Theor Appl Genet (2002) 106:84–91
DOI 10.1007/s00122-002-0998-x

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Microsatellite analysis of wheat chromosome 2D allows the reconstruction of chromosomal inheritance in pedigrees of breeding programmes

Received: 3 December 2001 / Accepted: 18 April 2002 / Published online: 12 July 2002

Abstract The dwarfing gene *Rht8* and the photoperiodic insensitivity gene *Ppd-D1* are linked on the short arm of chromosome 2D of bread wheat and play an important role in determining the geographic adaptation of modern wheat varieties. The genes are believed to originate from the old Japanese variety 'Akakomugi' and have been distributed throughout the world by diverse breeding programmes. Twelve microsatellite loci previously mapped on wheat chromosome 2D were used for a retrospective analysis of 59 wheat varieties with known pedigree, to trace the transmittance of the chromosomal region around these genes during extended breeding programmes. Within the range of the screened varieties 100 alleles were detected at the 12 microsatellite loci. For each microsatellite locus, a screen over varieties was performed to find the alleles corresponding to the parental variety 'Akakomugi'. A comparison of wheat varieties carrying the 192-bp allele, at locus *Xgwm261-2D* which is diagnostic for the presence of the gene *Rht8*, with the varieties without *Rht8*, showed linkage disequilibrium of 'Akakomugi' alleles for a segment of chromosome 2D which comprised at least 28 cM. Selection was accompanied with a linkage drag of 'Akakomugi' alleles in the neighbouring loci to *Rht8*. A diminution of the segment of chromosome 2D originating from 'Akakomugi' during several pedigree generations was observed. Varieties of the early generations were found to carry the whole short arm of chromosome 2D of 'Akakomugi', while the varieties of further selections possessed smaller segments including the diagnostic allele at locus *Xgwm261-2D*. Our results demonstrate that microsatellites can be successfully used for studying the inheritance of chromosomes within pedigrees of breeding programmes.

Communicated by J.W. Snape

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Keywords Dwarfing gene (*Rht8*) · Microsatellite · Pedigree · *Triticum aestivum* L.

Introduction

The development of highly productive, short-stemmed bread wheats that are resistant to lodging has remained one of the main breeding objectives for many years. To-date 21 genes influencing plant height in wheat have been found and assigned as *Rht* (reduced plant height) genes (McIntosh et al. 1998). The major semi-dwarfing genes *Rht-B1* and *Rht-D1* have GA insensitive alleles that occur in a majority of wheat varieties grown today. They have been shown to increase yields by up to 20% (Worland and Law 1986).

In Mediterranean countries, an alternative to the GA insensitive height-reducing genes is the GA sensitive dwarfing gene *Rht8* (Worland and Law 1986). This gene is located on chromosome 2D and can reduce plant height by up to 10 cm (Worland et al. 1998b). Over a long period of time, studying the distribution and genetic effects of this gene was limited by difficulties in recognising *Rht8* in varieties. Recently, tight linkage was found between wheat microsatellite WMS261 and the dwarfing gene *Rht8*. Microsatellite locus *Xgwm261-2D* is located 0.6 cM distal to *Rht8* on the short arm of chromosome 2D (Korzun et al. 1998). The close linkage permits the use of this microsatellite as a marker for detecting allelic variants at the *Rht8* locus. Three main alleles of *Xgwm261-2D* were found to be diagnostic for three different alleles of the *Rht8* gene. The 192-bp allele of *Xgwm261-2D* corresponds to a height-reducing phenotype of *Rht8*, the 174-bp allele correlates with a neutral phenotype of the gene while the 165-bp allele can be associated with height promotion (Worland et al. 1998b). A screen of more than 100 international wheat varieties using WMS261 allowed the determination of the distribution of the dwarfing gene *Rht8* in international breeding programmes, and the demonstration that *Rht8* was transmitted from initial crosses involving the Japanese variety 'Akakomugi' as the source of *Rht8*.

Relatively close linkage of the gene for photoperiodic insensitivity (*Ppd-D1*) to *Rht8* has been found (Worland and Law 1986; Worland et al. 1998a). The photoperiodic insensitivity gene *Ppd-D1* is extremely important in increasing the adaptability and yield in varieties from southern Europe. A selective advantage for the preservation of the linkage between *Ppd-D1* and *Rht8* has been observed in Italian, Yugoslavian and Russian breeding programmes. In north-western European varieties and CIMMYT wheats the absence of an adaptive advantage for the linkage *Ppd-D1/Rht8* led to the breakage of that linkage (Worland et al. 1998b).

