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Fifty new microsatellite loci for the wheat genetic map

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Abstract Hexaploid bread wheat (*Triticum aestivum*) has low levels of RFLP. Simple sequence repeats, however, show high levels of polymorphism and are therefore especially useful in intervarietal breeding applications. We present 53 newly mapped microsatellite loci for the wheat genetic map, 41 primary loci and 12 additional loci from these same primer pairs. Markers have been accredited with a quality score on a scale of 1–5 which describes the complexity of the amplification product profile from each primer pair.

Key words Wheat · Microsatellite markers · Genetic map

Introduction

Detailed genetic maps are now available for hexaploid bread wheat (*Triticum aestivum*, $2n = 6x = 42$) and include some 2000 loci either mapped directly or with inferred locations from the aligned genomes of related Triticum species. Details of the loci mapped in wheat can be found in the Wheat Gene Catalogue (Macintosh et al. 1995) and further updates published in the Annual Wheat Newsletter or in the GrainGenes database. The majority of the marker loci on these maps are restriction fragment length polymorphisms (RFLPs) which have limited use in intervarietal applications because of

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Table 1 Microsatellite repeat structures, chromosomal locations and expected product sizes

Locus	Microsatellite	Product size[b]
Xpsp3003-7D	$(CA)_{15}$	211[c]
1A		195
Xpsp3007-4D[a]	$(TG)_5$-$(TC)_d(TA)_5(CA)_{14}$	176
Xpsp3028-4A[a]	$(GA)_{2d}$	122
Xpsp3029-2A	$(AG)_{21}$	150
-6A		(245)
-6A		166
Xpsp3030-4B	$(AG)_{15}$	208
-2B		385
-3B		172
Xpsp3037-5B[a]	$(CT)_{16IMP}$	240
Xpsp3039-2A[a]	$(CT)_{30}$	152
Xpsp3058-6D	$(CCT)_3(TCT)_{12}$	189
-2D		290
-4A		165
Xpsp3065-5B[a]	$(CAG)_4$-$(AGG)_5$	266
Xpsp3078-3B	$(TG)_{11}$	178
-4B		184
Xpsp3079-6B[a]	complex	271
-4D[a]		298
-7D[a]		178
Xpsp3081-7B	$(TG)_{12IMP}$	123
Xpsp3094-7A	$(GT)_6(GC)_5$-$(GA)_{10}$	130
-7D[a]		198
Xpsp3142-2A[a]	$(CAA)_{12+}$	645
-4A[a]		(292)
Xpsp3200-6D[a]	$(AAG)_{16}$	170

[a] Markers which were not previously described in Bryan et al. (1997) or Devos et al. (1995)
[b] Product sizes are those expected in 'Chinese Spring' except where the figures are given in parentheses, which are the size of the product in 'Synthetic' where 'Chinese Spring' is null
[c] This marker has not been mapped

the low levels of polymorphism observed and the resources required for large-scale screening in breeding situations. Microsatellite markers, on the other hand, do display reasonable levels of polymorphism in wheat and are becoming available (Devos et al. 1995; Roder et al. 1995; Bryan et al. 1997). This paper supplements the information provided in Bryan et al. (1997) and provides map locations for many of the microsatellite loci previously described.

Materials and methods

The majority of the microsatellites mapped are described in Bryan et al. (1997). A re-evaluation of some microsatellite-containing clones previously discarded because of complex amplification patterns provided a further 12 markers (Table 1). Most of the microsatellite loci were mapped on 119 F_2 progeny (or F_3 derivatives/families) of a 'Chinese Spring' × 'Synthetic' population (Devos 1992). Two loci, *Xpsp3028* and *Xpsp3037*, were mapped using 'Chinese Spring' × SQ1, a doubled haploid population of 95 individuals (Quarrie et al. 1994).

Results

Primer design

Primer design is critical for the production of useful markers from microsatellite-containing clones, as demonstrated by the development of primers for clone PSR3081. The initial primers, which were designed by eye, produced a pattern of four fragments, all shown by nullisomic-tetrasomic analysis to be located on chromosome 7B, and later all mapped to the same locus on 7BL. Primers, redesigned using 'Oligo' primer analysis software (National Biosciences) and used with the same polymerase chain reaction (PCR) conditions reduced the marker to a single product (see Table 1). The design of primers for PCR is a rather empirical procedure which is exacerbated by the often small length of the flanking sequences available. Moreover, the large percentage of repetitive DNA in the wheat genome makes it likely that some primers will recognise multiple sites. It is possible that many of the complex patterns seen with some of the primers can be simplified by exploring other priming sites. A re-evaluation of primers for some previously discarded microsatellite-containing clones has also been productive and has provided 12 more loci, which are identified in Table 1. Table 1 also provides information not given in Bryan et al. (1997) for eight primers which amplify products mapping at multiple locations.

