Characterization of Inter-varietal Chromosome Substitution Lines of Wheat Using Molecular Markers

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Abstract: Several sets of wheat inter-varietal chromosome substitution lines (SLs) have been produced over the last fifty years at the CRI (formerly RICP) in Prague-Ruzyně, based on cytogenetic manipulations using aneuploids. Lines with defined genes have been obtained which significantly influence growth habit and flowering time and these have been used particularly in the study of the genetics and physiology of flowering. The sets of lines include substitutions of homoeologous group 5 chromosomes carrying *Vrn* genes that control vernalisation response, homoeologous group 2 chromosomes with *Ppd* genes controlling photoperiodic sensitivity, and some other substitutions, particularly those with chromosome 3B of the Czech alternative variety Česká Přesívka where a novel flowering time effect was located. Although the phenotypic and cytological analysis of substitution lines has been continually carried out during backcrossing generations, only the use of molecular markers can allow an unambiguous characterization to verify that substitutions are correct and complete. This analysis has allowed incorrect substitutions or partial substitutions to be identified and discarded. This paper summarizes the results of recent molecular checks of the substitution line collections at CRI.

Keywords: wheat; substitution lines; SSR markers

Bread wheat (*Triticum aestivum*) can tolerate changes in its basic chromosome number due to its allohexaploid constitution ($2n = 42$). Monosomics are the most common form of wheat aneuploids and they can be found at a frequency as high as 2–3% of the seed of commercial wheat varieties (Riley & Kimber 1961). This property was put into use for genetic analysis by the pioneering work of Professor E. Sears, who obtained a complete set of aneuploids including monosomics, nullisomics, tetrasomics and lines with telocentric chromosomes in a spring variety, Chinese Spring (Sears 1953, 1954). Sears also discovered how the monosomics could be used for the chromosomal localisation of genes, and how the monosomic constitution could be transferred to other varieties by crossing and backcrossing to the Chinese Spring series. Additionally, Sears developed the technology, via a backcrossing scheme to monosomics, for substituting individual chromosomes from one variety to another to create single chromosome substitution lines (SLs). This way of developing precise genetic stocks became widespread due to its utility as a method of wheat genetic analysis, despite the fact it was very laborious and time-consuming. To share the burden of stock development, an informal organisation known as the European Wheat Aneuploid Co-operative (EWAC) was established to co-ordinate the development and utilisation of wheat aneuploids, and many important wheat genes were identified and/or localised using these aneuploid techniques (Law

& Worland 1996; Worland 2001). The Chromosome Manipulation Group at CRI embraced this technology and developed several series of substitution lines for the study of genes influencing growth habit, flowering time and resistance to diseases of wheat (Košner 1987, 1992; Košner & Pánková 1998, 2002; Pánková & Košner 2004; Pánková & Bartoš 2006).

One disadvantage of using aneuploid techniques for the development of precise genetic stocks was the difficulties in verifying the correctness of substitution lines, although phenotypic and cytological observations could help. However, the era of molecular methods has brought a new level of knowledge to genetics and breeding and methodologies for verification using molecular markers. During the last four decades hundreds of genetic precise stocks including intervarietal substitution lines, alloplasmic lines and single chromosome recombinant lines have been developed, and now we have the opportunities to use molecular techniques for the verifications of these lines and for their utilization in detailed molecular analysis of plant processes (Börner *et al.* 2006; Landjeva *et al*. 2007). This paper reports on the use of molecular markers for the verification of the sets of single chromosome substitution lines developed at CRI.

MATERIALS AND METHODS

Molecular marker analyses were applied using polymorphic Single Sequence Repeat, SSR markers (Bassam *et al.* 1991). The advantage of SSR markers over other techniques for checking chromosome substitutions is their simple assay, reasonable levels of polymorphism in adapted crosses and co-dominant nature (Korzun *et al.* 1997; Pestsova *et al.* 2000; Salina *et al.* 2003). Publicly available microsatellite markers were used from various sources.

