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Isolation and characterisation of microsatellites from hexaploid bread wheat

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Abstract The development of large panels of simple-to-analyse genetic markers for tagging agronomically important genes and diversity studies in hexaploid bread wheat is an important goal in applied cereal genetic research. We have isolated and sequenced over 200 clones containing microsatellites from the wheat genome and have tested 153 primer pairs for genetic polymorphism using a panel of ten wheat varieties, including the parents of our main mapping cross. A subset comprising 49 primer pairs detects 76 loci, of which 74 can be unequivocably allocated to one of the wheat chromosomes. A relatively low frequency of the loci detected are from the D genome, and these loci show less polymorphism than those from the A and B genomes. Generally, the microsatellites show high levels of genetic polymorphism and an average of 3.5 alleles per locus with an average polymorphism information content (PIC), value of 0.51. The observed levels of polymorphism are positively correlated with the length of the microsatellite repeats. A high proportion, approximately two-thirds, of primer pairs designed to detect simple sequence repeat (SSR) variation in wheat do not generate the expected amplification products and, more significantly, often generate unresolvable PCR products. In general, our results agree closely with those obtained from other recent studies using microsatellites in plants.

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Introduction

In recent years the use of genetic mapping techniques based upon the polymerase chain reaction (PCR) methodology using simple sequence repeat (SSR) markers has begun to supercede the use of restriction fragment length polymorphisms (RFLPs), most notably in mammalian genome studies. For example, a recent mouse map now contains over 4000 microsatellite markers (Dietrich et al. 1994). However, microsatellite markers have been developed less rapidly in plants. The relatively slow adoption of microsatellite technology is due to the high development cost of SSR markers. The recent introduction of the amplified fragment length polymorphism (AFLP) technique (Vos et al.1995), with its capacity for the rapid generation of large numbers of markers, may also have affected the uptake of microsatellite-based markers in plants. It is also apparent that plant chromosomes carry microsatellites at relatively lower frequencies than mammals (Lagercrantz et al. 1993). Nevertheless, microsatellites are ubiquitous and have the potential to provide extremely polymorphic, codominant marker systems in plants (Akkaya et al. 1992; Morgante and Olivieri 1993; Wu and Tanksley 1993). Previous studies suggest that microsatellites occur at high enough frequencies to allow their isolation in relatively large numbers from plant genomes.

Hexaploid bread wheat shows relatively low levels of RFLP, most likely a result of its narrow genetic base (Chao et al. 1989). The formation of hexaploid wheat, in all likelihood, traces back to a single fortuitous hybrid only 10 000 years ago (Hillman 1972). This lack of marker polymorphism in hexaploid wheat necessitates the use of very wide crosses for efficient map generation. RFLPs also show a considerable degree of clustering on the genetic map, and it is possible that other

types of markers may show different patterns of genetic distribution across cereal genomes. There is an unquestionable need for more highly polymorphic genetic marker systems than RFLPs can provide, particularly in crosses and breeding schemes using adapted varieties. Simple sequence repeats have previously been shown to be useful as genetic markers in wheat (Devos et al. 1995; Röder et al. 1995). In order to develop markers for genetic mapping and diversity studies in hexaploid wheat and related species using microsatellites, we have isolated different microsatellite repeats from the wheat genome by hybridisation screens of small-insert genomic libraries. We have isolated a substantial number of SSRs and have screened them for genetic polymorphism using a panel of wheat varieties. Where possible these markers have been assigned to particular wheat chromosomes using aneuploid analysis. There have been some problems with the development of SSRs in wheat, associated with the extremely large genome size and high repetitive DNA content typical of cereal species. We compare our results with those obtained from other recent studies in grasses, most notably those of Röder et al. (1995).

Materials and methods

Genetic stocks/wheat varieties

The varieties used for the evaluation of polymorphism were chosen to represent material from wheat breeding programmes, as well as the parents of the main mapping cross used to construct the RFLP map in this laboratory: 'Cappelle-Desprez', 'Brigadier', 'Herzog', 'Hereward', 'Soissons', 'Timgalen', 'Hope', 'RL4137', and 'Chinese Spring' and a synthetic hexaploid (IPSR 1190903) hereafter referred to as 'Synthetic' (McFadden and Sears 1946; Sears 1976). The aneuploid stocks used for the determination of chromosomal origins of PCR products were the 21 nullisomic-tetrasomic lines of 'Chinese Spring' (Sears 1954).