Microsatellites or simple sequence repeats (SSRs) are found to be highly informative genetic markers and have become well utilised for genetic analysis and plant breeding (Gupta and Varshney 2000). To-date, many microsatellites are available on each chromosome of bread wheat (Röder et al. 1998a; Pestsova et al. 2000). In this study microsatellites were used to trace the transmittance of the chromosomal region around *Rht8* and *Ppd-D1* during extended breeding programmes, and also to estimate the size of the chromosomal pieces transferred from the parental variety to the progeny, along with the genes of interest.

Materials and methods

Plant material and DNA isolation

Fifty nine varieties of bread wheat with known pedigree information were selected for the study (see Table 1). Most of these varieties originated from the original cross between the Japanese variety 'Akakomugi' with a hybrid of the Dutch variety 'Wilhelmina' and the Italian landrace 'Riete'. Pedigrees of the wheats were published by Worland et al. (1998b) and Martynov et al. (1992). Seeds of the varieties were obtained from the John Innes Centre germplasm collection (JIC, Norwich, UK) and the IPK genebank collection (IPK, Gatersleben, Germany). DNA was extracted from one to five grains according to the procedure described by Plaschke et al. (1995).

Microsatellite analysis

Thirty microsatellite loci located on chromosome 2D were tested for polymorphism between parental varieties 'Akakomugi', 'Wilhelmina' and 'Riete'. Twelve microsatellite primer pairs revealing size polymorphisms are listed in Table 2, and their chromosomal location is shown in Fig. 1. The identification of wheat microsatellites (WMS), their location and primer sequences were previously described by Röder et al. (1998a), and the microsatellites isolated from *Aegilops tauschii* (GDM) were described by Pestsova et al. (2000). Unpublished primer sequences are available on request.

Polymerase chain reaction and fragment analysis

Polymerase chain reactions were performed as described by Röder et al. (1998a). Electrophoreses were carried out in 6% denaturing polyacrylamide gels on automated laser fluorescence (ALF) sequencers. Fragment sizes were calculated using the computer program Fragment Analyzer 1.02 (Pharmacia) by comparison with internal size standards. In case of the amplification of two alleles at one microsatellite locus the analysis was repeated to confirm heterogeneity of the varieties.

Results and discussion

Microsatellite analysis of chromosome 2D

Thirty microsatellite loci previously mapped on chromosome 2D were investigated for polymorphism between the parental varieties 'Akakomugi', 'Wilhelmina' and 'Riete'.

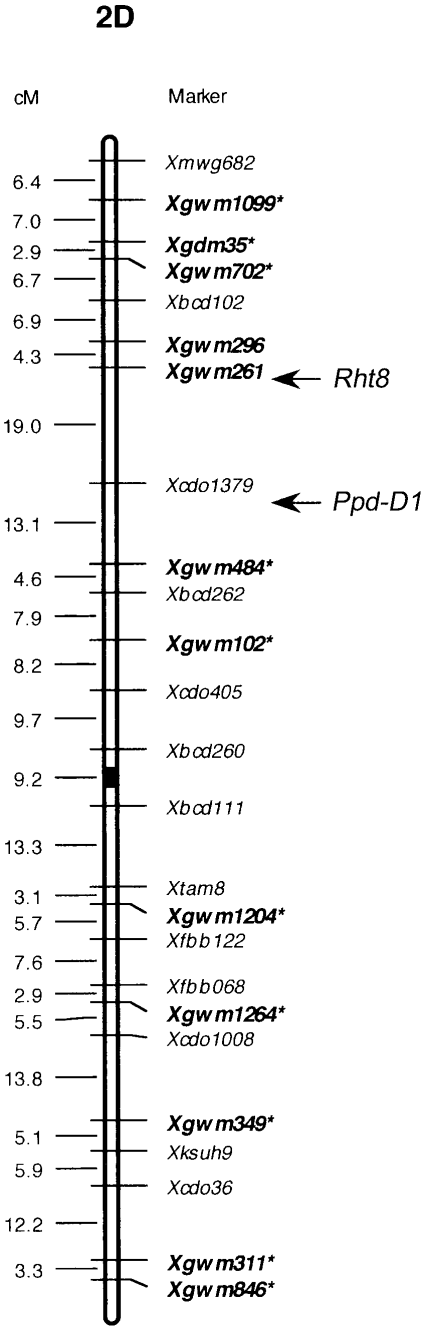

Fig. 1 Molecular linkage map of chromosome 2D of wheat. The centromere is indicated in *black*. The short arm of the chromosome is at the top. The microsatellite loci are indicated in *bold* and carry the lab designator "*gwm*" (Gatersleben wheat microsatellite) and "*gdm*" (Gatersleben D-genome microsatellite). Microsatellite loci integrated into the map with LOD<2.5 are marked by an *asterisk*