To carry out assays, genomic DNA was extracted from leaves of individual plants using commercial DNeasy Plant Mini kits (Qiagen) following the manufacturer's instructions. The polymerase chain reaction (PCR) was performed in a total volume of 15 µl in a Peltier thermocycler (MJ Research, Biorad). The individual reaction mixture contained 1.5 µl of 10× PCR buffer (containing $MgCl_2$), 1.5 µl of 2mM dNTP mixture, 1.5 µl of 2µM primer (total volume for both primers forward and reverse), 0.07 µl of Taq polymerase (5U/µl) and 50–100 ng of template DNA. PCR conditions were as follows: initial denaturation for 1 min at 94°C, then 30 cycles with 1 min at 94°C, 1 min at annealing temperature according to the primer, extension for 1 min at 72°C and final step at 72°C for 4 min. 3 µl of each PCR product was used and mixed with 3 µl formamide loading dye (20 mg of bromophenol blue, 20 mg of xylene cyanol, 0.4 ml of 0.5 m EDTA and 19.6 formamide in a total volume of 20 ml). After denaturation at 94°C for 90 s, samples were loaded and separated on 6% polyacrylamide gels in 1× TBE buffer under constant 80 W. Once the gel had been run, it was washed in a fixer (10% glacial acetic acid) for 20 min. Then it was washed with water (10 min) to remove any residual acetic acid. To stain amplified DNA, the gel was washed in a silver stain solution (silver nitrate with formaldehyde in water) for 30 min. The gel was developed in 60 g sodium carbonate, 3 ml of 40% formaldehyde and 300 µl of 0.1N sodium thiosulphate in a total volume of 2000 ml. When the bands were visible and of sufficient intensity, the development was stopped.

Molecular checks of substituted chromosomes and background variation were also carried out using primers for functional markers of the genes *Vrn-A1 (AP1)* and *Ppd–D1*, to test for the presence of sensitive or insensitive alleles to vernalisation or photoperiod, respectively (Fu *et al.* 2005; Beales *et al.* 2007).

RESULTS

Homoeologous group 2 substitutions

Aneuploid techniques were applied to develop a set of wheat substitution lines with chromosomes 2A and 2B from Chinese Spring, and 2D from Sonora, substituted into a winter background of the variety Zdar and a spring background of the variety Zlatka. This was aimed at producing lines for the study of the effects of genes for photoperiodic sensitivity (*Ppd-1* series*)*. During the backcrossing process, cytogenetic checks were applied, and the phenotype was checked for photoperiod sensitivity. However, molecular checks were applied only to these materials after 2003 and several backcrosses, and revealed a failure to obtain these expected chromosome substitutions (Table 1). Even those materials where the phenotypic assessments revealed a prevailing but segregating occurrence of changed photoperiodic sensitivity, were incorrect: The substitution line

Table 1. SSR alleles in substitutions of homoeologous group 2 chromosomes into the genetic backgrounds of spring variety Zlatka and winter variety Zdar; Sonora was the donor of chromosome 2D (*Ppd–D1)*; Chinese Spring was the donor of chromosomes 2B (*Ppd–B1)* and 2A (*Ppd–A1)*

Substitution line	SSR marker				Substitution	Recombination
Chromosome 2A	*gwm294*	*gwm445*	*gwm312*			
Zdar (CS2A) 1/02	Zdar	Zdar	Zdar		–	–
Zdar (CS2A) 2/02	Zdar	Zdar	Zdar		–	–
Zdar (CS2A) 3/02	Zdar	Zdar	Zdar		–	–
Zlatka (CS2A) 1/02	Zlatka	Zlatka	Zlatka		–	–
Zlatka (CS2A) 2/01	Zlatka	Zlatka	Zlatka		–	–
Chromosome 2B	*gwm388*	*gwm501*	*gwm526*			
Zdar (CS2B) 1/02	Zdar	CS	faint		+	+
Zdar (CS2B) 3/02	Zdar	CS	faint		+	+
Zlatka (CS2B) 2/01	Zlatka	Zlatka	Zlatka		–	–
Zlatka (CS2B) 1/01	Zlatka	Zlatka	Zlatka		–	–
Chromosome 2D	*gwm30*	*gwm539*	*gwm349*	*gwm301*		
Zdar (Sonora2D) 2/02	Zdar	Zdar	Zdar	Zdar	–	–
Zdar (Sonora2D) 1/02	Zdar	Zdar	Zdar	Zdar	–	–
Zlatka (Sonora2D) 1/02	Sonora	Sonora	Zlatka	Zlatka	+	+
Zlatka (Sonora2D) 2/02	Sonora	Sonora	Zlatka	Zlatka	+	+

Zlatka (Sonora 2D) exhibited a recombined rather than a complete chromosome substitution, caused by a possible mistake during previous backcrosses (Table 1). However, this line can still be useful if an additional check of the presence of alleles of the gene *Ppd–D1* reveals the presence of the substituted dominant allele.

