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Microsatellites confirm the authenticity of inter-varietal chromosome substitution lines of wheat (*Triticum aestivum* L.)

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Abstract Ninety-five wheat microsatellite markers (WMS) were used to verify the authenticity of the set of Saratovskaya 29/Yanetzkis Probat inter-varietal wheat chromosome substitution lines developed using Saratovskaya 29 as the recipient variety. Polymorphic markers were available for all chromosome arms except 4DS, 6DS and 7DS. Each chromosome substitution line was tested by 2–8 microsatellite markers. The results demonstrate that most of the lines are correct. Out of 21 lines tested 17 showed the expected microsatellite pattern of the donor variety. Two entire chromosomes, 1B and 7A, and two chromosome arms, 3AL and 6DS, were not substituted with Yanetzkis Probat in their respective lines. Three microsatellite markers located in the distal regions of chromosome arms 4AL, 3BS and 5BL in the corresponding substitution lines did not reveal the expected microsatellite pattern of the recipient variety. The possible causes of the incorrect substitution line development and the appearance of incorrect distal microsatellite markers are discussed. The data confirm the idea that microsatellite markers provide ideal tools for testing the authenticity of genetic stocks of wheat.

Keywords Wheat · Chromosome substitutions · Microsatellite markers

Introduction

Chromosome substitutions between wheat (*Triticum aestivum* L.) varieties provide an effective mean of analyzing the genetics of quantitative characters (Law and Worland 1973). They are widely used to study the influence of an individual chromosome on quantitative trait loci (QTLs) such as grain yield, bread-making quality, photoperiodic and vernalization response, plant height and disease resistance (Berke et al. 1992; Kato et al. 1999).

Credit is due to Sears (1953) and Kuspira and Unrau (1957) for the development of the first inter-varietal substitution lines of wheat. The task is laborious and time-consuming, requiring the prior creation of monosomic series. The majority of the first sets of chromosome substitutions were developed using Chinese Spring as recipient. However, Chinese Spring does not carry favorable characters, and it is therefore not used widely in agriculture. The production of inter-varietal substitution lines involving donor and recipient varieties with contrasting characters was needed to identify genes of agronomic importance. In 1966, the European Wheat Aneuploid Cooperative (EWAC) was established to coordinate the development of a wide range of genetic stocks of wheat and, thereby, to avoid duplications. Up to date about 45 monosomic series and 24 single-chromosome, inter-varietal substitution series have been developed (Börner et al. 1998). One set of substitution lines using Saratovskaya 29 and Yanetzkis Probat as recipient and donor, respectively, was produced in Novosibirsk, Russia. Spring wheat var. Saratovskaya 29 (S29) is widely used in agriculture and grows under various climatic conditions in Russia, Kazakhstan and the Baltic area. It is drought-resistant, weakly resistant to lodging and possesses other valuable technological properties. German var. Yanetzkis Probat (YP) is also a spring variety with contrasting qualities and increased grain protein content. This set of substitutions was efficiently used to estimate the genetic contribution of donor chromosomes to the manifestation of many productivity characters (Gaidalenok et al. 1995, Arbuzova et al. 1996).

Problems of ‘univalent shift’ and ‘univalent switch’ may arise during any one of the backcross stages of substitution development (Law and Worland 1973, 1996). Genetic, biochemical and molecular markers are helpful