Mapping

Forty-one primary microsatellite loci were mapped, i.e. those for which the 'Chinese Spring' allele matched

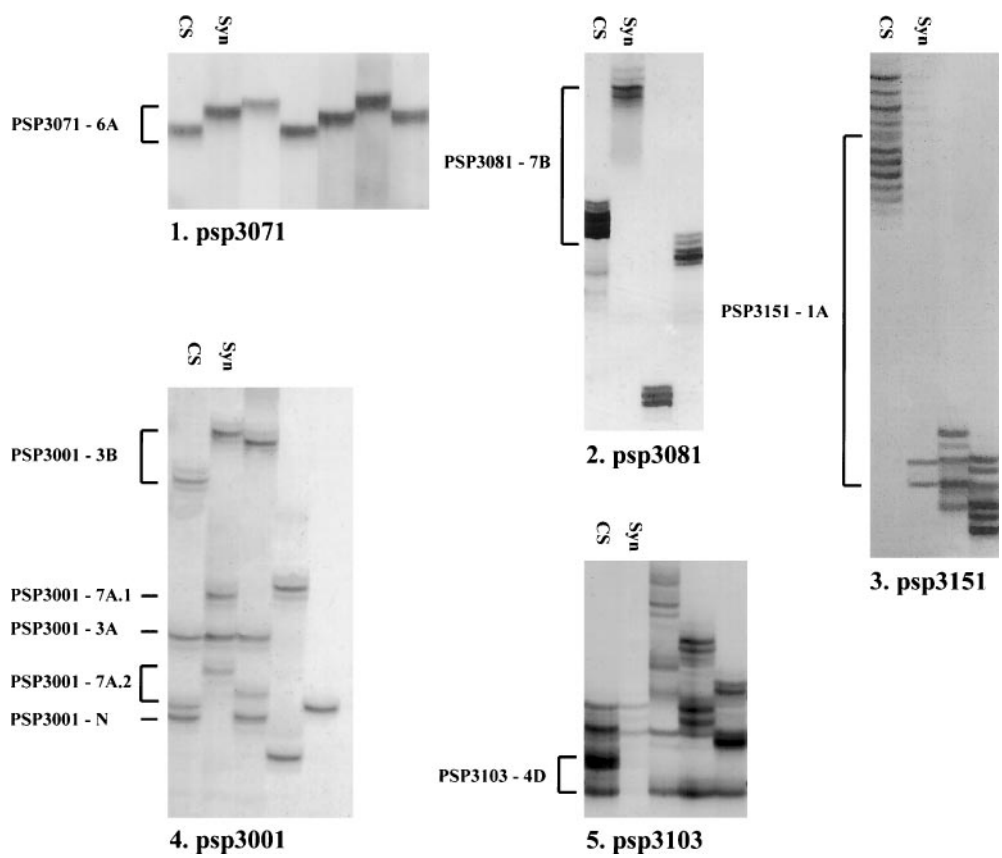

Fig. 1 Examples of microsatellite primer amplification products in different wheat varieties. Markers have been classified with a quality score which we feel best describes their usefulness: **1** single product recognising a single locus, little or no stuttering; **2** single product recognising a single locus, some stuttering, however easy to score; **3** single locus, profound and/or irregular stuttering, sometimes difficult to use; **4** multiple products and multiple loci, easy to score but sometimes difficult to relate polymorphic loci to those previously characterised and mapped; **5** multiple products and multiple loci, difficult to relate bands between genotypes.

