Development of inter-varietal chromosome substitution lines of wheat (*Triticum aestivum* L.) bearing a rye genetic marker and their verification with microsatellite markers

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Abstract

Two monosomic alien substitution lines (MAS lines, 2n=41=40+5R) of wheat *Triticum aestivum* L. cv. 'Saratovskaya 29' were used as recipients in the development of inter-varietal substitution lines for chromosomes 5A and 5D. In the MAS lines, chromosomes 5A or 5D of 'Saratovskaya 29' were replaced by homoeologous univalent chromosome 5R of rye *Secale cereale* L. cv. 'Onokhoiskaya', which bears the *Hp* marker gene coding for hairy peduncle. The donors included 18 spring and winter wheat varieties. The MAS lines were developed by crossing monosomic lines of 'Saratovskaya 29' for chromosomes 5A and 5D to a wheat–rye substitution line of 'Saratovskaya 29' 5R(5D) followed by cytological and morphological selection of plants with chromosome configuration 20II +5RI in metaphase I of pollen mother cells from F_1 and F_2 plants with slightly hairy peduncles. It was shown that MAS lines could be maintained during long-term propagation (18 generations). Use of MAS lines with the *Hp* marker gene allows acceleration and abbreviation of cytological analysis and elimination of the probability of 'univalent switch' in the course of the development of substitution lines. The method was applied to the development of 22 'Saratovskaya 29' lines with inter-varietal substitution for chromosomes 5A and 5D. Fourteen and thirteen microsatellite markers located in chromosomes 5A and 5D, respectively, were used to prove the authenticity of the inter-varietal substitution lines. According to these markers, 21 substitution lines from 22 studied were correct.

Key words: *Triticum aestivum* L. — *Secale cereale* L.— monosomic alien substitution lines — chromosome substitutions — microsatellite markers — genetic markers — *Hp* gene.

Inter-varietal chromosome substitution lines of wheat *Triticum aestivum* L. are applied to the genetic analysis of quantitative traits in wheat (Law and Worland 1973, Law et al. 1978). The common method of developing such lines involves monosomic or monotelosomic lines of the recipient variety as the recurrent parent. By now, monosomic line sets have been developed for more than 40 common wheat varieties (Worland 1988). However, with the use of monosomic lines, the substitution of a specified recipient chromosome by the homologous donor chromosome can be disturbed by either replacement of one chromosome by another chromosome of the 21-chromosome set ('univalent shift') or by a switch from the donor chromosome to the recipient chromosome ('univalent switch') (Person 1956). These deviations bring about either a line identical to the recipient or a substitution line with a recombined chromosome. These consequences can be avoided by using monosomic lines tagged with genetic markers. Unfortunately, few common wheat genetic markers are available. Marker genes can be provided by closely related and wild cereal species. For example, rye (*Secale cereale* L.) is known to have a species-related character, hairy peduncle, which is controlled by genes located in the long arm of chromosome 5RL (*Hp* or *Ha2*), as well as in chromosomes 6R(*Ha3*) and 3R(*Ha1*) (Chang 1975, Schlegel et al. 1986, Schlegel et al. 1998, Korzun et al. 1996). There is still little experience in using alien marker genes for inter-varietal chromosome substitution.

The correct replacement of the recipient chromosome by the donor chromosome in substitution lines should be verified. Nowadays, molecular markers can be used for this purpose. This was efficiently demonstrated in many cereal species. Molecular markers (RAPD, RFLP, and STS) are used for mapping new genes and QTL, as well as for genotyping and certification of currently existing cereal varieties. Microsatellites, or SSR markers, represent one of the most promising molecular marker types for common wheat. It has been shown that microsatellites are more

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polymorphic and, consequently, more informative than RFLP-based markers in analyzing wheat varieties and reconstructed genomes (Prasad et al. 2000, Huang et al. 2003). Three complete sets of common wheat substitution lines ('Cappelle-Desprez'/ 'Bezostaya 1', 'Chinese Spring'/ 'Synthetic', and 'Saratovskaya 29'/'Yanetzkis Probat') were identified and characterized by microsatellite analysis (Korzun et al. 1997; Pestsova et al. 2000a; Salina et al. 2003).