Chromosome 3B substitutions

Substitutions of chromosome 3B of Česká Přesívka, a Czech alternative wheat variety, were developed in the genetic backgrounds of a series of Czech varieties – Fenman, Košutka, Sandra, Vala, Zdar, Zlatka, and also some others. This followed initial studies that had revealed that chromosome 3B of Česká Přesívka (CP 3B) was carrying a new gene that influenced the flowering time (FT) of wheat (Košner 1987; Košner & Pánková 2002). The SSR checks revealed that most substitutions were correct, but that some were recombinant chromosomes between CP 3B and the recipient parent (Table 2).

The substitution lines for the CP3B chromosome manifested enhanced sensitivity to photoperiod, and so additional checks for the presence of alleles of the gene *Ppd–D1* (288 bp = photoperiod insensitive allele *Ppd-D1*; 414 bp = photoperiod sensitive allele *Ppd-D1)* were carried out to discover if the sensitivity was caused by the background presence of insensitive alleles. Some variation was found with the variety Košutka substitutions (Table 2). The substitution lines with verified substitutions of the CP 3B have been used for the mapping and characterization of effects of the new FT gene (Pánková *et al.* 2008, submitted).

Homoeologous group 5 substitutions

Substitution lines with homoeologous group 5 chromosomes carrying the dominant alleles at the *Vrn-A1*, *Vrn-B1* and *Vrn-D1* loci for vernalisation requirement, substituted into the genetic backgrounds of three wheat varieties differing in earliness (Zdar, Vala, Košutka), were obtained with the aim to study the effect of different genes

Table 2. SSR alleles in substitutions of chromosome 3B of Česká Přesívka (CP) into the genetic backgrounds of spring varieties Fenman, Sandra, Zlatka, and winter varieties Košutka, Vala, Zdar; the correctness of the genetic background was checked using the functional marker for alleles of *Ppd-D1* (I (288 bp) = photoperiod insensitive allele *Ppd-D1*; S (414 bp) = photoperiod sensitive allele *Ppd-D1)*