Table 1 Varieties of wheat, their origin and genotypic data for 12 microsatellite loci on chromosome 2D; nd = no data

Variety	Origin	Microsatellite locus[a]												
		Photoperiodic response[b]	'Akakomugi' in pedigree	*Xgwm 1099*	*Xgdm 35*	*Xgwm 702*	*Xgwm 296*	*Xgwm 261*	*Xgwm 484*	*Xgwm 102*	*Xgwm 1204*	*Xgwm 1264*	*Xgwm 349*	*Xgwm 311*	*Xgwm 846*
Group 1: Varieties carrying the gene *Rht8* based on microsatellite data															
Akakomugi	Japan	I	+	1	1	1	1	1	1	1	1	1	1	1	1
Haya Komugi	Japan	nd	nd	1	1	1	1	1	1	1	0	1	1	0	0
Argelato	Italy	I	+	0	1	1	1	1	1	1	1	0	0	1	1
Marzotto	Italy	nd	+	1	1	1	1	1	1	1	0	0	0	0	0
Campodore	Italy	I	+	1	1	1	1	1	1	1	0	0	0	0	0
Villa Gloria	Italy	nd	+	1	1	1	1	1	1	1	0	1	0	0	0
Mara	Italy	I	+	1	1	1	1	1	1	1	0	0	0	0	0
Sava	Yugoslavia	I	+	1	1	1	1	1	0	0	0	1	1	0	1
Aquila	Italy	nd	+	1	1	1	1	1	0	0	0	1	0	0	0
Ardito	Italy	I	+	1	1	1	1	1	0	0	1	0	0	0	0
Salto	Italy	I	+	1	1	1	1	1	0	0	1	0	0	0	0
Sanja	Yugoslavia	I	+	0	1	1	1	1	0	0	1	1	0	0	0
San Pastore	Italy	I	+	1	1	1	1	1	0	0	0	1	0	0	0
Siete Cerros	Mexico	I	–	0	1	1	1	1	0	0	0	0	0	0	0
Superzlatna	Yugoslavia	I	+	0	1	1	1	1	0	0	1	1	0	0	0
Orlandi	Italy	I	+	1	1	1	1	1	0	0	0	1	0	1	1
NS646	Yugoslavia	nd	+	1	1	1	1	1	0	0	0	1	0	0	0
Fortunato	Italy	I	+	0	0	0	1	1	1	0	0	1	1	0	0
Avrora	Russia	I	+	0	0	0	1	1	0	1	0	1	0	0	0
Dneprovskaya	Ukraine	nd	+	0	0	0	1	1	0	1	0	1	0	0	0
Kavkas	Russia	I	+	0	0	0	1	1	0	0	0	0	0	0	0
Damiano	Italy	I	+	0	0	0	1	1	0	0	0	1	0	0	0
Jugoslavia	Yugoslavia	I	+	0	0	0	1	1	0	0	0	1	0	0	0
Radusa	Yugoslavia	nd	+	0	0	0	1	1	0	0	0	1	0	0	0
Skopljanka	Yugoslavia	I	+	0	0	0	1	1	0	0	1	0	0	0	0
Bezostaya 1	Russia	I	+	0	0	0	1	1	0	0	0	1	0	0	0
Salvia	Italy	nd	+	1	1	1	0	1	1	1	0	0	0	0	0
Funo	Italy	nd	+	0	1	1	0	1	0	0	0	1	0	1, 0	1, 0
Orso	Italy	nd	+	0	1	1	0	1	0	0	0	1	0	0	0
Farnese	Italy	I	+	0	1	1	0	1	0	0	1	0	0	0	0
Zitnica	Yugoslavia	I	+	0	0	0	0	1	0	0	0	1	1	0	1, 0
Produttore	Italy	I	+	0	0	0	0	1	0	0	0	1	0	0	0
Group 2: Varieties without the gene *Rht8* based on microsatellite data															
Frontana	Brazil	I	+	0	0	0	1	0	1	1	0	0	0	0	0
Quaderna	Italy	I	+	0	0	0	0	0	1	1	1	0	1	0	0
Mentana	Italy	I	+	0	0	0	0	0	1, 0	1	0	0	0	0	0
Autonomia	Italy	I	+	0	0	0	0	0	1	1	1	0	0	0	0
Chris	USA	I	+	0	0	0	0	0	1	1	0	1	0	1	1
Chaimite	Portugal	I	–	0	0	0	0	0	1	0	1	0	0	1	1
Frontiera	Brazil	I	–	0	0	0	1	0	0	1	0	0	0	1	1
New Thatch	USA	R	–	0	0	0	0	0	1	0	0	1	0	0	0
Bob white	Mexico	R	+	0	0	0	1	0	0	0	0	1	0	0	0
Maringa	Brazil	I	+	0	0	0	0	0	0	1	0	0	0	0	0
Red Coat	USA	nd	–	0	0	0	0	0	0	1	0	1	0	0	0
Saitama 27	Japan	I	–	0	0	0	0	0	0	0	0	1	0	1	1

