Theor Appl Genet (2000) 101:1291–1298

A.P. Davierwala · W. Ramakrishna · P.K. Ranjekar
V.S. Gupta

Sequence variations at a complex microsatellite locus in rice and its conservation in cereals

Received: 10 March 2000 / Accepted: 14 April 2000

Abstract In an attempt to study changes associated with microsatellites in rice, the DNAs of cultivated rice, including indica and japonica varieties, and wild rice genotypes were amplified by the polymerase chain reaction with primers flanking the $(GATA)_n$ and $(AC)_n$ repeats at a microsatellite-containing locus OS1E6 (Genebank accession number AFO16647) previously reported from a *Pst*I rice (var. Malkolam) genomic library in pUC18. Eight alleles of varying sizes were obtained which were cloned and sequenced. Sequencing data indicated that the size variations of the different alleles were due to differences in the repeat number as well as to sequence variations in the region flanking the microsatellite motifs. In order to study the presence of this complex microsatellite-containing locus of rice in different cereals, their DNAs were amplified using primers flanking the OS1E6 locus. It was found that this locus was present in the various cereal genotypes analyzed, indicating its conservation across different cereal members.

Keywords Microsatellite · Sequence variation · Genetic diversity · Cereals

Introduction

Microsatellites or simple sequence repeats (SSRs) are 2–5 bp in length and are widely dispersed throughout the nuclear genome of eukaryotes (Tautz and Renz 1984). They are considered to be the most informative molecular genetic markers (Tautz et al. 1986; Tautz 1989) for DNA fingerprinting and varietal identification (Ramakrishna et al. 1994; Rongwen et al. 1995; Olufowote et al. 1997; Udupa et al. 1999), genome mapping (Chen et al. 1997; McCouch et al. 1997), gene tagging and gene diagnostics (Blair and McCouch 1997; Caskey et al. 1992), and studies of population dynamics (Yang et al. 1994). Recently, microsatellites are being exploited at a rapid pace in crop plants and, as a result, marker-assisted breeding is considered to play an important role in agriculture. Microsatellites exhibit a high degree of polymorphism mainly due to variation in the copy number of the basic motif. In order to detect variations due to repeat-number change among different genotypes, it is essential to develop sequence-tagged microsatellite markers by screening a genomic library with microsatellites followed by the selection and sequencing of the clones containing the microsatellite inserts and synthesizing primers flanking the microsatellite motif (Litt and Luty 1989; Weber and May 1989). These primer pairs can amplify locus-specific DNA fragments and are of great use in genomic, systematic, evolutionary and ecological studies even between different laboratories. SSRs have been successfully amplified in different plant species such as *Arabidopsis thaliana* (Treuren et al. 1997), citrus (Kijas et al. 1995) and soybean (Maughan et al. 1995; Powell et al. 1995).

In our laboratory the utility of microsatellites, such as $(GATA)_4$, $(GACA)_4$, $(GGAT)_4$, $(CAC)_5$, $(GAA)_6$ and $(TG)_{10}$, and inter-simple sequence repeats has been shown in profiling rice germplasm (Gupta et al. 1994; Ramakrishna et al. 1994; 1995; Joshi et al. 2000). We have also studied the repeat expansion at a microsatellite locus, RM122 containing the dinucleotide $(GA)_n$ in various rice cultivars and related wild forms (Ramakrishna et al. 1998). Further we have studied the evolutionary and polymorphic organization of the *knotted-1* homeobox in cereals (Deshpande et al. 1998, 1999). Although different cereals have evolved from a common ancestor, there are vast differences in their genome sizes (Bennett and Smith 1991). The massive expansion in the size of some of these cereal genomes is due to a large amplification of

Communicated by P.M.A. Tigerstedt

A.P. Davierwala · W. Ramakrishna[1] · P.K. Ranjekar · V.S. Gupta (✉)
Plant Molecular Biology Unit, Division of Biochemical Sciences,
National Chemical Laboratory, Pune 411008, India
e-mail: vidya@ems.ncl.res.in
Tel.: 91-20 5893173/5893034, Fax: 91-20 5884032