Library construction/microsatellite isolation

Small insert libraries of the wheat variety 'Chinese Spring' were constructed as follows. DNA (1–10 μg) was digested with *Sau*3AI, and fragments of the desired size range (i.e. 300–500 bp) were size-selected and purified from agarose gels using Prepagene (Biorad) or DEAE paper (Schleicher and Schuell). Purified DNA fragments were ligated into the *Bam*HI site of M13 mp18 or mp19. Ligations were transformed into JM101/JM105 by electroporation (Biorad Gene Pulser). M13 plaques were screened with oligonucleotide probes labeled with γ-[^{32}P] using T4 polynucleotide kinase. Positive plaques were suspended in 1 ml of L-broth and rescreened using serial dilutions from the original plaque suspension. Three dilutions from each clone (10^{-7}, 10^{-8}, 10^{-9}) were plated in ordered arrays onto seeded bacterial lawns in 24-cm-square petri dishes at a density of 70 clones per petri dish and then rescreened with the original probe oligonucletide. Both single positive plaques and phage DNA were isolated by standard small-scale procedures. DNA sequencing was performed with either the AutoRead Sequencing kit (Pharmacia) analysed with an Automated Laser Fluorescence (ALF) DNA sequencer (Pharmacia) or with the Applied Biosystems Prism DNA sequencing kit analysed with an Applied Biosystems ABI373A Automated DNA sequencer.

PCR screening of positive clones

In order to minimise the amount of unnecessary sequencing, a PCR screen was used to check each putative positive SSR-bearing clone for the presence and position of a microsatellite. This enabled the determination of the approximate size of the clone, as well as the position of the SSR within the clone. (PCR screens were performed using three primer pairs, i.e. Reaction no. 1: M13 Forward + M13 Reverse; Reaction no. 2: M13 Forward + SSR primer A, Reaction no. 3: SSR primer B + M13 Reverse). SSR primers A and B are the microsatellite probe repeat sequence and its complementary sequence, respectively. For those clones selected by simultaneous hybridisation with two different oligonucleotide probes, it was necessary to perform the PCR screen using five reactions per clone (i.e. as above, but with two pairs of reactions using different microsatellite repeat primers). This procedure effectively determined whether the clone contained a microsatellite, the position of the microsatellite within the clone and, in some cases, the type of microsatellite. Following Senior and Heun (1993), an arbitrary 8-base-pair tail (5′-TAGCCTAG-3′) was added to the 5′-terminus of the simple sequence repeat oligonucleotide PCR primer.

PCR amplification and product analysis

DNA was extracted from fresh or freeze-dried plant tissue as described by Sharp et al. (1988). PCR primers were designed from the microsatellite flanking sequences by inspection, and primers were chosen to be between 18 and 28 nucleotides in length and with annealing temperatures in the range 58°–65°C. DNA (100 ng) was PCR-amplified in 30-μl reactions containing: 10 m*M* TRIS-HCl, pH 8.3, 2.5 m*M* $MgCl_2$, 10 m*M* KCl, 200 nM of each primer, 200 μ*M* of each dNTP and 2.5 units *Taq* DNA polymerase (Stoffel fragment, Perkin-Elmer). Cycling conditions were as follows: an initial denaturation step of 4 min at 94°C, followed by 30 cycles of 30-s denaturation at 95°C, 1-min annealing at 61°C (unless where mentioned otherwise) and 1-min extension at 73°C. After cycling, the reactions were incubated for 5 min at 73°C.

An aliquot of each PCR reaction was first run on agarose gels to confirm that successful amplification had occurred. Following this, the PCR products were separated either on 6% non-denaturing polyacrylamide or, more commonly, on standard denaturing sequencing gels. For non-denaturing gels, samples were mixed with 1/10 volume of loading buffer (20% Ficoll, 0.25% Orange G) and run on 1-mm-thick 6% polyacrylamide (acrylamide : Bis 29 : 1) gels in 1 × TBE buffer at 200 V for 2–3 h, with the products visualised by ethidium bromide staining. Sequencing gels (6% polyacrylamide (19 : 1 acrylamide : Bis), 8 *M* urea) were run under standard conditions, and the products were visualised by silver staining (Promega Inc) or by use of autoradiography, where γ-[^{32}P]-labeled oligos were used as primers.

