and all the lines where only the 6B chromosome was detected were more susceptible than the other progeny.

Natural infection in the field

Under field conditions the ‘AMP12’ line was resistant to leaf rust infection throughout its development, from tillering to waxy ripeness. Although a few uredospore colonies appeared on the lower leaves when the plants were close to maturity, no colonies were observed on the flag leaves except when the leaves were aging. The susceptible genotype planted next to the ‘AMP12’ line was strongly infected with leaf rust during the whole vegetation period.

The level of field resistance to leaf rust was examined in the F₄ generation of ‘AMP12’ × CO4 progeny carrying the 6G(6B) substitution. The majority of the progeny were more resistant than the parental wheat cultivars MIR808, Fleischmann-481 and Mv14, and many plants had resistance equal to that of ‘AMP12’.

NEW SCIENTIFIC RESULTS

1. Among the microsatellite markers mapped to the 6B chromosome of wheat, the convertibility of 42 was tested on the 6G chromosome of *T. timopheevii*. Twelve of the 42 markers were found to be polymorphic for chromosomes 6G and 6B and can thus be used to trace the presence of the 6G chromosome or chromosome segments in consecutive generations.
2. The *T. timopheevii* chromosomes were identified using the pSc119.2 probe and also with the repetitive probes Afa family and (GAA)₇. In the course of cyclic translocation, the Afa family probe was able to detect a segment of the 6A^t chromosome on the short arm of 4G.
3. The 6G and 6B chromosomes were successfully distinguished by means of FISH using the pSc119.2, Afa family and (GAA)₇ probes.
4. The 6G chromosome was also identified with the help of polymorphic molecular markers and FISH in the wheat genomic background.
5. Plants carrying the 6B.6G translocation were identified using polymorphic microsatellite markers from among the progeny of a cross between ‘AMP12’, a 6G(6B) substitution line carrying the 6G chromosome of *T. timopheevii*, and Chinese Spring CO4, which contains the *Ph1* suppressor gene.
6. FISH was applied to screen 6B.6G translocations for rearrangements involving the exchange of the short arms of 6B and 6G.

7. Greenhouse and field experiments on the leaf rust resistance of progeny carrying various types of 6B.6G translocations or the 6G(6B) substitution revealed a clear relationship between resistance and the presence of the 6G chromosome of *T. timopheevii*.

CONCLUSIONS AND RECOMMENDATIONS

1. The majority of the microsatellite markers mapped to the 6B chromosome of wheat could be converted to the 6G chromosome of *T. timopheevii*. Five of the 42 markers gave products of different sizes on the 6B and 6G chromosome. Together with a further seven markers present as null alleles, a total of 12 microsatellite markers were thus suitable for detecting the presence of the 6G chromosome of *T. timopheevii* or of segments of this chromosome in the wheat genomic background.
2. FISH can also be used to identify the 6G chromosome in the wheat background. Due to the homoeology between the B and G genomes it is difficult to detect the 6B.6G translocation by means of genomic *in situ* hybridisation. In progeny carrying a 6B.6G translocation where the satellite and part of the short arm of the 6G chromosome were exchanged for part of the 6B chromosome, the chromosome rearrangement could be identified using FISH. However, many progeny were heterozygous for the translocation, but this could not be detected with the dominant microsatellite markers. The joint application of microsatellite markers and FISH was thus required to distinguish which of the progeny with this type of translocation carried it in homozygous form. The separation of the gliadin storage proteins that resulted in specific bands in the course of acidic polyacrylamide gel electrophoresis only allowed the substitutions to be detected in the present work. If more precise results are to be achieved, allowing the detection of the translocation, the samples need to be run on a gel with better resolution or using plant material taken from the actual parental plants.
3. The *T. timopheevii* chromosomes can be identified using a combination of the repetitive probes pSc119.2, Afa family/(GAA)₇ and pTa79. Among the species-specific intergenomic translocations, only the incorporation of 6A¹ into the 4G chromosome could be detected with FISH. The possible presence of diverse chromosome rearrangements in *T. timopheevii* gene bank accessions exhibiting no polymorphism when analysed with microsatellite markers or FISH may be detectable using GISH.
4. Chinese Spring CO4 contains the suppressor of the Ph 1 gene that intensely regulates the pairing of homoeologous chromosomes, thus allowing these chromosomes to pair in the

progeny of crosses made with this genotype, resulting in the development of plants recombinant for the 6G-6B chromosomes. However, these progeny plants may also carry the suppressor gene, with the consequence that further chromosome rearrangements may arise. Backcrosses or the development of doubled haploid lines are thus necessary in translocation lines carrying useful genes to ensure that the translocation is stable in the progeny.

5. The 'AMP12' line proved to be resistant to leaf rust in the case of both artificial inoculation and field infection. A moderate level of infection was observed after heading. As the cultivars from which this line was bred (MIR808, Fleischmann-481, Mv14) were very susceptible under field conditions, it seemed likely that this improved resistance could be attributed to the 6G chromosome. This was backed up by the results published by other authors investigating the 6G chromosome of *Triticum timopheevii*. Progeny arising from crosses of this line with Chinese Spring CO4 had varying levels of leaf rust resistance or susceptibility, but were rarely as susceptible as the control cultivars. In experiments on artificial inoculation in the greenhouse, more than 70% of progeny carrying various sizes and types of 6B.6G translocations were resistant to the fungus, but the remaining 30% were susceptible or very susceptible. In the case of the progeny sown in the field experiment only the previous generation was analysed, and these plants may have been heterozygous with respect to the presence of the 6G chromosome. After crossing with the very susceptible genotype Chinese Spring CO4, other chromosomes may have been rearranged in addition to the 6G-6B rearrangement, which could have resulted in a decline in resistance in many cases. As phenotypic traits do not indicate which chromosome each plant carries, this can only be determined afterwards. It would be worth carrying out further tests on the disease resistance of progeny proved by means of FISH and microsatellite marker analysis to be homo- and heterozygous for the 6B.6G and 1BL.1RS translocations. The resistance of the 'AMP12' line could be caused by complex effects that have not yet been investigated. The supposedly unfavourable effects of Chinese Spring CO4 could perhaps be avoided by means of backcrossing or the development of doubled haploid lines. The breakage of the 6G chromosome and the subsequent development of 6G.6B translocations could also be induced using ionising radiation, but this method has the disadvantage that other rearrangements might also take place between other non-homoeologous chromosomes.