Present address:
[1] Department of Biology, H339 Hansen Building,
Purdue University, West Lafayette, Indiana 47907, USA

repetitive DNA, which evolves rapidly and hence diverges substantially with speciation (Moore et al. 1993). Among cereals, rice has the smallest genome size and is considered to be an ideal crop to study cereal genome evolution and transpecies crop improvement. We were, therefore, interested to examine whether noncoding microsatellite-containing loci are also conserved/variable in different cereals. In the present study, we have analyzed inter- and intra-specific variations at a complex locus containing tetra- and di-mer microsatellites using various cultivars and a landrace of rice, a number of cereals, and related wild germplasm. Homologous loci from different species may or may not contain the same copy number of repeat motifs and these too may be at the same position; hence, we have further sequenced these loci from representative *Oryza* species in order to study sequence divergence in these genotypes.

Materials and methods

Plant material and DNA extraction

Seeds of different cultivars and wild forms of rice were obtained from the Directorate of Rice Research, Hyderabad, and the Agricultural Research Station, Vadgaon, India. Seeds of pearl millet and maize were procured from the Indian Agricultural Research Institute, New Delhi, while seeds of wheat were obtained from the Agharkar Research Institute, Pune, India. Seeds of barley, oat, rye and *Aegilops* were made available by USDA ARS, National Small Grains Collection, Aberdeen, USA.

Total genomic DNA was extracted from leaves of rice, barley, maize, oat, rye and wheat by the CTAB method (Rogers and Bendich 1988), while pearl millet DNA-extraction was by the method described by Sharp et al. (1988).

Locus-specific PCR of different rice genotypes

A plasmid clone, OS1E6 (Genebank accession number AFO16647), identified by screening a partial *Pst*I genomic library of rice (var. Malkolam) in pUC18 in our previous studies (Chowdari et al. 1998), contained three microsatellite repeats namely $(GATA)_n$, $(AC)_n$ and $(AG)_n$. Primers flanking the $(GATA)_n$ and $(AC)_n$ repeats were synthesized and used for locus-specific amplification by PCR in various rice genotypes. The sequence of the primers used in PCR was as follows:
Primer 1:5′ AGCAGTAGAGGGAGATGA 3′
Primer 2:5′ TCTGTCATGCAGCCATGC 3′

Each reaction was performed in a 10 µl volume (Wu and Tanksley 1993) containing 1 µCi each of α^{32}P dATP and α^{32}PdCTP, 25 µM each of dATP and dCTP, 200 µM each of dGTP and dTTP, 1 × reaction buffer (10 mM TRIS-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, pH 8.3), 500 µM of Spermidine, 10 ng of each primer, 0.24 U of Taq DNA polymerase (Boehringer Mannheim, Germany) and 50 ng of DNA. DNA amplifications were carried out in a Perkin Elmer Cetus thermal cycler wherein an initial denaturation of 2 min was performed at 94°C followed by 40 thermal cycles of denaturation for 1 min at 94°C, primer annealing at 55°C for 2 min and extension at 72°C for 1.5 min with a final extension at 72°C for 5 min. The amplification products were separated on 6% denaturing polyacrylamide gels containing 7 M Urea and 0.5×TBE buffer (pH 8), electrophoresed at 1500 V and visualized by autoradiography.

Cloning and sequencing of PCR products

Alleles of different sizes, obtained by amplification of wild rice and cultivars, were cloned and sequenced after carrying out amplification reactions in a larger volume. Each reaction was performed in a 100 µl volume containing 200 µM of each dNTP, 1× reaction buffer (10 mM TRIS-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, pH 8.3), 500 µM of Spermidine, 100 ng of each primer, 2.5 U of *Taq* DNA polymerase (Boehringer Mannheim, Germany) and 500 ng of DNA using the amplification conditions as described earlier. The PCR products were loaded on 1.4% agarose gels and eluted using a gel extraction kit (QIAGEN, USA). The ligation reaction was carried out at 16°C overnight in 20 µl with 2 U of T4 DNA ligase (USB, UK), 50 ng of pMOS blue T-vector (USB, UK) in 660 mM of Tris HCl pH 7.6, 66 mM of $MgCl_2$, 100 mM of DTT and 660 mM of ATP. The ligated mixture was transformed into XL1-Blue competent cells and the recombinant clones obtained were screened for inserts, where positive clones were sequenced by Sanger's dideoxy chain-termination method using a Sequenase version 2.0 DNA sequencing kit (USB, UK).