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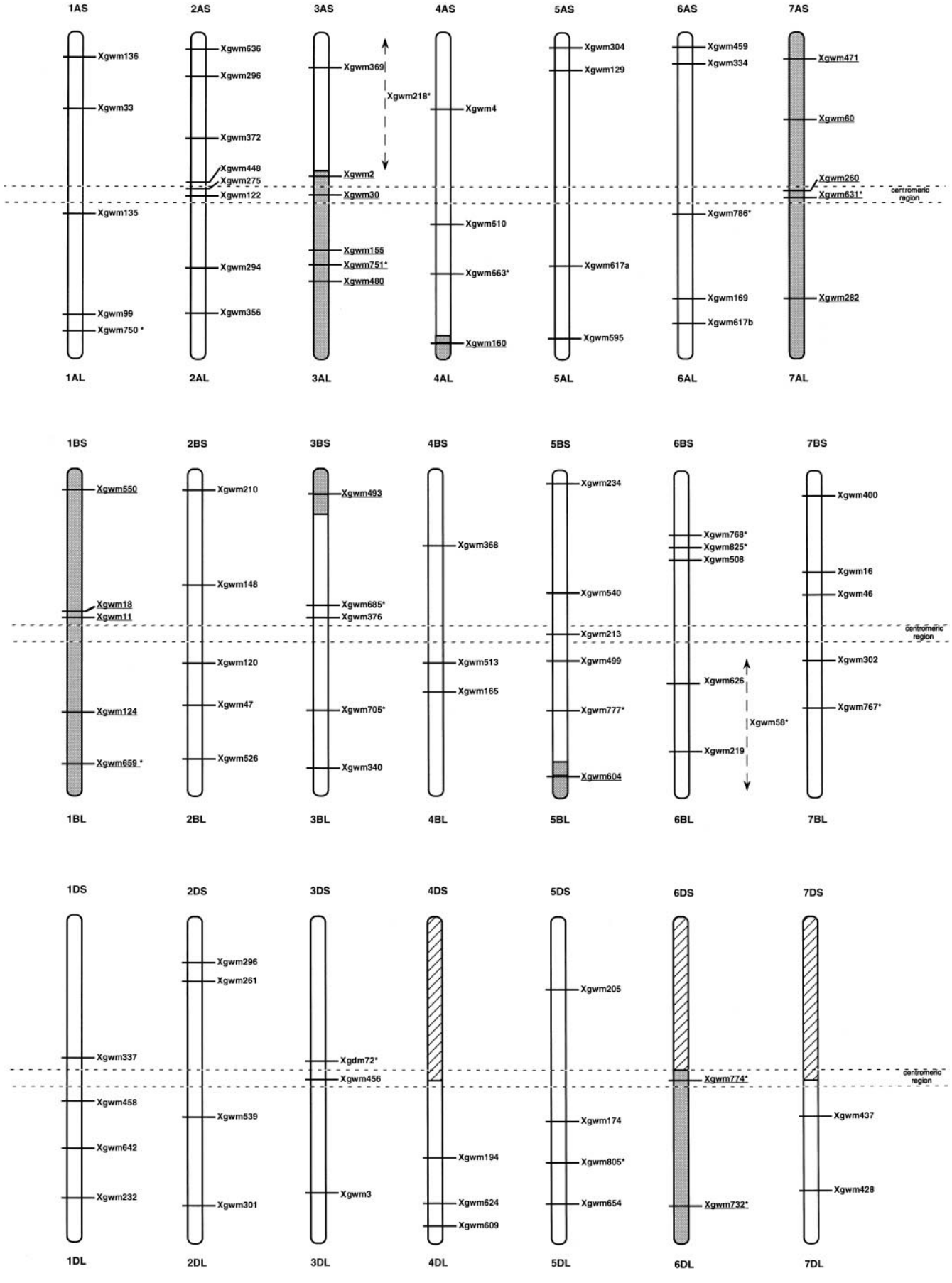
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Xgwm786*
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Xgwm617b
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Xgwm550
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Xgwm11
Xgwm124
Xgwm659 *
1BL
2BS
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Xgwm148
Xgwm120
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Xgwm526
2BL
3BS
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Xgwm340
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4BS
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Xgwm165
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Xgwm768*
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Xgwm219
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7BS
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centromeric region
1DS
Xgwm337
Xgwm458
Xgwm642
Xgwm232
1DL
2DS
Xgwm296
Xgwm261
Xgwm539
Xgwm301
2DL
3DS
Xgdm72*
Xgwm456
Xgwm3
3DL
4DS
Xgwm194
Xgwm624
Xgwm609
4DL
5DS
Xgwm205
Xgwm174
Xgwm805*
Xgwm654
5DL
6DS
Xgwm774*
Xgwm732*
6DL
7DS
Xgwm437
Xgwm428
7DL
centromeric region

in circumventing these problems. However, most of the available substitution lines have been developed before the advent of the versatile molecular and biochemical markers. Therefore, further tests of the authenticity of the cytogenetically developed substitution lines are needed.