Estimation of polymorphism information content

We have estimated the polymorphism information content (PIC), assuming homozygosity of the wheat varieties, following Anderson et al. (1993):

$$PIC_i = 1 - \sum_{j=1}^{n} p_{ij}^2$$

where p_{ij} is the frequency of the jth pattern for marker i, and the sum is made over n patterns.

Chromosome assignment of SSRs

PCR amplifications were performed with DNA from a set of 21 nullisomic-tetrasomic lines of 'Chinese Spring'. The microsatellite loci were assigned to chromosomes corresponding to the nullisomic-tetrasomic template for which either no PCR product was obtained, or one of the PCR products was missing, provided that the templates for all of the other 20 lines generated the relevant amplification product.

Table 1 Locus names, repeat types, repeat lengths, PIC values, numbers of alleles and NT localisations

Marker	Expected PCR product size in CS	Repeat type and length	PIC (no. of alleles)	Location	Additional loci (PIC, no. of alleles)
PSP3001	207	$(CA)_{24}$imp.[b]	0.78 (5)	3B	4D (0.18,2), 3A (0.48,2), 7A (0.42,2) 7A (0.42,2) N[c] (0.42,3)
PSP3003	211	$(CA)_{15}$	0.66 (4)	7D	1A (0.46,3)
PSP3004	266	$(CA)_{10}$	0.48 (2)	1D	–
PSP3009	221	$(CA)_{34}$ imp.	0.66 (4)	6B	–
PSP3012	230	$(GT)_9$	0.42 (2)	3B	–
PSP3013	266	$(TG)_9$	0.34 (3)	7B	–
PSP3019	219	$(CAA)_8$	0.18 (2)	3D	–
PSP3023	88	$(GTT)_8$	0 (1)	2A	–
PSP3027	164	$(GA)_{17}$	0.74 (5)	1A	–
PSP3029	166	$(AG)_{17}$	0.32 (2)	2A	2D (0.32,2), 6A (0.48,2), 6A (0.66,3)
PSP3030	208	$(AG)_{15}$	0.76 (6)	4B	3B (0.46,3), 2B (0.18,2)
PSP3033	192	$(AG)_{22}$	0.76 (5)	7B	–
PSP3034	198	$(CT)_{12}(AT)_{18}$	0.84 (7)	7B	–
PSP3035	170	$(CT)_{12}$	0.54 (3)	7D	3B (0.18,2)
PSP3045	124	$(TC)_{19}$	0.18 (2)	7D	–
PSP3047	184	$(TC)_{18}(TC)_9$imp.	0.64 (4)	3A	–
PSP3050	160	$(CT)_{12}(AT)_{13}$	0.76 (6)	7A	–
PSP3058	189	$(TTC)_{13}$	0.34 (3)	6D	2D (0.18,2), 4A (0.18,2)
PSP3059	118	$(CAG)_5$	0 (1)	3B	–
PSP3061	127	$(CAG)_7$	0 (1)	4A	–
PSP3063	138	$(GAC)_{11}$	0.48 (2)	7A	–
PSP3070	396	$(CA)_{13}$	0.54 (3)	6A	7D (0.18,2)
PSP3071	257	$(TC)_{14}$	0.74 (4)	6A	–
PSP3078	174	$(CA)_{11}$	0.54 (3)	3B	4B (0.69,4), 4B (0,1)
PSP3080	228	$(AC)_{10}$	0.54 (3)	3A	–
PSP3081	182	$(TG)_{12}$imp.	0.72 (5)	7B	7B (0.58,4), 7B (0.62,3), 7B (0.66,4)
PSP3082	203	$(CA)_{12}$	0 (1)	N	7A (0.34,3)
PSP3088	168	$(TG)_{15}$	0.64 (4)	2A	–
PSP3090	166	$(GA)_{13}$	0 (1)	5D	–
PSP3094	130	$(GT)_6$ $(GC)_5$-$(GA)_{10}$	0.76 (5)	7A	–
PSP3100	167	$(GA)_{20}$	0.48 (4)	1B	–
PSP3103	162	$(GA)_{13}$imp.	0.54 (4)	4D	4D (0.78,5)
PSP3107	137	$(GA)_{14}$	0.66 (4)	2B	2B (0,1), 2B (0.34,3)
PSP3112	175	$(GT)_{11}$	0.18 (2)	4D	–
PSP3113	163	$(CA)_{17}$	0.7 (4)	7D	–
PSP3114	116	$(CA)_{12imp.}(TA)_4$	0.58 (3)	7A	–
PSP3119	94	$(TG)_{18}$	0.6 (5)	4A	7B (0.58,4), 7B (0,1)
PSP3123	178	$(TC)_{16}$	0.46 (3)	7D	–
PSP3124	144	$(TC)_{18}$imp	0 (1)	4D	–
PSP3131	144	$(GAA)_{13}$imp.	0.58 (4)	6B	–
PSP3136	185	$(CA)_{18}$	0.58 (3)	1A	–
PSP3137	119	$(GA)_{16}$	0.72 (5)	1D	–
PSP3139	?[a]	(GA)	0.46 (3)	6B	–
PSP3144	170	$(TC)_{12}$imp.	0.74 (5)	3B	–
PSP3151	139	$(TG)_{28}$	0.82 (7)	1A	–
PSP3152	221	$(GT)_{10}(GA)_{21}$	0.76 (5)	6A	–
PSP3153	211	$(TTG)_{12}$	0.62 (3)	2A	–
PSP3159	170	$(CT)_{15}(TG)_6$	0.54 (3)	4A	–
PSP3163	129	$(TC)_5.(TG)_{11}$	0.18 (2)	4B	4B (0.64,3)