Locus specific amplification in cereals

The primers employed for the amplification of different rice genotypes were used to amplify the DNA of barley, maize, oat, pearl millet, rye, wheat and their wild relatives. The PCR reaction was performed in a 10 µl volume as described earlier.

Data analysis

The sequences of the amplified and cloned products were aligned using the CLUSTAL W (1.60) multiple alignment package (Thompson et al. 1994).

Results

Sequence variations at a complex microsatellite-containing OS1E6 locus in rice

In order to assess the genetic diversity in different wild and cultivated rice genotypes at the complex microsatellite-containing locus OS1E6, we amplified 11 different rice genotypes at that locus. PCR-amplification of different wild and cultivated genotypes of rice using primers flanking the OS1E6 locus resulted in one common allele of size 220 bp (which was the expected size of the allele in the original OS1E6 clone obtained from the Malkolam genomic library) in all the rice genotypes studied. In addition to this, several other alleles were also obtained in the different rice genotypes examined. Among the *Oryza sativa* varieties studied, in addition to the 220-bp allele, the rice cultivars Basmati-370 and Taichung-65 and the landrace, Tambdarambhog resulted in alleles of sizes 226 bp, 268 bp and 192 bp, respectively. In the wild rice species studied, *Oryza rufipogon* (AA genome) gave a single band of 220 bp, while *Oryza officinalis* (CC), *Oryza granulata* (GG), *Oryza minuta* (BBCC) and *Oryza latifolia* (CCDD) showed alleles of sizes 224 bp, 227 bp, 228 bp and 234 bp, respectively, in addition to the common band at 220 bp.

Since OS1E6 was a complex locus containing two different microsatellite repeats, $(GATA)_n$ and $(AC)_n$, we decided to determine the microsatellite sequence responsible for size variation among the different alleles and