This paper presents the results of using monosomic alien substitution lines (MAS lines) harbouring rye chromosome 5R with the *Hp* gene for developing wheat lines with inter-varietal substitution of chromosomes of homologous group 5. The correct replacement of wheat chromosomes was verified by using microsatellite markers.

Materials and Methods

Plant material: Twelve lines of wheat cv. 'Saratovskaya 29' ('S29') with inter-varietal substitution of chromosome 5A and ten substitution lines for chromosome 5D were obtained. The following spring wheat varieties were used as chromosome 5A donors: 'Novosibirskaya 67', 'Grekum 114', 'Yanetzkis Probat', 'Atlas 66', 'Skala', 'Bezenchukskaya 98', 'Buryatskaya 34', 'Kzyl Bas', 'Kzyl Shark', 'Kometa', 'Sonora 64' and 'Siete Cerros 66'. The chromosome 5D donors included spring varieties 'Novosibirskaya 67', 'Grekum 114', 'Yanetzkis Probat', 'Atlas 66', 'Hybrid 21', 'Diamant' and 'Chinese Spring' and winter varieties 'Ul'yanovka', 'Mironovskaya 808' and 'Skorospelka 35'. For the first time, two MAS lines of 'S29' with rye chromosome 5R (2n=41=40+5R) developed by Prof. O.I. Maystrenko (unpublished data) were used as recipients. In the MAS lines, chromosome 5A or 5D of 'S29' was replaced by homoeologous chromosome 5R from rye cv. 'Onokhoiskaya', bearing the marker gene for hairy peduncle (*Hp*). Meiotic chromosome configuration was examined at metaphase I (MI) in pollen mother cells (PMCs) using the 2% acetocarmine smear method. The 'S29'5R(5D) wheat–rye line, developed by Shchapova and Kravtsova (1982), was used as the donor of chromosome 5R. After eight backcrosses, monosomic plants were selfed in each substitution line and disomic plants were isolated. 'Univalent shift' was checked with 5AL and 5DL ditelosomic lines of 'S29'.

Development of monosomic alien substitution lines with rye chromosome 5R: To develop the 'S29'5R(5A) MAS line, an cv.'S29' monosomic for chromosome 5A was crossed to the 'S29'5R(5D) alien substitution line. A triple monosomic with 19**II** + 1(5R)**I** + 1(5A)**I** + 1(5D)**I** in MI of PMCs was selected from the F_1 hybrid plants and selfed. Traits controlled by genes in rye chromosome 5R (*Hp*) and wheat chromosome 5A (*Q*, speltoid-ear inhibitor and *B1*, inhibitor of the awned condition) were used to isolate monosomics with 20 bivalents and one 5R univalent having speltoid and awned ears (absence of 5A) and hairy peduncle (presence of 5R) from F_2. In subsequent experiments, these plants gave rise to a MAS line in which one chromosome 5R of cv. 'Onokhoiskaya' rye was substituted for chromosome pair 5A of cv. 'S29' wheat.

The 'S29'5R(5D) MAS line was obtained by simple crossing of cv.'S29'mono5D with the 'S29'5R(5D) disomic alien substitution line followed by cytological and morphological selection of F_1 plants having 20 bivalents and one univalent for 5R in MI of PMCs together with hairy peduncle. The monosomic plants had less hairy peduncles than the male line.