Table 1 (continued)

Variety	Origin	Microsatellite locus[a]												
		Photoperiodic response[b]	'Akakomugi' in pedigree	*Xgwm 1099*	*Xgdm 35*	*Xgwm 702*	*Xgwm 296*	*Xgwm 261*	*Xgwm 484*	*Xgwm 102*	*Xgwm 1204*	*Xgwm 1264*	*Xgwm 349*	*Xgwm 311*	*Xgwm 846*
Mironovskaya	Ukraine	R	+	0	0	0	0	0	0	0	0	0	0	1	1
Ciano 67	Mexico	I	+	0	0	0	0	0	0	0	0	0	0	0	1
Kenya	Kenya	nd	–	0	0	0	0	0	0	0	0	0	0	1, 0	1
Leonardo	Italy	I	+	0	0	0	0	0	0	0	1	1	0	1, 0	1
Penjamo 62	Mexico	I	+	0	0	0	0	0	0	0	0	0	0	0	1
Norin 1/Brevor 14	USA	nd	–	0	0	0	0	0	0	0	0	0	0	0	0
Lerma Rojo	Mexico	I	–	0	0	0	0	0	0	0	1	0	0	0	0
Glennson 81	Mexico	I	+	0	0	0	0	0	0	0	1	0	0	0	0
Veery-S	Mexico	I	+	0	0	0	0	0	0	0	0	0	0	0	0
Brevor	USA	nd	–	0	0	0	0	0	0	0	1	0	0	0	0
Inallettabile 95	Italy	nd	–	0	0	0	0	0	0	0	0	1	0	0	0
Gabo	Australia	I	–	0	0	0	0	0	0	0	0	0	0	0	0
Lutescens 17	Russia	nd	–	0	0	0	0	0	0	0	0	1	0	0	0
Wilhelmina	Netherlands	R	–	0	0	0	0	0	0	0	0	0	0	0	0
Riete	Italy	R	–	0	0	0	0	0	0	0	0	0	0	0	0

[a] The order of the microsatellite loci in the table corresponds to their order on the genetic map presented in Fig. 1. Alleles of parental variety 'Akakomugi' and all alleles with the same size in other varieties are assigned as 1, alleles with different size are designated as 0

[b] I = photoperiod insensitive varieties; R = photoperiod responsive (according to Worland et al. 1998b)

Table 2 Number of alleles (number of unique alleles) at different microsatellite loci on chromosome 2D among 59 varieties of bread wheat

Microsatellite locus	Number of alleles
Xgwm1099	12 (1)
Xgdm35	4
Xgwm702	8 (2)
Xgwm296	7 (1)
Xgwm261	3
Xgwm484	12 (6)
Xgwm102	6 (1)
Xgwm1204	12 (4)
Xgwm1264	6 (2)
Xgwm349	11 (2)
Xgwm311	12 (3)
Xgwm846	7 (1)

Twelve of them revealed intervarietal polymorphism of the amplified fragments (Fig. 1). The variety 'Akakomugi' had no common alleles with the other two varieties 'Wilhelmina' and 'Riete'. At the same time, 'Wilhelmina' and 'Riete' had common alleles at four out of the 12 loci. The selected microsatellites were used for analysis of 59 varieties of wheat with known pedigrees originating from Italy, Yugoslavia, Mexico, Russia and some other countries (Table 1).

Within the range of the screened varieties, 100 alleles were detected at the 12 microsatellite loci (Table 2). The number of alleles per microsatellite locus varied from three at locus *Xgwm261-2D* to 12 at loci *Xgwm311-2D*, *Xgwm484-2D*, *Xgwm1099-2D* and *Xgwm1204-2D*. Twenty three alleles were unique, occurring only in one of the analysed varieties.