	Chromosome 2D	Chromosome 3B						
	Ppd-D1 allele	*cfd4*	*barc164*	*cfa2170*	*wmc326*	*barc77*	substitution	recombination
Fenman 13/98	S		Fenman	Fenman	Fenman	Fenman	–	
Fenman (CP3B) 1/96	S		CP	CP	CP	CP	+	–
Fenman (CP3B) 21/98	S		CP	CP	CP	CP	+	–
Košutka 04	I		Košutka	Košutka	Košutka	Košutka	–	–
Košutka 06	I		Košutka	Košutka	Košutka	Košutka	–	–
Košutka (CP3B) 2/96	I		CP	CP/Košutka	CP	CP	+	–
Košutka (CP3B) 3/96	I		CP	CP/Košutka	CP	CP	+	–
Košutka (CP3B) 21/97	S		CP	CP/Košutka	CP	CP	+	–
Košutka (CP3B) 22/97	I		CP	CP/Košutka	CP	CP	+	–
Košutka (CP3B) 23/97	I		Košutka	CP/Košutka	Košutka	Košutka	–	–
Košutka (CP3B) 22/98	S		CP	CP/Košutka	CP	CP	+	–
Košutka (CP3B) 6/99	I		Košutka	CP/Košutka	Košutka	Košutka	–	–
Košutka (CP3B) 11/04	S			CP	CP	CP	+	–
Košutka (CP3B) 12/04	I			CP	CP	CP	+	–
Košutka (CP3B) 13/04	I			CP	CP	CP	+	–
Košutka (CP3B) 14/04	I			CP	CP	CP	+	–
Sandra 23/04	S		Sandra	Sandra	Sandra	Sandra	+	–
Sandra 35/05	S		Sandra	Sandra	Sandra	Sandra	+	–
Sandra (CP3B) 1/98	S		CP	CP	CP/Sandra	CP	+	–
Sandra (CP3B) 2/98	S		CP	CP	CP/Sandra	CP	+	–
Sandra (CP3B) 2/03	S		CP	CP	CP/Sandra	CP	+	–
Sandra (CP3B) 6/04	S		CP	CP	CP/Sandra	CP	+	–
Vala 2/05	I	Vala	Vala		Vala	Vala	–	–
Vala 22/04	I	Vala	Vala		Vala	Vala	–	–
Vala (CP3B) 1/04	I	CP	CP		CP	CP	+	–
Vala (CP3B) 2/04	I	CP	CP		CP	CP	+	–
Vala (CP3B) 23/98	I		CP	CP/Košutka	CP	CP	+	–
Zdar 25/04	S		Zdar	Zdar	Zdar	Zdar	–	–
Zdar 6/05	S		Zdar	Zdar	Zdar	Zdar	–	–
Zdar (CP3B) 2/97	?		Zdar	CP	CP	CP	+	+
Zdar (CP3B) 2/99	S		Zdar	Zdar	Zdar	CP	+	+
Zdar (CP3B) 3/99	S		Zdar	Zdar	Zdar	CP	+	+
Zdar (CP3B) 4/99	S		Zdar	Zdar	Zdar	CP	+	+
Zdar(CP3B) 1/98	S		CP	CP	CP	CP	+	–
Zdar(CP3B) 1/99	S		Zdar	CP	CP	CP	+	+
Zdar(CP3B) 2/98	S		CP	CP	CP	CP	+	–
Zdar(CP3B) 21/98	S		CP	CP	CP	CP	+	–
Zlatka 22/05	S		Zlatka	Zlatka	Zlatka	Zlatka	–	–
Zlatka 26/04	S		Zlatka	Zlatka	Zlatka	Zlatka	–	–
Zlatka (CP3B) 1/97	S		CP	CP/Zlatka	CP	CP	+	–
Zlatka (CP3B) 2/06	S		CP	CP/Zlatka	CP	CP	+	–
Zlatka (CP3B) 2/97	S		CP	CP/Zlatka	CP	CP	+	–
Zlatka (CP3B) 4/06	S		CP	CP/Zlatka	CP	CP	+	–
CP 22/04	S		CP	CP	CP	CP	–	–
CP 24/04	S		CP	CP	CP	CP	–	–

Table 3. SSR alleles in intervarietal substitutions of wheat homoeologous group 5 chromosomes carrying dominant *Vrn-1* alleles: Zlatka was the donor of chromosome 5A (*Vrn-A1)*; Česká Přesívka (CP) was the donor of chromosome 5B (*Vrn-B1)*; Chinese Spring (CS) was the donor of chromosome 5D (*Vrn-D1)*; the spring allele of the gene *AP1,* designated *S*, signals the presence of the dominant spring *Vrn-A1* allele; it was expected in substitutions for chromosome 5A only; the winter allele of the gene AP1, designated *W*, was expected when the other homoeologous chromosomes, 5B and 5D, were substituted; OT = off type, the band is not from either parent; no amp = no amplification