DNA isolation and microsatellite analysis: Total genomic DNA of each substitution line was isolated from 5-day old seedlings according to Plaschke et al. (1995). For microsatellite analysis, 14 and 13 markers located in chromosomes 5A and 5D, respectively, were chosen. Primer sequences, fragment sizes in the 'Opata' and 'Synthetic' genotypes and the chromosome arm locations of microsatellite markers were reported by Röder et al. (1998, unpublished results) and Pestsova et al. (2000b). Polymerase chain reaction (PCR) was performed as described by Röder et al. (1995). Amplified fragments were analyzed in an automated laser fluorescence (ALF) sequencer (Pharmacia); fragment sizes were calculated by using the computer program Fragment Manager Version 1.2 and by comparison with internal size standards.

Results

Cytological and morphological analysis of monosomic alien substitution lines

In the MAS lines of cv. 'S29' wheat, a pair of chromosomes 5A or 5D was replaced by a homoeologous univalent 5R of cv.'Onokhoiskaya' rye. The monosomic plants had 20 wheat chromosome pairs and one alien rye chromosome. The MAS plants were viable. They showed good tillering and formed large ears with large seedsets. The 'S29'5R(5A) and 'S29'5R(5D) MAS lines were actually nullisomics for wheat chromosomes 5A or 5D. Wheat nullisomics 5A and 5D are sterile; therefore, a single chromosome 5R can make up for the absence of the homoeologous wheat chromosome and restore the fertility of nullisomics for these chromosomes. Selfing of alien monosomics gave rise to di-, mono-, and nullisomic plants. The monosomic and disomic plants can be distinguished according to the expression of the *Hp* rye marker gene. Observation of 18 selfing generations of the MAS lines and cytological examination of MI in PMCs revealed clear differences between all aneuploid types. The 20II + 2(5R)II disomics had hairier peduncles (two *Hp* doses) than the 20II + 1(5R)I monosomics (one *Hp* dose), whereas the peduncles of the nullisomics (20II) were not hairy at all. Peduncle hairiness varied in the monosomic plants from sparse to occasional hairs. The nullisomic plants grew slower. They were weak and sterile.

Like 5A monosomics, monosomic and disomic plants of the 'S29'5R(5A) MAS line had speltoid ears. It is conjectured that rye chromosome 5R bears a gene suppressing the speltoid condition, but it is weaker than the wheat *Q* gene (Bielig and Driscoll 1970). Moreover, the absence of the dominant inhibitor of the awned condition *B1* from cv.'S29' as a result of 5A nullisomy brought about long awns throughout the ear in monosomic and disomic plants. Plants of line 'S29'5R(5D) had awnless ears.

Cytological analysis showed a high misdivision frequency of univalent rye chromosome 5R. Of 70 F_3-F_{18} examined plants of two MAS lines, 18% had telocentric chromosomes 5R (tI), 2% had telocentric bivalents (tII), and 2% had isochromosomes 5R. It was shown that the formation of the telocentric chromosome for the long arm of chromosome 5RL did not affect the manifestation of the hairy peduncle trait, because the *Hp* gene was located just in the long arm. The 5RL monotelocentric was fertile, and it could be used as a recipient in inter-varietal chromosome substitution programme.

Long-term selfing (18 generations) of the MAS lines showed that the 5R(5D) substitution decreased viability and fertility. Of the total of 440 sown seeds of the corresponding line, 12% did not germinate and 20% of seedlings died at the 1–2 leaf stage. Of 400 sown mono5R(5A) seeds, 3% did not germinate and less than 6% of seedlings died.