NB. Information concerning the locus of predicted size (i.e. presumed to be from locus equivalent to the cloned sequence) is given in fourth and fifth columns, with information on additional loci in the rightmost column

[a] For PSP3139 the repeat length is not known since the sequencing reaction was unable to proceed through the repeat.

[b] 'imp' indicates imperfect repeat

[c] 'N' indicates that the PCR fragment was not localised to a single wheat chromosome

Results

Cloning data and frequency of microsatellites in libraries

The M13 libraries were probed with six different microsatellite sequences: $(CA)_{16}$, $(GA)_{16}$, $(CAA)_{10}$, $(GAA)_{10}$, $(ACG)_{10}$, $(CAG)_{10}$. Approximately 2000 positive plaques were picked from the primary screens of approximately 700 000 M13 clones. Of these, 1500 positive clones were re-screened and, wherever possible, single plaques isolated. From these hybridisations, it was possible to plaque-purify approximately 700 M13 clones. These plaques were then screened by PCR to check the presence, nature and position of the microsatellites within the clones. The addition of an 8-bp tail to the microsatellite repeat primer appears to help anchor the SSR PCR primer and to restrict slippage of the SSR primer along the microsatellite array, resulting in more discrete PCR products (data not shown). Only positive clones with a satisfactory location of the SSR were selected for DNA sequencing.

Sequencing data

Of 277 M13 clones sequenced, 222 were found to contain a microsatellite in the sequenced portion of the clone. Use of the PCR screen obviated the need for sequencing a further 430 clones. Flanking primers from 153 sequences (69%) were synthesised. The majority of microsatellite sequences contain either a $(CA)_n$ or a $(GA)_n$ repeat (approx. 75%), which indicates that, as might be expected, the frequency of these types of dinucleotide repeats is significantly higher than that of the selected trinucleotide repeats in the wheat genome. Approximately 15% of clones contained more than one type of microsatellite repeat, and usually the different repeat types were adjacent, or very nearly so, in the clones.