Eleven varieties out of 59 (18.6%) were found to be heterogeneous at microsatellite loci. The varieties 'Frontana', 'Autonomia', 'Maringa', 'Kenya' 'Brevor' and 'Dneprovskaya' were heterogeneous for one locus, 'Funo', 'Zitnica' and 'Leonardo' for two loci, and 'Mentana' for three loci. Maximum heterogeneity was found for the Mexican variety 'Penjamo 62' which carried two alleles at six loci. Since common wheat is a self-pollinated crop the presence of two alleles at one locus is probably a result of DNA isolation from several seeds and reflects the presence of unfixed alleles in the varieties.

Recently, several studies were performed to investigate the uniformity of common wheat cultivars at microsatellite loci (Chebotar et al. 2002; Freeman et al. 2002). High levels of non-uniformity were revealed compared to the standard morphological assessment. Our data are in agreement with these results.

In most cases the differences between the two alleles in heterogeneous varieties consisted of more than two motifs. Therefore microsatellite mutations are an unlikely explanation for such differences. We rather suppose that the main reasons for microsatellite heterogeneity are non-completed inbred processes during variety development or outcrossing events during variety multiplication and cultivation.

For each microsatellite locus a screen over wheat varieties was performed to find the alleles corresponding in

their size to the variety ‘Akakomugi’. The results of this screen are shown in Table 1 where ‘Akakomugi’ alleles were assigned as 1 while other alleles were designated as 0. The order of the microsatellite loci in the table corresponds to their order on the genetic map (Fig. 1). It was observed that wheat varieties related to ‘Akakomugi’ carried more ‘Akakomugi'' alleles than unrelated ones. The old Japanese land-race ‘Haya Komugi’ shared nine alleles out of 12 with ‘Akakomugi’. Obviously these varieties are closely related to each other. Variety ‘Argelato’ also carried nine ‘Akakomugi’ alleles. This variety could have obtained the alleles from two sources, variety 'Mara' or variety ‘Orlandi’, both of them having the parental variety ‘Akakomugi’ in their pedigrees.

From the original cross between the parental varieties ‘Akakomugi’, ‘Wilhelmina’ and ‘Riete’ four varieties were selected: ‘Villa Gloria’, ‘Damiano’, ‘Mentana’ and ‘Ardito’. ‘Villa Gloria’ carried eight ‘Akakomugi’ alleles and seven of them occurred on the short arm of chromosome 2D. This variety obviously received the whole chromosomal arm 2DS from ‘Akakomugi’. Varieties ‘Ardito’, ‘Damiano’ and ‘Mentana’ shared with ‘Akakomugi’ six, three and two alleles, respectively.

Alleles with the same sizes as ‘Akakomugi’ were also found in varieties not related to ‘Akakomugi’ with a frequency of 13.6%. For example, the Mexican variety ‘Frontiera’ carried four alleles of the same size as ‘Akakomugi’ but this variety had no relation to ‘Akakomugi’. Similarly, the American varieties ‘Newthatch’ and 'Red Coat' both carried two alleles identical to ‘Akakomugi’. In these cases the alleles identical to ‘Akakomugi' were mainly observed on the long arm of chromosome 2D. The finding of common alleles in unrelated varieties could be explained by the low level of polymorphism within the genome of bread wheat. However, the relatively high rate of mutations in microsatellites should be taken into account. It could lead to independent mutations for new microsatellite alleles with the same number of repeats.

All wheat varieties were divided into two groups based on the analysis of microsatellite locus *Xgwm261-2D* (Table 1). The first group consisted of 32 varieties carrying the 192-bp allele at the locus *Xgwm261-2D* that is diagnostic for the presence of the gene *Rht8*. All varieties in this group, except the Mexican variety ‘Siete Cerros’ and the old Japanese land-race ‘Haya Komugi’, belonged to South European and former Soviet Union breeding programmes and could be traced in their pedigrees to the parental variety ‘Akakomugi’. The members of this group could also be defined as varieties subject to a selection pressure for the gene *Rht8*, and possibly *Ppd-D1*, during their development. The second group included 27 varieties from different breeding programmes carrying two other alleles at the locus *Xgwm261-2D*. It consisted of 12 varieties which could be traced to ‘Akakomugi’ and 15 varieties which have no relationship to ‘Akakomugi.