Use of the rye genetic marker for developing wheat substitution lines

Substitution lines were developed by an unusual method, because MAS lines with rye chromosome 5R were used as the recipient instead of monosomic wheat lines. This allowed selection of female and male monosomic plants according to the expression of the *Hp* gene. Figure 1 presents the expected aneuploid types arising during multiple backcrossing in the course of the development of lines with inter-varietal replacement of chromosomes 5A and 5D. Female monosomic plants were selected according to the phenotype with weak peduncle hairiness, resulting from the dose effect of the *Hp* gene. Chromosomes 5A or 5D were inherited from donor varieties. During backcrossing, they were always univalent, having no homologue. Male plants with hairless peduncles (monosomics with the donor chromosome, (20II + 1I) were selected from F_1 and crossed with monosomic plants of the corresponding MAS lines. The mixed backcross progeny consisted of 5A or 5D monosomics (20II + 1I), 5R monosomics (20II + 5RI), and disomics with one wheat and one rye chromosomes (20II + 1I + 1-5RI). From this progeny, plants with rye chromosome 5R were discarded according to the hairy peduncle trait, which corresponded to the *Hp* marker gene. From BC_1–BC_8, monosomics for the wheat donor chromosome without hairy peduncles were selected as male plants. Nullisomics were most often inviable and sterile.

After backcrossing, we performed a test for 'univalent shift'. Cytological analysis of F_1 hybrids between monosomic plants of BC_8 bearing donor chromosome 5A or 5D and ditelosomic 'S29' lines for chromosomes 5AL