PCR results and levels of genetic polymorphism

Primers from only 49 of the 153 clones amplify a PCR product of the predicted size from var. 'Chinese Spring', the source of the M13 clones. Table 1 gives information concerning repeat types, lengths and expected PCR product size for these 49 SSRs. The remaining primers either produce no detectable PCR products, incorrectly sized PCR products or a larger number of bands that cannot be genetically analysed with ease. This is based upon, in most cases, the use of a single primer pair for each microsatellite sequence, and it is probable that the use of different primers could generate single resolvable PCR products of the correct size. From Table 1, it can be seen that several clones contain more than one type of microsatellite repeat. Table 1 also shows the number of alleles and the levels of observed polymorphism for the 49 SSRs. Where more than one polymorphic locus has been identified, the information has been given for each locus separately. Figure 1 shows examples of polymorphic PCR products from 3 of these primer sets on silver-stained sequencing gels. Of the 49 primer pairs 14 (29%) detect more than 1 locus. Overall, the 49 primer sets detect 76 loci, with an overall average PIC of 0.46, an average number of alleles of 3.1 per locus and an average of 1.5 loci per primer pair. The additional loci generally generate smaller PCR products that are less polymorphic than the locus originally cloned from 'Chinese Spring'. The loci of predicted size show an average PIC of 0.51 and 3.5 alleles per locus, whereas the additional loci show average PICs of 0.36 and 2.4 alleles per locus. If the 6 'predicted' loci (12%) which are monomorphic are excluded from the data, the average PIC rises to 0.58 and the average number of alleles to 3.8 per locus.

Localisation of microsatellites to chromosomes

The chromosomal locations of microsatellite loci is shown in Table 1. Figure 2 shows an example of the

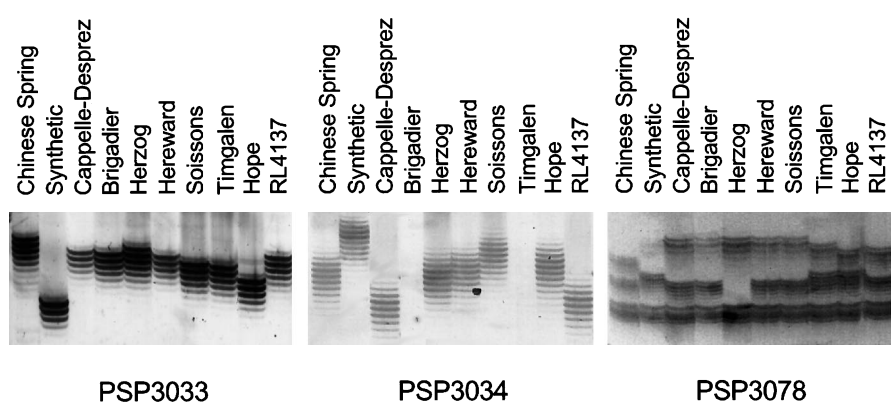

Fig. 1 Variability of microsatellites amplified by PCR in a set of ten wheat accessions. Samples have been run on 6% denaturing polyacrylamide gels and visualised by silver staining

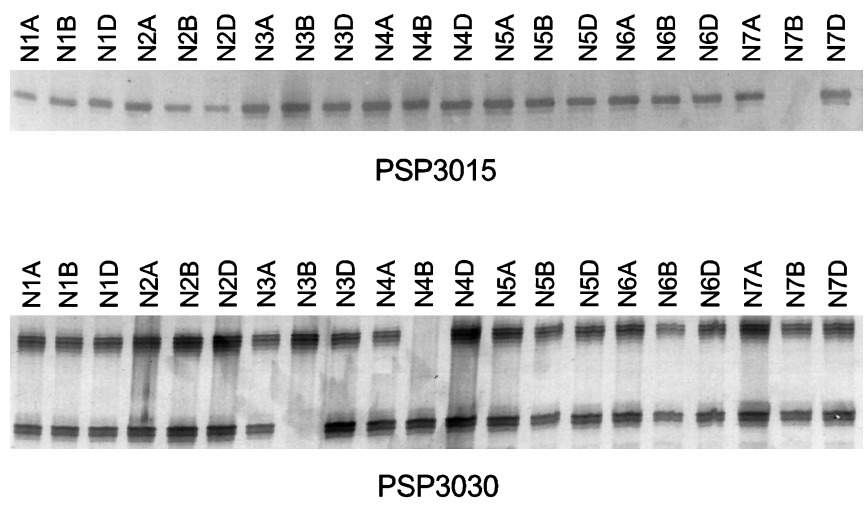

Fig. 2 Localisation of microsatellites amplified by PCR to chromosomes using wheat nullisomic-tetrasomic lines. *Top panel* Single locus for marker PSP3015 localised to wheat chromosome 7B. *Lower panel* Two loci for marker PSP3030 localised to wheat chromosomes 3B and 4B