Substitution line	Top of short arm				Long arm		
Chromosome 5A	*barc10*	*gwm205*	*gwm156*	*barc186*	*barc151*	*wmc110*	*AP1*
Zdar (Zlatka5A) 1/99	Zdar	Zdar	–	–	Zdar	Zdar	W
Zdar (Zlatka5A) 3/99	Zdar	Zdar	–	–	CS	Zdar	S
Zdar (Zlatka5A) 4/99	Zdar	Zdar	–	–	CS	Zdar	S
Zdar (Zlatka5A) 5/99	Zdar	Zdar	–	–	Zdar	Zdar	S
Košutka (Zlatka5A) 3/02	Kos	–	Kos	–	Zlatka	–	W
Košutka (Zlatka5A) 13/04	Kos	–	Zlatka	–	Zlatka	–	S
Košutka (Zlatka5A) 21/00	Kos	–	Zlatka	–	Zlatka	–	S
Košutka (Zlatka5A) 24/00	Kos	–	Zlatka	–	Zlatka	–	S
Vala (Zlatka5A) 1/03	Vala	Vala	Vala	Vala	no amp	–	W
Vala (Zlatka5A) 2/03	Vala	Vala	Vala	Vala	Vala	–	W
Vala (Zlatka5A) 2/00	Vala	Vala	Vala	Vala	Vala	–	W
Vala (CS5D) 21/1/98	Vala	Vala	Vala	CS	Vala	–	W
Vala (CS5D) 21/2/98	Vala	Vala	Vala	CS	Vala	–	W
Zdar (CS5A) 1/03	Zdar	Zdar	Zdar	–	Zdar	Zdar	W
Chromosome 5B	*barc21*	*barc109*	*gwm67*	*gwm371*	*wmc289*		*AP1*
Zdar (ČP5B) 3/01	–	CP	–	CP	Zdar		W
Zdar (ČP5B) 2/00	–	CP	–	Zdar	Zdar		W
Zdar (ČP5B) 1/00	–	CP	–	CP	Zdar		W
Zdar (ČP5B) 3/02	–	CP	–	Zdar	Zdar		W
Košutka (ČP5B) 11/04	Kos	Kos	–	Kos	–		W
Košutka (ČP5B) 12/04	Kos	Kos	–	Kos	–		W
Košutka (ČP5B) 1/01	Kos	Kos, CP	–	CP	–		W
Vala (ČP5B) 1/03	–	CP	no amp	CP	–		W
Vala (ČP5B) 2/03	–	CP	CP	Vala	–		W
Vala (ČP5B) 4/99	–	CP	CP	CP	–		W
Chromosome 5D	*gwm190*	*gwm583*	*wmc215*		*barc144*		*AP1*
Zdar (CS5D) 1/02	Kos?	OT	OT		OT		W
Zdar (CS5D) 11/04	no amp	Zdar	Zdar		–		S
Zdar (CS5D) 1/99	Zdar	Zdar	Zdar		–		S
Zdar (CS5D) 22/00	Zdar	Zdar	Zdar		–		S
Košutka (CS5D) 1/03	CS	CS	CS		CS		W
Košutka (CS5D) 2/03	CS	CS	CS		CS		W
Košutka (CS5D) 3/03	CS	CS	CS		CS		W
Vala (CS5D) 2/03	–	OT	Vala		Vala		W
Vala (CS5D) 2/01	–	OT	Vala		CS		W
Vala (CS5D) 24/03	–	OT	Vala		Vala		no amp

Table 4. SSR alleles in reciprocal substitutions of wheat chromosome 5A between the winter wheat variety Bezostaya 1 (Bez) and Mironovskaya 808 (Mir)

Substitution line	*Xgwm261 2D*	*Xgwm304 5A*	*Xgwm186 5A*	*Xgwm156 5A*	*Xgwm291 5A*	Substitution	Recombination
Mir (Bez5A) 13/04	Mir	Bez	Bez	Bez	Bez	+	–
Mir (Bez5A) 21/05	Mir	Mir	Mir	Mir	?	–	–
Mir (Bez5A) 11/04	Mir	Mir	Mir	Mir	?	–	–
Mir (Bez5A) 12/04	Mir	Bez	Bez	Bez	Bez	+	–
Bez (Mir5A) 12/04	Mir	?	Mir	Mir	Mir	+	–
Bez (Mir5A) 6/04	Bez	Mir	Mir	Mir	Mir	+	–
Bez (Mir5A) 3/04	Bez	Mir	Mir	Mir	Mir	+	–
Bez (Mir5A) 4/04	Bez	Mir	Mir	Mir	Mir	+	–
Bez (Mir5A) 1/04	Bez	Mir	Mir	Mir	Mir	+	–

Vrn-1 on the growth habit and yield of wheat. Although the selection of the expected phenotypes of substitution lines was done continuously during backcrossing, the SSR molecular checks of the lines revealed a lot of abnormalities that often occurred due to mistakes during the early crosses or following recombination caused by mistakes during the series of backcrosses (Table 3).

To distinguish between the alleles of homoeologous genes *Vrn-A1*, *Vrn-B1* and *Vrn-D1* present, a functional marker, *AP1*, detecting the presence of different *Vrn-A1* alleles, was used in the molecular checks, in addition to the SSR markers. The spring allele of the gene *AP1,* designated *S* in Table 3, should signal the presence of the dominant spring *Vrn-A1* allele. It was expected in substitutions for chromosome 5A only. The winter allele of the gene *AP1*, designated *W*, was expected when the other homoeologous chromosomes, 5B and 5D were substituted. Only four out of the set of checked lines showed the correct substitutions: Vala (ČP5B) 4/99 and three lines of Košutka (CS5D) (Table 3).