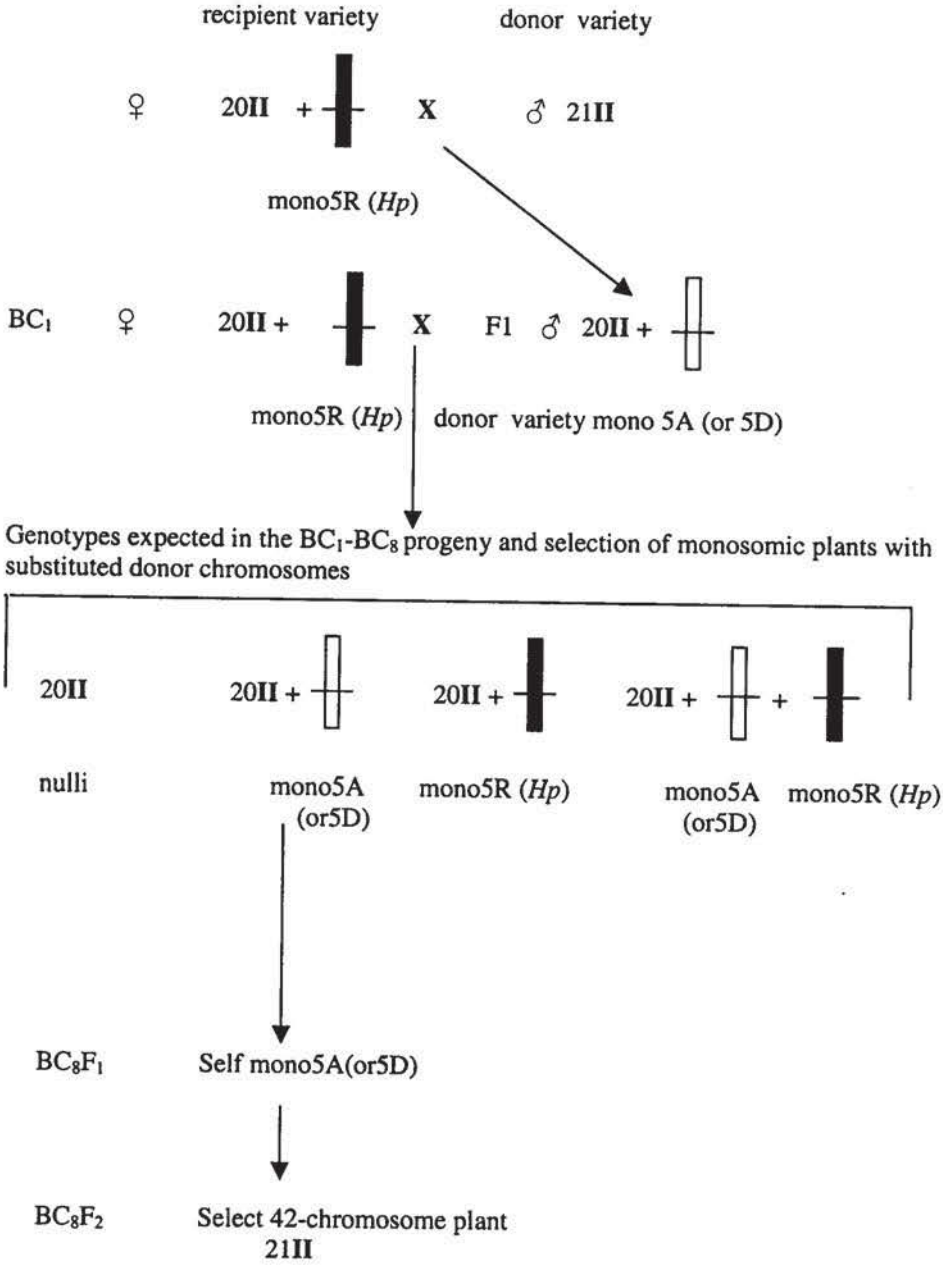

Figure. 1: Scheme of development of wheat lines with inter-varietal chromosome substitution with the use of MAS lines tagged with the rye *Hp* gene. The recipient variety is 'Saratovskaya 29'. Black bars indicate rye chromosome 5R; open bars, wheat chromosomes.

and 5DL showed the absence of univalent replacement from each line. The F_1 hybrids had the chromosome composition 20''+t' in MI of PMCs.

Microsatellite analysis

The correct replacement was checked by the use of microsatellite markers with known map location on wheat chromosomes 5A and 5D. Study of inter-varietal polymorphism in 18 parent varieties showed that the number of polymorphic markers varied from 4 to 14 in chromosome 5A and from 3 to 9 in 5D. In most parental varieties, polymorphic markers were evenly distributed over chromosomes and located in both arms (Figure 2).

Correct replacement was confirmed for all the 12 lines with substitution for chromosome 5A, as well as for the 10 lines (of cv. 'S29'-delete) with substitution for chromosome 5D. However, check of the line obtained from cv. 'Novosibirskaya 67' wheat showed that eight markers located in the long arm were not polymorphic. Thus, it remained unknown whether the whole chromosome 5D was replaced or only its short arm.

Discussion

The conventional method of developing wheat substitution lines involves monosomic lines as recipients. It requires comprehensive cytological examination of a great number of samples, including chromosome count, because monosomics, owing to random univalent segregation, produce gametes with different chromosome numbers (Sears 1954). The preservation of monosomic lines is very laborious, and the more so is their use for the development of substitution lines.

Use of cytologically tagged monotelosomic lines of a recipient variety allows tracing of correct donor univalent chromosomes. This cuts the time required for inter-varietal chromosome substitution and facilitates cytological studies (Law and Worland 1973).