localisation of three microsatellite loci to different chromosomes of hexaploid wheat. For several of the SSR primer pairs, it has been possible to identify the chromosomal origin of more than 1 PCR product. Overall, it has been possible to assign 74 of the 76 loci to a particular wheat chromosome. These loci are spread across the three wheat genomes as follows: A genome – 27 loci (36%), B genome – 29 loci (38%), D genome – 18 loci (24%). Interestingly, the fewer D-genome loci also show lower levels of polymorphism (average PIC of 0.38) than those from the A or B genomes (average PICs of 0.52 and 0.49, respectively). In only a single case (PSP3001) does one of the additional loci appear to originate from a homoeologous chromosome to the expected locus and, obviously, this could be due to chance. In six cases more than 1 PCR product appears to originate from the same chromosome, suggesting the possibility of duplicated chromosome-specific sequences containing microsatellites. It is not yet clear if these multiple products map to the same locus. In three cases the PCR products are not absent in any of the NT lines, suggesting amplification from a duplicated sequence at two or more chromosomal locations in the wheat genome.

Discussion

Identification of useful microsatellite loci

In this paper we describe the isolation of a substantial number of microsatellites from the wheat genome. During our SSR isolation procedure, over 50% of putative positive clones were lost or discarded during the secondary screening/plaque-purification process. Approximately 60% of the plaque-purified clones were rejected following the PCR screen, obviating the need for the sequencing of over 400 clones. Of 277 clones sequenced, 124 were deemed unsuitable for primer synthesis (55 contained no SSR in the sequenced part of the clone, 69 contained an SSR that was too short or too close to one end of clone). Therefore, as a proportion of the number of positive clones from the first screen, only 10% of the isolated SSRs were selected for primer synthesis. Owing to our stringent selection criteria, we have been able to make PCR primers against a high proportion (55%) of the sequenced clones or 69% of clones containing a microsatellite. The success rates of our primers in detecting analysable (32%) PCR products is similar to that (36%) obtained by Röder et al. (1995). In both studies, approximately one-third of the microsatellites to which primers have been synthesized are of direct use as genetic markers in hexaploid wheat. In our case, these results are based upon the synthesis of a single primer pair for each microsatellite. Overall, this means that, from the original hybridisation screen, only 3% of the positive clones give rise to a useful genetic marker. This high attrition rate of positive clones emphasises the need to make the isolation of microsatellites more efficient.

Given our experience of large attrition rates of positive clones, it may be advisable in the future to opt for efficient SSR enrichment strategies in order to reduce expenditure of time and resources in clone purification (Karagyosov et al. 1993; Kandpal et al. 1994). Future SSR isolation programmes should combine an efficient enrichment strategy with one that avoids the cloning of highly repetitive flanking sequences from the wheat genome. The use of methylation-sensitive restriction enzymes, such as *Pst*I and *Hpa*II, during the enrichment and cloning processes could possibly be used to address this problem (Cheung et al. 1992).

SSR polymorphism

In this study the degree of polymorphism is consistent with that observed by Röder et al. (1995). These authors gave average PIC values of 0.54 among a set of 12 wheat varieties. Based on a set of ten varieties, our microsatellites show an average PIC value of 0.51, which increases to 0.58 if the 6 monomorphic loci are omitted. The lower PICs obtained in the present study may be related to the shorter length of the microsatellite repeats. Röder et al. (1995) report dinucleotide repeats that are considerably longer (i.e. average of 27 repeats in Röder et al. versus 16 in this study). This difference in repeat lengths may be due to the different types of SSR probe used in the library hybridisation screens (i.e. end-labeled oligonucleotide probe versus randomly labeled concatenated repeat), differences in the stringency of hybridisation or differences in the type of phage vectors used. However, given the large difference in microsatellite length, the average PIC values are very similar, suggesting that, beyond a critical length the level of polymorphism exhibited by SSRs does not increase dramatically. Moreover, the mean number of alleles seen by Röder et al. (i.e. 3.2) in their sample of 12 varieties is similar to that from our set of ten genotypes (i.e. 3.5). We have tested a few of our SSRs on larger sets of more diverse material and larger numbers of alleles have been detected (data not shown). The level of polymorphism seen in this study is considerably higher than that shown by RFLPs, although we do not have an estimate based on the same set of varieties used in this study. The data of Chao et al. (1989), when subjected to a PIC analysis, gives an average figure of 0.06.