A set of six reciprocal SLs for homoeologous group 5 chromosomes was developed using the aneuploid techniques with the aim of studying the effects of recessive *vrn* alleles on flowering time and frost resistance of wheat. The presence of multiple recessive alleles of *Vrn-A1*, *Vrn-B1* and *Vrn-D1* was detected using this set of substitution lines (Košner & Pánková 1998, 2001). The substitutions were found to be correct in most of the lines with substitutions of the 5A and 5B chromosomes (Tables 4 and 5). The checks of the reciprocal substitutions for chromosome 5D will be the subject of further analysis.

DISCUSSION AND CONCLUSIONS

An extensive series of SSR checks is being carried out on the precise genetic stocks that have been

Table 5. SSR alleles in reciprocal substitutions of wheat chromosome 5B between the winter wheat variety Bezostaya 1 (Bez) and Mironovskaya 808 (Mir)

Substitution line	*barc4 5B*	*Xgwm213 5B*	*Xgwm408 5B*	*barc59 5B*	Substitution	Recombination
Bez (Mir5B) 1/02	Mir	Mir	Mir	Mir	+	–
Bez (Mir5B) 2/05	Mir	Mir	Mir	Mir	+	–
Mir (Bez5B) 1/05	Bez	Bez	Bez	Bez	+	–
Mir (Bez5B) 21/05	Bez	Bez	Bez	Bez	+	–
Mir (Bez5B) 3/05	Bez	Bez	Bez	Bez	+	–
Mir (Bez5B) 4/05	Bez	Bez	Bez	Bez	+	–

produced by applying wheat aneuploid techniques at the CRI (formerly the RICP) in Prague over the last fifty years. Studies to date have revealed that although many of the substitutions are correct, others are not, and that there are several SLs that are, in fact, recombinant substitutions between the donor and recipient chromosomes rather than complete substitutions. Some others are completely wrong and have reverted to the recipient chromosome. These abnormalities indicate that despite the care taken during the development of SLs, through cytological and phenotypic assessment, mistakes have occurred. Law and Worland (1972) outlined the problems that can occur in the development of SLs, termed 'shift' and 'switch', and it seems likely that these phenomena have occurred in the development of these different series of SLs to different extents in the different sets. The studies here also highlight the importance of applying molecular checks during and after the process of SL development.

Many of the SLs for the substitutions of homoeologous group 2 chromosomes are incorrect, giving the possibility of only using the Zlatka (Sonora 2D) substitution line for further studies. But this is possible only if the presence of the gene *Ppd-D1* or other genes of interest is confirmed using functional markers for this locus. The substitutions of chromosome CP3B were found to be mostly correct, and these substitution lines can be utilised in molecular studies and mapping of genes of interest.

Several substitutions of homoeologous group 5 chromosomes carrying dominant *Vrn-1* genes, substituted into three different genetic backgrounds of winter wheat, have been verified. It is expected again that the use of functional markers for different alleles of the genes *Vrn-A1*, *Vrn-B1*, and *Vrn-D1* could help with the selection, by inter-chromosomal characterization, of some lines where recombination had occurred and identify those useful for further study. The set of reciprocal substitution lines for homoeologous group 5 chromosomes between two winter varieties, Bezostaya 1 and Mironovskaya 808, has shown correct reciprocal substitutions for chromosomes 5A and 5B.

The molecular checks of the sets of substitution lines were very useful in describing in detail the constitutions of the substitution lines, and allowed selection of valuable intervarietal substitutions for the further genetic analysis of important traits, even when the chromosomes are recombinants. Some of the validated lines, particularly those with the substitution of chromosome 3B in different genetic backgrounds, are available and are already being used for the mapping of a new flowering time gene that was firstly described by the research group of chromosome manipulations, Prague-Ruzyně. After the substitutions have been validated for the homoeologous group 5 chromosomes of wheat, both with dominant and recessive alleles of the genes *Vrn-1*, the production of recombinant lines will be started to allow a more detailed genomic and proteomic study of the genetics and physiology of vernalisation and frost tolerance. These precise materials will be a resource of information concerning genes of interest to plant breeders. The data also confirm that microsatellite markers provide an ideal tool for testing the authenticity of genetic stocks of wheat (Korzun *et al.* 1997; Pestsova *et al.* 2000; Salina *et al.* 2003).

List of symbols

FT – flowering time
PCR – polymerase chain reaction
SL – substitution line

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