The analysis of the 12 microsatellite loci showed that varieties from group 1 carried ‘Akakomugi’ alleles at two to nine loci, and varieties from group 2 contained ‘Akakomugi’ alleles at zero to five loci. As could be expected, the mean number of loci carrying ‘Akakomugi’ alleles in group 1 (5.3) exceeded the mean number of the loci in group 2 (1.8).

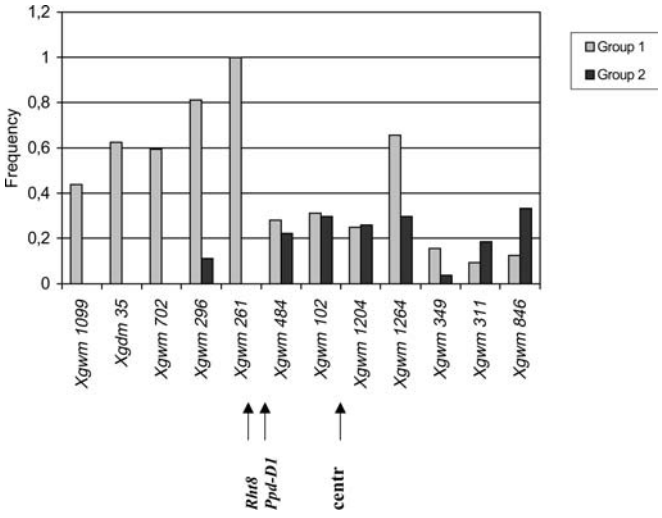

Fig. 2 Frequencies of ‘Akakomugi' alleles at 12 microsatellite loci of chromosome 2D within the two groups of wheat varieties. Group 1 carries the ‘Akakomugi' allele 192-bp at the locus *Xgwm261-2D*; group 2 does not

For both groups of wheat varieties, the frequencies of ‘Akakomugi’ alleles at each microsatellite locus were calculated (Fig. 2). The results showed that microsatellites fell into two categories depending on their location relative to *Xgwm261-2D*. Concerning the microsatellite loci located proximally on 2DS and those on 2DL the frequencies of ‘Akakomugi’ alleles were comparable in groups 1 and 2. For microsatellite loci located distally from *Xgwm261-2D*, the frequency of ‘Akakomugi’ alleles in group 1 was much higher. Group 2 did not contain any ‘Akakomugi’ alleles at the distal loci *Xgwm1099-2D, Xgdm35-2D* and *Xgwm702-2D*.

Group 1 is composed of varieties subject to a selection for the gene *Rht8*. Our results show that the selection for the gene also involved other regions of chromosome 2D, resulting in linkage drag of ‘Akakomugi’ alleles on the distal part of chromosome arm 2DS. The chromosomal fragment showing linkage disequilibrium for ‘Akakomugi’ alleles comprised at least 28 cM (Figs. 1, 2). At the same time, the loci in the proximal part of 2DS and obviously the loci on 2DL were not influenced by selection and recombined independently. However, the closest proximal locus adjacent to *Xgwm261-2D* was located in a genetic distance of 32.1 cM. Therefore, if a similar linkage drag of ‘Akakomugi’ alleles would occur proximally to the gene *Rht8* we were not able to detect it.

We compared our results with the data on the physical distribution of microsatellites in the genome of wheat (Röder et al. 1998b). The loci *Xgwm296-2D* and *Xgwm484-2D* flanking *Rht8* on the genetic map (Fig. 1) were physically located in the distal 60% of the chromosome arm 2DS. Since we did not find any linkage dis-