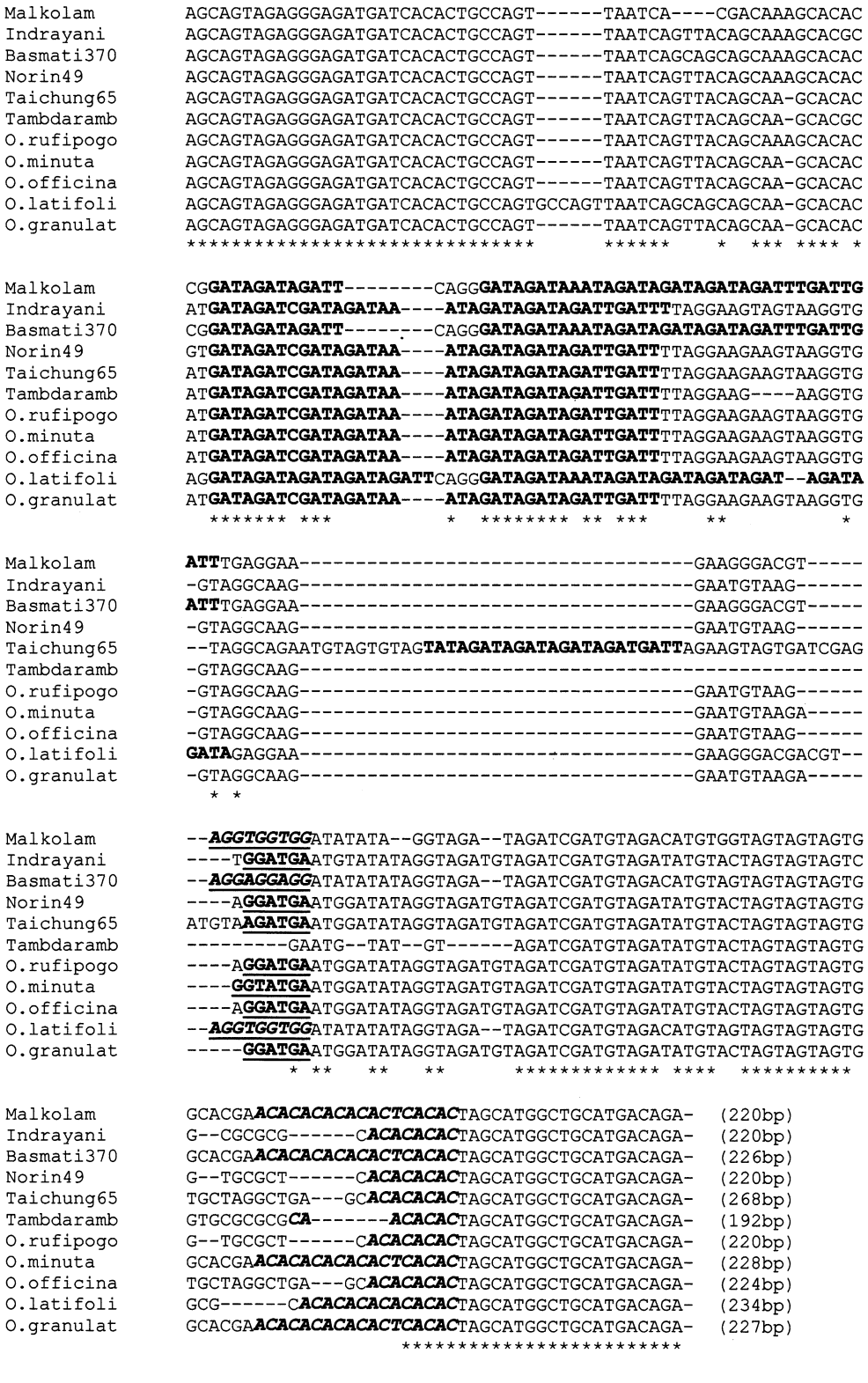

Fig. 1 Multiple sequence alignment, using the CLUSTAL W package, at the OS1E6 locus in genotypes of cultivated and wild rice. Gaps during alignment are indicated by *dashes*. *Stars* denote the nucleotides that are identical. $(GATA)_n$ repeats are highlighted in *bold*, while $(AC)_n$ repeats are in *bold italics* and the remaining repeats are *underlined and in bold*

also to examine whether alleles of the same size had the same sequence. We, therefore, eluted, cloned and sequenced the alleles of varying sizes from different wild and cultivated rice and performed multiple sequence alignment using the CLUSTAL W package (Fig. 1) As seen in this figure, alleles of different sizes showed variations in both the repeat regions as well as the flanking sequences. The 220 bp allele of Indrayani, Norin-49 and *O. rufipogon* showed a similar sequence composition. With the exception of one additional nucleotide (T) in *O. minuta*, the sequences of *O. minuta* and *O. granulata* were also similar.

Table 1 Sequence variation at the $(GATA)_n$ and $(AC)_n$ repeat regions and a 3rd site at the complex microsatellite-containing OS1E6 locus