The aim of the study reported here was to verify the authenticity of the Saratovskaya 29/Yanetzkis Probat (S29/YP) set of substitutions using microsatellites. To date, many microsatellite markers are available on each wheat chromosome (Röder et al. 1998a), and they reveal a high level of polymorphism between closely related wheat varieties (Plaschke et al. 1995). They have been applied to test the authenticity of a set of Capelle-Desprez/Bezostaya 1 substitution lines (Korzun et al. 1997).

Materials and methods

Plant material and DNA isolation

Intervarietal substitution lines S29/YP were obtained from Novosibirsk, Russia. A complete set of 21 substitution lines was developed using various aneuploids of wheat var. S29, which were developed by Maystrenko (1971).

Monotelocentrics of S29 were used for the development of substitution lines of chromosomes 1A, 1D, 3A, 3B, 3D, 5A, 5D, 6A, 6B, 6D, 7B and 7D. Substitutions of chromosome 2A, 2B, 2D, 4A, 4B and 4D were performed using monosomic lines of S29. Substitution lines 1B and 7 A were obtained using both monosomics and nullisomics of S29, and substitution line 5B was produced using monosomic and isosomic lines. The development of substitution lines from monosomics was accompanied by selfing between backcross generations to avoid switch. Disomics were extracted after eight backcrosses (Gaidalenok et al. 1995).

Total genomic DNA of each line was isolated from individual plants according to Anderson et al. (1992). Wheat var. Chinese Spring was also included in the analysis. DNA of Chinese Spring was extracted from whole seeds (Plaschke et al. 1995).

Microsatellite markers

The isolation of wheat microsatellite markers has been described by Röder et al. (1998a). DNA of S29, YP and Chinese Spring were amplified with 172 primer pairs representing wheat microsatellites (WMS). Ninety-five markers revealing polymorphism of the amplified loci between vars. S29 and YP were chosen for the analysis of substitution lines. The chromosomal location of the polymorphic markers are shown in Fig. 1. Of these, 77 microsatellite markers have been already published (Röder et al. 1998a), and 18 primer pairs (marked with asterisk) are unpublished (primer sequences are available upon request).

Polymerase chain reaction (PCR) and fragment analysis

PCR reactions and fragment detection were performed as described by Röder et al. (1998a). Fragment analysis was carried out on automated laser fluorescence (ALF) sequencer (Pharmacia), and fragment sizes were calculated using the computer program FRAGMENT MANAGER 1.2 (Pharmacia) by comparison with internal size standards.

Fig. 1 Schematic chromosomal location of polymorphic microsatellite markers based on the genetic microsatellite map of wheat (Röder 1998a). Seventy-seven microsatellite markers have been published by Röder et al. (1998a), and 18 primer pairs (marked with an *asterisk*) are unpublished (primer sequences are available upon request). Markers showing incorrect recipient fragments are *underlined*. □ Correct substitution, ■ incorrect substitution, ▨ no polymorphic markers available

Results

One hundred and seventy-two primer pairs were amplified with DNA of wheat vars. S29, YP and Chinese Spring to produce intervarietal polymorphism of amplifying fragments. In total, 95 microsatellite markers (55%) were polymorphic between S29 and YP, and these were used to test the authenticity of the substitution lines.

Polymorphic markers cover all of the 21 wheat chromosomes, and their number per chromosome varies from 2 on chromosomes 6D and 7D to 8 on chromosome 2A, with an average of 4.5 per chromosome. The size of the amplifying products was in the range of 70–320 bp, and the differences between the parental varieties varied from 2 bp to 46 bp. Markers are randomly distributed along the chromosomes and located on all of the chromosome arms except 4DS, 6DS and 7DS. The chromosomal distribution of polymorphic microsatellite markers is shown in Fig. 1.

Of the 21 substitution lines tested, 12 (1A, 1D, 2A, 2B, 2D, 3D, 4B, 5A, 5D, 6A, 6B, 7B) showed the expected microsatellite pattern of YP for both chromosome arms. Only the long arms of substituted chromosomes 4D and 7D were tested, and they were also found to be authentic.

Three substituted chromosomes, 4A, 5B and 3B, gave the correct donor varietal patterns for 3–5 markers per chromosome, while they gave the incorrect recipient varietal patterns for 1 marker per chromosome (Fig. 1). These incorrect markers have been located in the distal region of the chromosomes (Röder et al. 1998a).