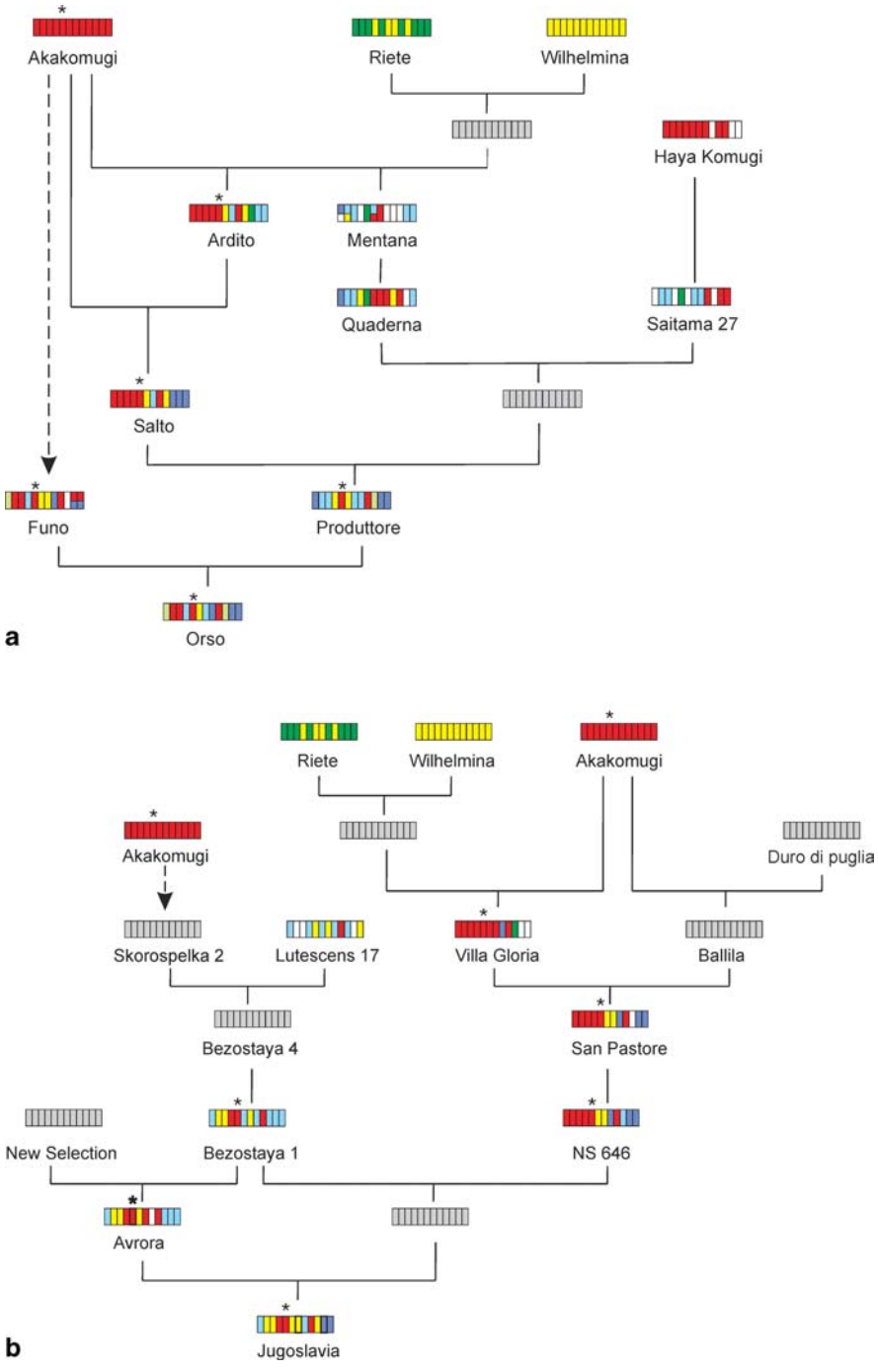

Fig. 3 Pedigrees of wheat varieties and genotypic data for 12 microsatellite loci on chromosome 2D. The loci are arranged in accordance with the map location presented in Fig. 1. Different alleles at the microsatellite loci are marked with *colours*, alleles corresponding to 'Akakomugi' are in *red* and the alleles which occur in the presented pedigrees only once are in *white*. The *grey colour* designates untested varieties. The allele at locus *Xgwm261-2D* that is diagnostic for the presence of *Rht8* gene is marked by an *asterisk*

equilibria of 'Akakomugi' alleles for the region proximal to *Xgwm484-2D* we can conclude that the segment of the 'Akakomugi' chromosome affected by selection for the *Rht8* gene comprises less than 60% of the physical length of 2DS.

Worland et al. (1998b) found a selective advantage for the preservation of the linkage between *Ppd-D1* and *Rht8* in the South European and the former Soviet Union breeding programmes. A linkage map of chromosome 2D constructed earlier using single chromosome recombinant lines of the varieties 'Cappelle' and 'Mara' put the photoperiodic response gene *Ppd-D1* 20.9 cM proximal to the gene *Rht8* (Worland et al. 1998a). Therefore the gene *Ppd-D1* should be located in the 32.1 cM gap between the loci *Xgwm261-2D* and *Xgwm484-2D.* Additional loci and further investigations will be necessary to confirm, at the molecular level, the preservation of the linkage between *Ppd-D1* and *Rht8* in the breeding programmes.