No effect on levels of polymorphism due to the different types of microsatellite can be seen from our data. The PIC values of $(CA)_n$ repeats average 0.54, while the $(GA)_n$ repeats average 0.53. The seven microsatellites containing two or more different types of dinucleotide repeats have an average PIC of 0.63. None of these average PICs is statistically different from one another. The dinucleotide repeats show an average PIC of 0.55, which is somewhat higher than that shown by the trinucleotide repeats (i.e. 0.28). However, the average repeat length of the trinucleotide repeats is only ten, and three of these microsatellites are monomorphic on our panel of varieties.

The level of polymorphism, as estimated by the number of alleles, shows a positive correlation with the length of the microsatellite repeat from the cloned 'Chinese Spring' sequence. For example, the observed number of alleles for the 49 'expected' loci show a highly significant positive correlation ($r = 0.61$, $P < 0.001$) with the repeat lengths based on the 'Chinese Spring' sequence. The PIC values show a similar relationship with repeat length. If the data of Röder et al. (1995) are subjected to the same analysis no significant correlation is obtained. It is likely that the relationship between repeat length and level of polymorphism is essentially logarithmic in nature, whereby the level of polymorphism attains a maximum once the repeats are greater than a certain length.

Additional products generated by microsatellite primers

The SSR primers generally detect additional loci. The additional loci show lower levels of polymorphism (average PIC of 0.36, 2.4 alleles per locus) and the PCR products are generally smaller in size than those from loci correponding to the 'expected loci'. This raises the possibility that the additional loci may contain a shorter microsatellite repeat than that present in the clones from Chinese Spring, or even no repeat at all. Sometimes the observed size difference between PCR products from different loci exceeds the length of the microsatellite in Chinese Spring, which implies that the microsatellite may be absent or that the flanking sequence has been deleted or rearranged. We feel that it is important to analyse the DNA sequences of these additional products in order to understand the basis of multilocus microsatellite amplification in hexaploid wheat. A common problem when using a set of unrelated material for the analysis of microsatellites is the unequivocal identification of alleles for the same locus for primers detecting multiple loci, especially where the products from the different loci are of a very similar size.

In this study a number of the 153 synthesised primer pairs give rise to a larger number of PCR products than can be resolved or analysed with ease. In several cases these products were observed as smears or stuttered ladder patterns on polyacrylamide gels. Obviously, with the high levels of repetitive sequences present in the wheat genome, it is likely that a relatively large number of microsatellites will be present in flanking DNA sequences that are themselves repetitive sequences. During the isolation of SSRs we have isolated clones which appear to contain short repetitive motifs in the flanking DNA (data not shown). It is important to establish the proportion of wheat microsatellites which are embedded in repetitive flanking DNA and discover if such microsatellites can be rendered useful by judicious primer selection. However, again, the use of methylation-sensitive restriction digests for the initial library construction may reduce the proportion of non-useful amplification products.

Primer transportability

We have seen very little evidence of homoeologous amplification in this analysis, which somewhat contrasts with the results of Röder et al. (1995), who reported at least three cases of homoeologous amplifi-

cation. It is not yet known if SSRs isolated from wheat represent homoeologous sequences and whether the failure of primers to amplify across the three wheat genomes is due to polymorphism in the flanking DNA. There are important questions concerning the transferability of PCR primers raised against genomic sequences, across the three wheat genomes and beyond, into other grass genomes. The data of Röder et al. (1995) suggest that these types of PCR marker may not transfer well to other species in the way that RFLPs have been shown to do very effectively. Our results also show a low level of transportability across the three wheat genomes and to other cereal genomes (data not shown). These results contrast greatly with results obtained using PCR primers raised against cDNAs, which exhibit very high levels of transportability across cereal genomes (unpublished results). This also contrasts with data from mammalian microsatellite studies, whereby primers raised against one species often work on others (Schloetterer et al. 1991). This question of marker transfer between species is assuming an ever increasing level of significance in genetic studies in grasses and other organisms, given the current level of investment in comparative genetic mapping. The development of PCR-based markers, which are expensive to develop, with the ability to transcend species barriers in grasses is likely to be an important area of marker development in the coming years.

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