Two substitution lines, 1B and 7A, showed the same size of amplifying products as recipient var. S29. Both these lines were analyzed using 5 markers. Thus, there was reason for concluding that a ‘shift’ or ‘switch’ had occurred during the development of the lines. The same conclusion was made for chromosome arm 6DL tested by 2 markers.

Four markers located on the long arm of the substituted chromosome 3A demonstrated the incorrect substitution, while 2 markers of 3 on the short arm gave the correct donor varietal pattern (Fig. 1). This chromosome seems to have resulted from recombination in the proximal region of the short arm during the development of the substitution line.

Discussion

Maystrenko et al. (1988) observed ‘switch’ and ‘shift’ during the development of S29 monosomics at a frequency of 2 in 21 lines (9.5%). The highest level of ‘shift’, 30%, has been observed by Röbbelen (1968) dur-

ing the production of a monosomic set in var. Wachtel. In other monosomic sets, 'shift' has occurred at a lower frequency. Thus, four 'univalent shifts' have occurred during the development of Capelle-Desprez monosomics and two during the development of the 21 substitution lines into Bersee after eight generations of backcrossing (Law and Worland 1973). Law and Worland (1973) calculated the 'switch' and 'shift' probability for substitution lines based on the data obtained for the Bersee monosomics, where the total 'switch' and 'shift' frequency is 2.9%. They estimated that 1–5 of the 21 substitutions into this variety may be incorrect. A higher univalent replacement probability during the development of S29/YP substitution lines based on the higher frequency of 'switch' and 'shift' observed in S29 monosomics (9.5%) may be expected.

Tests on a set of S29/YP substitution lines by microsatellite markers demonstrated that most substitutions in the set were correct. Microsatellites also showed that two entire chromosomes and two chromosome arms were substituted incorrectly. This level of 'switch' and 'shift' could be expected. Incorrect substitution chromosomes 1B and 7A were obtained using monosomic and then nullisomic lines of S29. The development of substitution lines using monosomics in the absence of cytological and genetical markers requires selfing between each backcross generation and, hence, takes double the time for the production of the line. Under these conditions, a 'switch' cannot occur, but the probability of misclassification errors increases. However, substitution lines 3A and 6D were produced using monotelocentric lines of S29 which were readily recognized in both mitotic and meiotic cell preparations. The obtained results indicate that cytological markers can not exclude completely errors in the development of a substitution line. Incorrect cytological counting as well as taking pollen from the wrong parent and a low-frequency but unavoidable 'univalent shift' in the male parent could result in the development of incorrect substitution lines. In the case of incorrect substitutions, the previous backcross generation should be tested for the presence of the donor chromosomes.

Three substituted chromosomes (4A, 3B and 5B) were found to carry several markers specific to the donor chromosome and one marker specific to the recipient chromosome. The occurrence of recipient sequences on the donor chromosome may be explained by nonhomologous recombination or their transfer together with transposable elements. These events are usually observed at a low frequency. All 'incorrect' markers occur in the distal region of the chromosomes composed of the restriction fragment length polymorphism (RFLP) markers on the genetic map (Röder et al. 1998a). As a result of suppressed recombination in the centromeric regions and increased recombination in the telomeric regions, microsatellite markers localize more distally in the physical map than in the genetic map (Röder et al. 1998b). Therefore, it is possible that the incorrect fragments localize in the telomeric region of the chromosomes in the regions enriched with repeated sequences. Telomeric arrays have been found to be more variable than any other known region of the plant genome (Broun et al. 1992; Pestsova et al. 1998). The frequency of genomic changes such as nonhomologous recombination may be increased in such regions. This possibility requires further studies.

Another explanation for the occurrence of fragments of recipient DNA on the donor chromosome may be reciprocal translocations distinguishing the parental genomes. No large reciprocal translocations were observed during the development of the set of S29/YP substitution lines. Small translocations that were difficult to discern cytologically might have given rise to a recombinant substituted chromosome in the substitution line produced.

The obtained data confirm the idea that microsatellite markers add power to the geneticist's toolkit in tests of the authenticity of genetic stocks.

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