Pedigree analysis

A comparison of the microsatellite data with known pedigree information allowed the investigation of the inher-

itance of individual microsatellite alleles at different loci on chromosome 2D. The pedigrees of the studied varieties of wheat were published by Worland et al. (1998b) and the complete information about the parents was known for 46 varieties. Unfortunately, not all varieties in the pedigrees were available. Therefore in 18 of the 46 cases both parent varieties were analysed, while in the other 28 cases only one parent was analysed.

The analysis of the 28 varieties with a lack of microsatellite information for one of the parents showed that 55% of the alleles at the 12 microsatellite loci could be traced to the known parent. This result is in a good agreement with the theoretically expected equal ratio of parental alleles. However, the analysis of 18 varieties with complete microsatellite data for both of the parents revealed some discrepancies between microsatellite and pedigree data. Figure 3a and b present the pedigrees of varieties 'Orso' and 'Jugoslavia'. Both varieties have 'Akakomugi' in their pedigrees.

The analysis of the varieties selected from the initial cross between 'Akakomugi' and a hybrid of 'Wilhelmina' and 'Riete' showed that progeny 'Villa Gloria' (Fig. 3a) and 'Ardito' (Fig. 3b) contained nine parental alleles, 'Damiano' possessed seven parental alleles (data not shown) and 'Mentana' carried only four parental alleles from 12 (Fig. 3b). 'Mentana' was also heterogeneous and carried different alleles at three microsatellite loci.

The further selected variety 'Jugoslavia' carried 11 parental alleles of 'Avrora', 'Bezostaya 1' and 'NS 646' (Fig. 3a), and one inconsistent allele at locus *Xgwm349-2D*. At this locus 'Jugoslavia' carried an allele of 225 bp whereas all parental varieties have an allele of 227 bp. The difference of 2 bp could be explained by a single mutation event in the microsatellite sequence.

'Produttore (Fig. 3b) carried 'Salto', 'Saitama 27' and 'Quaderna' alleles at 11 loci out of 12. The lack of coincidence occurred again at locus *Xgwm349-2D*. 'Produttore' possessed an allele of 209 bp while the parental varieties carried alleles of 235 bp, 199 bp and 203 bp, respectively. Variety 'Orso' which originated from 'Produttore' and 'Funo' inherited the 209-bp allele of 'Produttore' at locus *Xgwm349-2D*. All other alleles of variety 'Orso' were in accordance with the pedigree.

Two possible explanations for the observed inconsistencies are the heterogeneity of varieties or the instability of microsatellites. As discussed above, a considerable level of heterogeneity exists in varieties. Therefore the inconsistencies may in the first place be a result of heterogeneities of the parental lines used for breeding.

The pedigrees belong to the South European and the former Soviet Union breeding programmes, and it was postulated that selection for the genes *Rht8* and *Ppd-D1* took place during the production of varieties (Worland et al. 1998a). For the pedigrees, the allele of 192 bp at the locus *Xgwm261-2D* is marked by an asterisk in order to trace the varieties carrying *Rht8* (Fig. 3). It was observed that selection was often accompanied by a linkage drag of 'Akakomugi' alleles in the neighbouring loci to *Rht8*. A diminution of the piece of chromosome 2D originating from 'Akakomugi' during several generations of selection was found. Varieties of the early generations like 'Villa Gloria', 'Ardito'; 'San Pastore' and 'Salto' carried the whole short arm of chromosome 2D of 'Akakomugi'. The varieties of further selections like 'Produttore', 'Funo' 'Orso' and 'Jugoslavia' possessed only from one to three 'Akakomugi' alleles on 2DS including the diagnostic allele at *Xgwm261-2D*.

A benefit of using microsatellites with a known chromosomal location for retrospective genotypic analysis of pedigrees was recently demonstrated in barley (Russell et al. 2000). The analysis of 28 microsatellite loci located on different barley chromosomes allowed the assessment of the level of diversity of European spring barley over time and the detection of regions of the barley genome that have been selected preferentially.

The high level of polymorphism revealed by microsatellites allowed us to use them for the study of transmittance of individual loci as well as chromosomal pieces during extended breeding programmes. Our results show that microsatellites can be successfully used for studying the inheritance of chromosomes within pedigrees of breeding programmes.

Acknowledgements We would like to dedicate this research to the memory of Dr. A.J. Worland (JIC, Norwich, UK) who initiated the current study. We thank Dr. A. Börner (IPK, Gatersleben, Germany) and Dr. V. Korzun (Lochow-Petkus GmbH, Einbeck, Germany) for valuable comments during preparation of the manuscript. This work was supported by a grant of the Deutsche Forschungsgemeinschaft (grant no. Ro 1033/1-5). The experiments comply with current German laws.

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