Group	No. of repeats	Sequence at repeat region
(A) At the $(GATA)_n$ repeat		
Group 1 (Indrayani, Tambdarambhog, Norin-49, Taichung-65, *O. rufipogon*, *O. officinalis*, *O. minuta*, *O. granulata*)	9	GATA GATC $(GATA)_2$ AATA $(GATA)_2$ $(GATT)_2$
Group 2 (Malkolam, Basmati-370)	12	$(GATA)_2$ GATT CAGG $(GATA)_2$ AATA $(GATA)_3$ GATTT $(GATT)_2$
Group 3 (*O. latifolia*)	14	$(GATA)_2$ $(GATA)_2$ GATT CAGG $(GATA)_2$ AATA $(GATA)_3$ $(GATA)_3$
(B) At the $(AC)_n$ repeat		
Group 1 (Indrayani, Tambdarambhog, Norin-49, Taichung-65, *O.rufipogon*, *O.officinalis*)	4	$(AC)_4$ [Tambdarambhog CA $(AC)_3$]
Group 2 (Malkolam, Basmati-370, *O. minuta*, *O. granulata*)	9	$(AC)_6$ TC $(AC)_2$
Group 3 (*O. latifolia*)	7	$(AC)_7$
(C) At the third motif		
Group 1 (Indrayani, Norin-49, Taichung-65, *O. rufipogon*, *O. officinalis*, *O. minuta*, *O. granulata*)		GGATGA [Taichung-65 AGATGA], [O.minuta GGTATGA]
Group 2 (Malkolam, Basmati-370, *O. latifolia*)		AGGTGGTGG

All the sequences shown in Fig. 1 basically contained two stretches of repeats, $(GATA)_n$, and $(AC)_n$. Analysis of the GATA repeat region revealed three types of repeats in the different rice allele sequences studied. As shown in Table 1, Indrayani, Tambdarambhog, Norin-49, Taichung-65, *O. rufipogon*, *O. officinalis*, *O. minuta* and *O. granulata* formed the first group containing nine GATA-like repeats in a continuous stretch, while Malkolam and Basmati-370 comprised the second group containing 12 GATA like repeats in a discontinuous stretch (interrupted by a CAGG motif) and *O. latifolia* with 14 GATA repeats made up the third group. In all these rice genotypes, the GATA repeat was an imperfect repeat containing motifs such as GATT, GATC and AATA. In addition, in Malkolam, Basmati-370 and *O. latifolia* the GATA repeat region was discontinuous due to a CAGG motif within the GATA repeat.

A close analysis of the AC repeat region revealed the presence of three types of motifs, as seen in Table 1. Indrayani, Norin-49, Taichung-65, *O. rufipogon* and *O. officinalis*, containing four perfect AC repeats, constituted the first group of AC repeats. In addition, Tambdarambhog, containing 3 AC and 1 CA motif, was also included in the first group. All the wild rice and cultivars included in group 1 of the GATA repeats, with the exception of *O. minuta* and *O. granulata*, were also present in the first group of AC repeats. The second group of AC repeats comprised Malkolam, Basmati-370, *O. minuta* and *O. granulata*, all of which contained nine imperfect AC repeats, while *O. latifolia* containing seven perfect AC repeats formed the third group.

In addition to these two main regions, GATA, and AC, a third site comprising different microsatellite sequences was also observed. As seen in Table 1, Indrayani, Norin-49, Taichung-65, *O. rufipogon*, *O. granulata* and *O. minuta* contained the motif GGATGA. In Taichung-65 the motif changed to AGATGA, due to a point mutation, while in *O. minuta*, due to the insertion of a T, the motif obtained was GGTATGA. All the rice genotypes which fell into the first group of GATA repeats also contained the GGATGA motif. Malkolam, Basmati-370 and *O. latifolia*, which formed the second and third groups of GATA repeats, contained the motif AGGTGGTGG at this site.

Taichung-65 showed the presence of an additional $(GATA)_n$ motif, 37 nucleotides after the first stretch of $(GATA)_n$. This region contained six GATA motifs preceded by a $(TGTAG)_2$ motif (Fig. 1).