To elaborate simple and precise methods of identification of common wheat chromosomes, it is important to find genes suitable as markers for certain chromosomes. Moreover, use of genetic markers permits scientists to minimize the volume of cytological analysis required for developing substitution lines or dispense with it at all. In doing so, it is essential that the recipient variety should have such markers for the majority of chromosomes. As there are still no common wheat varieties with genetic or cytological markers for each chromosome, it is necessary to look for genes controlling morphological traits, which would be suitable as markers for recognition of monosomic plants. Several major morphological genes are known in species of the genus *Triticum*: *s* (sphaerococcoid), *q* (speltoid ear type), *C* (compact spike), *bh* (branch spike) and *P* (long glume). They alter the phenotype of the whole plant and the ear dramatically, which allows their use as genetic markers for certain chromosomes (McIntosh et al. 1998). The locations of these genes in chromosomes and the modes of their inheritance are known. However, in order to use genetic markers for chromosome substitution, a complete monosomic set should be obtained, in which a univalent chromosome should bear a dominant marker trait. Such a monosomic would be a perfect recipient form for chromosome substitution (Law and Worland 1973). At present, a few monosomic sets bearing morphological marker genes are available (Tsujimoto 2001). Scientists of the Institute of Cytology and Genetics (Novosibirsk, Russia) obtained a monosomic line of cv. 'S29' wheat for chromosome 7A having the dominant *P(Eg)* gene for long awns, introduced from *T. polonicum* L., and monosomics for chromosomes 3D, 3B, and 3A, tagged with the *S1*, *S2*, and *S3* genes, controlling the sphaerococcoid trait (Arbuzova et al. 1996, Arbuzova et al. 1998). Unfortunately, there are few morphological markers in wheat.