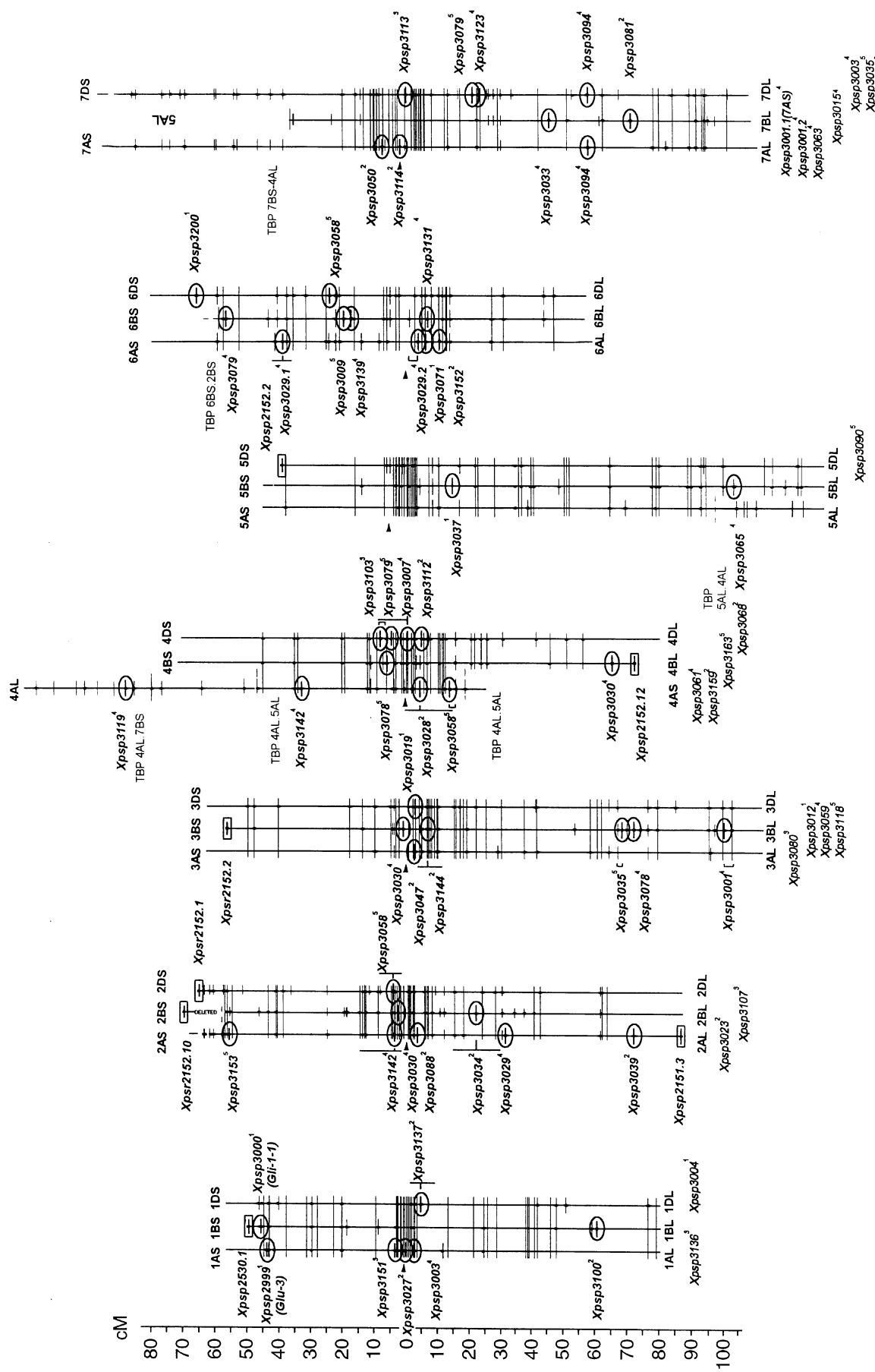

Fig. 2 A hexaploid wheat genetic map including 51 new microsatellite loci. Mapped *Xpsp* loci are identified with a *circle*. Two further microsatellite loci, *Xpsp2999(Glu3)* and *Xpsp3000(Gli-1-1)*, reported by Devos et al. (1995) are included. The map also shows the location of seven telomeres mapped by Mao et al. (1998) and the chromosomal locations of a further 21 microsatellites which we were unable to map within chromosomes. This map can be aligned with the JIC RFLP map by reference to Gale et al. (1995)

exactly the molecular weight expected from the DNA sequence. A further 12 loci were provided by additional products produced by these same primer pairs. Although these are loci recognised by microsatellite primers, it is not known whether these the PCR products also contain microsatellite repeats. The fragment size in 'Chinese Spring' for these additional loci is given in Table 1.

Consideration of multiple band PCR profiles of some microsatellites raises the issue of quality of individual microsatellite markers. Microsatellites, or more specifically the primers used to identify them, vary considerably in their ease of use, which is likely to be very important for large-scale screening applications or when several markers are to be multiplexed for simultaneous use. Based on the degree of stuttering (the amplification of several artifactual fragments from a single locus, with both greater and lesser numbers of repeat units than the template fragment) observed on sequencing gels and the number of fragments produced from distinct loci, which is a particular problem in polyploids such as wheat, we have devised a scale of 1 (best) to 5 (useful but only with care), which is explained by example in Fig. 1, and used to anotate the mapped microsatellites in Fig. 2 where the individual loci are associated with the quality scores.

Conclusions

Of the 53 loci, most (21) were mapped in the A genome and fewest (13) in the D genome. The map in Fig. 2 shows the location of the *Xpsp* loci and may be compared with the more extensive RFLP maps reported by Gale et al. (1995). Although there are many fewer points there is no reason to speculate that the distribution of microsatellite and RFLP loci are different.

It can be seen that in only one case have probable homoeologous loci been mapped (*Xpsp3094*, 7A and 7D). This confirms that the value of microsatellite markers will be in the main restricted to intra-specific analysis. Microsatellite markers are likely to be of limited use for comparative analyses, or even introgression studies involving wild species related to wheat. Nevertheless, the locus specificity and high level of polymorphism associated with microsatellites must make them the marker of choice for practical wheat breeding today. This project, together with the work of the Gaterleben group, has provided a reasonable start at coverage of the wheat genome.

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The microsatellite markers are available as primer pairs for academic research applications. Requests should be addressed to Dr. M.D. Gale.

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