Conservation of the OS1E6 locus in cereals

Since the OS1E6 locus was found to be present in all the wild and cultivated rice species studied, it was of interest to check if this locus was also conserved across different cereals. Hence, we amplified the DNA from 15 diverse genotypes of wheat, maize, barley, oat, pearl millet, rye and their wild relatives using OS1E6 flanking primers (Fig. 2). As seen in Fig. 2, a common band of 220 bp was present in all the cereals used in our study except for *Hordeum bogdanii* (lane 9). In all the wild rice and cultivars analyzed, this common band of

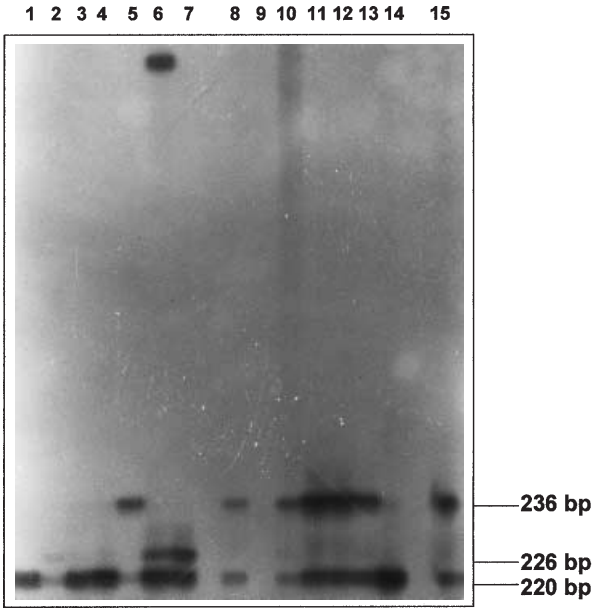

Fig. 2 Radioactive PCR-amplification of the OS1E6 locus from different cereals. The genomic DNA of several cereals was amplified and loaded on 6% denaturing polyacrylamide gels. *Lanes 1 to 15* are as shown in Table 2

220 bp was also found to occur indicating the presence of this allele in all the cereals examined. In addition to this common allele, several other alleles were also detected. A cultivated tetraploid wheat, *Triticum dicoccum*, showed another allele of 226 bp (lane 2), while a wild, diploid wheat, *Aegilops tauschii*, had a band of 236 bp (lane 5). Maize cultivar Sikkim Primitive and its distant wild relative, *Chionachne koenigir* var. Chio-3, showed the presence of an allele at 226 bp (lanes 6 and 7, respectively) with *Chionachne* also containing a third band of very high molecular weight (lane 6). Barley genotypes *Hordeum marinum* and *Hordeum vulgare*, three oat genotypes *Avena sativa*, *Avena fatua* and *Avena vaviloviana* and rye had a second allele of 236 bp (lanes 8 10, 11, 12, 13 and 15, respectively). *H. bogdanii* showed no amplification, indicating the presence of a null allele at this locus.

Thus, four totally different alleles were observed when the OS1E6 locus was amplified from 15 different cereals, indicating its conservation during evolution (Table 2).

Genetic diversity in cereals using primers flanking a mapped microsatellite locus in rice

Since the rice OS1E6 locus was conserved in different cereals, we extended this analysis to check if other mapped loci of rice were also conserved in cereals. PCR-amplifications were performed in diverse cereals at a $(GA)_n$-harboring microsatellite locus RM122 (Wu and Tanksley 1993), which we had earlier amplified and sequenced from different wild and cultivated rice genotypes (Ramakrishna et al. 1998). A common band of 227 bp was observed in all the cereals, i.e. maize, barley, oat, pearl millet and rye, except wheat. In addition, maize, barley and pearl millet showed bands of higher molecular weight. In wheat, *Triticum dicoccoides* revealed the presence of four different bands while *Triticum durum* landrace Narsimgarh 111 and *Triticum aestivum* cv Chinese spring had only one common band of 243 bp, which was also present in *T. dicoccoides*. This band of 243 bp was not observed in any of the other cereals studied. *T. dicoccum* and *Ae. tauschii* did not show any amplification at this locus, possibly indicating the presence of a null allele in both of them. On the other hand, *H. bogdanii*, which showed no amplification at the OS1E6 locus, gave two bands when amplified with primers flanking the RM122 locus. Amplification of the RM122 locus from 15 different cereals thus resulted in the identification of seven alleles of varying sizes (Table 2).