Another opportunity of facilitating the tracing of a univalent chromosome is provided by alien marker genes from taxonomically distant species (Anderson and Driscoll 1967). The best genetic markers are alien genes that follow the simple Mendelian laws of inheritance, clearly manifest themselves in the common wheat genotype, and produce no cytological deviations when introduced into the recipient variety. In addition, it is desirable that a single gene should tag different wheat chromosomes. This approach can be implemented with the *Hp* marker gene from rye (*S. cereale*), because it allows developing numerous lines with rye chromosome 5R substituted for different wheat chromosomes

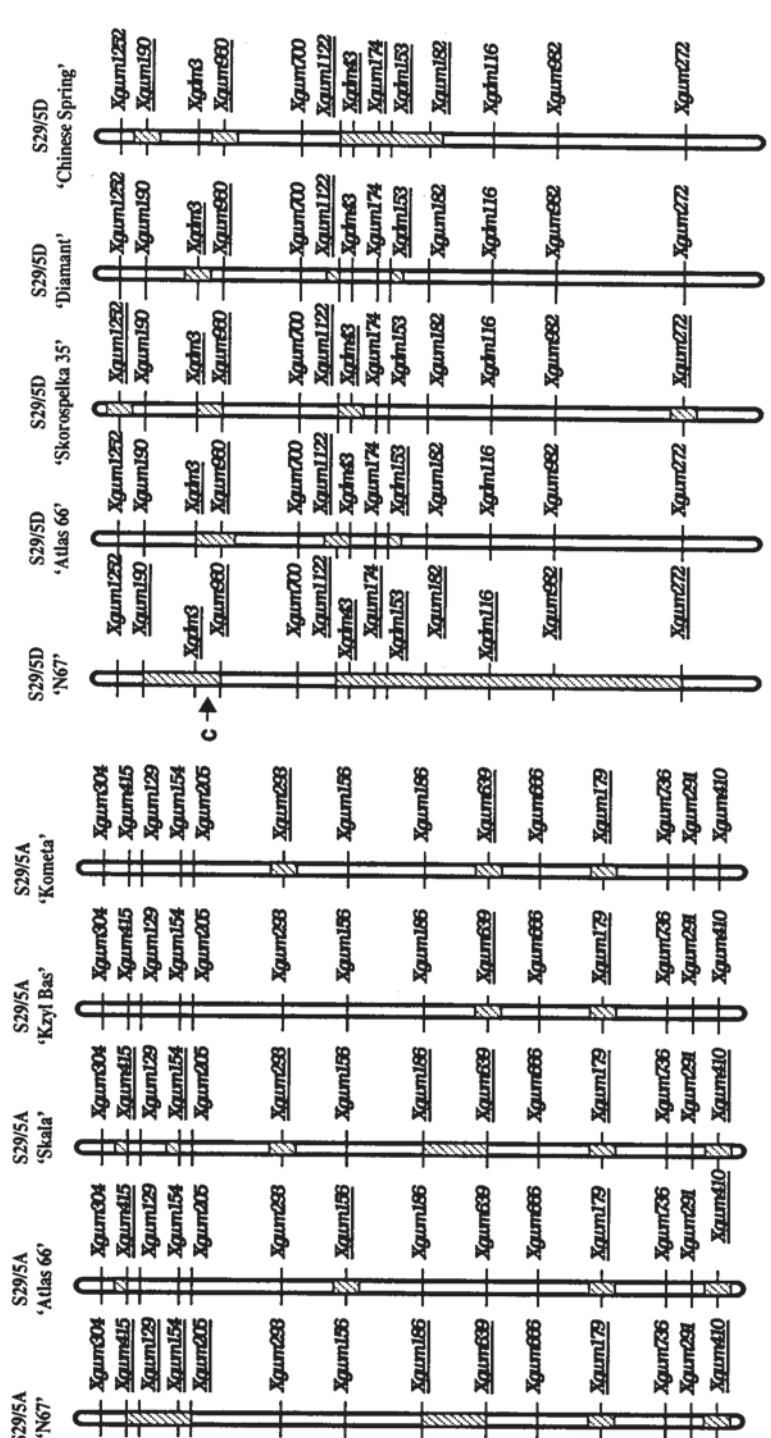

Figure 2: Comparative presentation of microsatellite marker polymorphisms of substitution lines for chromosome 5A (a) and chromosome 5D (b). Hatched boxes indicate stretches between nonpolymorphic markers.

and lines with translocations tagged with the alien gene (Schlegel et al., 1998). Chromosome 5R can replace chromosomes of homologous group 5. Also, lines that harbour a fragment of chromosome 5RL with the *Hp* gene in arms of several wheat chromosomes, namely, 4BL, 5BS, 5DL, 6DL, and 6BL were reported (Driscoll and Sears 1965, Sears 1967, Bielig and Driscoll 1970).

Here we present our study of MAS lines with rye chromosome 5R and their potential as recipients in chromosome substitution programme. Our method of developing lines with inter-varietal chromosome substitution is based on the expression of the *Hp* gene for hairy peduncle, which offers several advantages. First, MAS lines are characterized by an easily detectable morphological trait, whose expression allows distinguishing mono- and disomic plants. This was also observed by Bielig and Driscoll (1970). Therefore, laborious cytological examination, necessary for selection of parental forms in the course of multiple backcrosses can be facilitated or even dispensed with by making use of this gene. In addition, use of MAS lines tagged with an alien gene eliminates the chance of switch between the donor and recipient chromosomes in the backcrosses, because monosomic plants with the univalent donor chromosome can be easily distinguished from monosomic plants with rye chromosome 5R according to peduncle hairiness. Generally, programme involving common monosomics demand alteration of backcrosses and monosomic selfing to eliminate 'univalent switch', which doubles the time required for developing substitution lines. Application of our method permitted us to develop 22 substitution lines of cv. 'S29' wheat in which chromosome pairs 5A or 5D were replaced by the homologue of the donor variety.

Testing of the substitution lines with microsatellite markers showed that all of them were correct. Only one substitution line for chromosome 5D of the donor 'Novosibirskaya 67' had no polymorphic microsatellites comparing with cv. 'S29'. The absence of univalent shift, previously shown by ditelosomic analysis, was confirmed by microsatellite analysis of all substitution lines. Thus, use of the rye genetic marker for tagging wheat chromosomes overcame errors during chromosome substitution. Earlier molecular analysis of three substitution line sets ('S29'/ 'Yanetzkis Probat', 'Chinese Spring'/ 'Synthetic', and 'Cappelle-Desprez'/ 'Bezostaya 1') showed that cytogenetic markers did not ensure precise substitution (Korzun et al. 1997, Pestsova et al. 2000a, Salina et al. 2003). Microsatellite analysis showed that only 15–18 lines of 21 lines of each set were correct, whereas the rest had errors in the substitution of whole chromosomes or their arms.

Thus, for the first time MAS lines with rye chromosome 5R were used for developing lines with inter-varietal chromosome substitution. Such lines can be used, as well as other aneuploid lines, for genetic studies as an alternative to conventional wheat monosomic lines.

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