Table 2 List of different cereals used in the present study along with the number of alleles amplified from each at the OS1E6 and RM122 loci

Strain. no.	Cereal	Genotype	Accession Number	Number of alleles of OS1E6 locus	Number of alleles of RM122 locus
1	Wheat	*Triticum dicoccoides*	3515	1	4
2	Wheat	*Triticum dicoccum* var. Ex-33	33b	2	–
3	Wheat	*Triticum durum* landrace Narsimgarh 111	1633	1	1
4	Wheat	*Triticum aestivum* cv Chinese spring	226	1	1
5	Wheat	*Aegilops tauschii*	PI 21098	2	–
6		*Chionachne koenigir* var Chio3	DK93	3	3
7	Maize	*Zea mays* cv Sikkim Primitive	DK94/6714	2	2
8	Barley	*Hordeum marinum*	PI 200341	2	2
9	Barley	*Hordeum bogdanii*	PI 269406	–	2
10	Barley	*Hordeum vulgare* ssp. *vulgare*	CLHO 7382	2	1
11	Oat	*Avena sativa*	CLAV 1110	2	1
12	Oat	*Avena fatua*	CLAV 2526	2	1
13	Oat	*Avena vaviloviana*	PI 412733	2	1
14	Pearl millet	*Pennisetum glaucum* cv 841B	–	1	2
15	Rye	*Secale cereale* ssp. *ancestrale*	CLSE 107	2	1

Discussion

Sequence variations in the repetitive and non-repetitive region: a cause for allelic polymorphism at microsatellite loci

It is now well-established that microsatellite-containing sequences, i.e. non-coding sequences, evolve at a faster rate as compared to coding sequences. Consequently, a large number of informative nucleotide variations can be detected on studying a shorter stretch of DNA while comparing closely related species. $(GATA)_n$ is the most-frequent tetranucleotide reported in rice with about 270 $(GATA)_n$ motifs in the entire genome, while the microsatellite $(AC)_n$ is the second highest in frequency in the rice genome (Panaud et al. 1995; McCouch et al. 1997). Since the OS1E6 locus contains both $(GATA)_n$ and $(AC)_n$ repeats, we thought that it would be interesting to exploit this locus to analyze fine variations in the rice genome. In this context, we showed that PCR-amplification of the OS1E6 locus from different wild rice and cultivars resulted in eight alleles varying in size from 192 bp to 268 bp. Moreover, the *O. sativa* primers showed amplification in other *Oryza* species at the same PCR stringency level indicating that these sequences might be located in the conserved region of the genome. However, the alleles obtained in different *Oryza* species were of different sizes and no two species had a minimum of two alleles of the same size.

To the best of our knowledge there is very little information available regarding the sequences of different alleles of a microsatellite locus within the same species or between different species. With this background in mind the sequence variation of different alleles at the OS1E6 locus observed in our analysis revealed that an increase in the number of AC repeats was responsible for the increase in length of the alleles of *O. minuta* and *O. granulata*. The increased size of the Taichung-65 allele, on the other hand, was due to an insertion of a $(TGTAG)_2$ motif followed by a stretch of GATA repeats containing six imperfect motifs. However, for the other wild rice and cultivars studied, mere variations in the repeat region could not account for the varying sizes of the alleles. In these rice genotypes, differences in allele sizes were due to variations in the adjoining non-repetitive region as well. In the case of Malkolam, Basmati-370 and *O. latifolia*, there was an increase in repeat number with a subsequent deletion in the sequence immediately following the $(GATA)_n$ repeat. In addition, in *O. latifolia* there was also an expansion of the AC repeat, followed by the deletion of sequences just preceding this repeat, thus resulting in an allele of the observed size of 234 bp. In the case of Tambdarambhog, the decreased size of the allele was due to a deletion in the region between the GATA and AC repeats. In addition, in Malkolam, Basmati-370 and *O. latifolia* the GATA repeat was interrupted by the insertion of a CAGG motif.

Inter-specific variation at a microsatellite-containing intergenic region in the mitochondrial genome of the genus *Pinus* was due to expansion and contraction of the repeat motif (Soranzo et al. 1999). Phelps et al (1996) demonstrated microsatellite repeat variation within the *y1* gene of maize and teosinte. Studies of microsatellites in the TNF (tumor necrosis factor) region (in the major histocompatibility complex of humans and primates) and in chromosome 4 indicated that the interspecific differences in allele length were not always due to changes in repeat number, but also due to insertions and deletions in the sequences flanking the repeats and interruption of perfect repeats which were correlated with microsatellite stability (Blanquer-Maumont and Crouau-Roy 1995; Garza et al. 1995; Crouau-Roy et al. 1996). Deka et al. (1995) also reported that, in humans, the increase or decrease in allele size was not in increments of 2 bp but could involve insertions/deletions of single nucleotides or might be due to the involvement of some other more-complex phenomena. Another school of thought suggested that the most-likely mechanism for evolution of simple sequence repeats was slipped-strand mispairing (Levinson and Gutman 1987; Charlesworth et al. 1994). The significant correlation observed between the average number of repeats and the amount of variation obtained in our analysis indicates that replication slippage may be the molecular mechanism responsible for generating variability at the OS1E6 locus in rice.

Microsatellite loci: a tool for comparative genome mapping in cereals

Comparative genetic studies using rice, wheat, maize, oat, sorghum, foxtail millet and sugarcane have demonstrated that gene content and gene order are highly conserved between species within the grass family, both at the map and megabase levels (Devos and Gale 1997). Since microsatellites are non-coding sequences, their conservation between species is less marked. However, since all the plant genomes contain repetitive regions we wanted to see if the OS1E6 locus of rice was conserved in different cereals. We found that the OS1E6 locus was highly conserved in all the cereals studied except *H. bogdanii*, where no band was obtained on amplification with primers flanking the OS1E6 locus. This indicated that in *H. bogdanii* either the OS1E6 locus was absent or that there were mutations at the primer-binding site. In order to test that the conservation of the microsatellite-containing OS1E6 locus was not an isolated phenomenon, we amplified the DNAs of different cereals with RM122 flanking primers, and found that the $(GA)_n$-containing locus was also conserved in almost all the cereals studied. *T. dicoccum* and *A. tauchii* were the only two cereals that did not produce amplification at the RM122 locus, indicating that this locus was not conserved in these two wheat genotypes. The other wheat species, viz. *T. dicoccoides*, *T. durum* landrace Narsimgarh 111 and *T. aestivum* cv Chinese spring, showed amplification of this locus but the size of the alleles obtained was higher than expected indicating that the RM122 locus was selectively conserved in wheat. All the

other cereals, viz. maize, barley oat, pearl millet and rye, yielded at least one allele of the expected size of 227 bp, indicating that the $(GA)_n$-containing RM122 locus was well-conserved in these cereals.

Roder et al (1995) showed that accessions of barley and rye could be amplified using primers flanking wheat microsatellites, while the $(GGC)_n$-containing microsatellite locus, RTL011, from rice could be amplified from maize and bamboo (Zhao and Kochert 1993) indicating that some microsatellite loci are present in conserved regions of the genome. Primers flanking repeats in the tobacco chloroplast genome were observed to amplify DNA from members of the family Solanaceae and some of these primers were also functional in most other angiosperms (Weising and Gardner 1999).

Being non-coding sequences, additions and deletions in microsatellites occur on a rapid time scale and, hence, their conservation across different genera is thought to be low. However, we showed that these microsatellite-containing loci were conserved in different cereals and could, therefore, be used as tools for comparative mapping with a common set of primers. Since we found that microsatellite loci were conserved across different species and genera, a large proportion of polymorphic microsatellites could be cross-amplified and further used to search for economic-trait loci.

Acknowledgements The authors acknowledge the financial support from the Rockefeller Foundation, USA. A.P.D acknowledges the Council of Scientific and Industrial Research, New Delhi, India, for a Senior Research Fellowship. The experiments described in this manuscript comply with the current laws of the country.

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