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ORIGINAL PAPER

Development and genetic mapping of SSR markers in foxtail millet [*Setaria italica* (L.) P. Beauv.]

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Abstract SSR markers are desirable markers in analysis of genetic diversity, quantitative trait loci mapping and gene locating. In this study, SSR markers were developed from two genomic libraries enriched for (GA)n and (CA)n of foxtail millet [*Setaria italica* (L.) P. Beauv.], a crop of historical importance in China. A total of 100 SSR markers among the 193 primer pairs detected polymorphism between two mapping parents of an F_2 population, i.e. "B100" of cultivated *S. italica* and "A10" of wild *S. viridis*. Excluding 14 markers with unclear amplifications, and five markers unlinked with any linkage group, a foxtail millet SSR linkage map was constructed by integrating 81 new developed SSR markers with 20 RFLP anchored markers. The 81 SSRs covered nine chromosomes of foxtail millet. The length of the map was 1,654 cM, with an average interval distance between markers of 16.4 cM. The 81 SSR markers were not evenly distributed throughout the nine chromosomes, with Ch.8 harbouring the least (3 markers) and Ch.9 harbouring the most (18 markers). To verify the usefulness of the SSR markers developed, 37 SSR markers were randomly chosen to analyze genetic diversity of 40 foxtail millet accessions. Totally 228 alleles were detected, with an average 6.16 alleles per locus. Polymorphism information content (PIC) value for each locus ranged from 0.413 to 0.847, with an average of 0.697. A positive correlation between PIC and number of alleles and between PIC and number of repeat unit were found [0.802 and 0.429, respectively ($P < 0.01$)]. UPGMA analysis revealed that the 40 foxtail millet cultivars could be grouped into five clusters in which the landraces' grouping was largely consistent with ecotypes while the breeding varieties from different provinces in China tended to be grouped together.

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Introduction

Foxtail millet [*Setaria italica* (L.) P. Beauv.] has been grown in China for a long history. Archaeological evidence indicated that foxtail millet was cultivated in some sites near the Yellow River before ca. 5000–6000 BC (Li and Wu 1996). Because grains of foxtail millet are enriched for various amino acids and nutritive minerals and the crop possesses some advantageous traits, e.g. high photosynthesis efficiency and drought tolerance, foxtail millet was the most important crop in northern China in ancient times. Even now the annual growing area of foxtail millet in China still reached about 1 million ha (http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567). Due to its smaller genome size which is close to the rice genome (Sivaraman and Ranjekar 1984) and the nature of relatively strict self-pollination, foxtail millet is suitable for genetic and molecular manipulation. However, in-depth research on foxtail millet is scarce since it has become a minor cereal crop since the 1980s. A lot of the previous studies focused on uncovering geographical origin of foxtail millet and

exploring the evolutionary relationships between foxtail millet and its wild relative (*S. viridis*) by archaeology, morphology, cytology and isozymes (Li et al. 1945; De Wet et al. 1979; Küster 1984; Kawase and Sakamoto 1984; Jusuf and Pernes 1985; Li et al. 1995). Particularly, DNA markers such as RAPD, RFLP and AFLP were used in this kind of studies (Fukunaga et al. 1997, 2002; Schontz and Rether 1998, 1999; Li et al. 1998; Le Thierry d'Ennequin et al. 2000; Fukunaga and Kato 2003).

Genetic linkage maps are important for gene tagging, quantitative trait loci (QTL) mapping, marker-assistant selection and research on comparative genomics. For foxtail millet, the only available genetic linkage map was constructed by Wang et al. (1998) using RFLP markers, which provided foundation for genetic analysis of some traits. Doust et al. (2004, 2005) used the map to locate QTL for vegetative branching and inflorescence architecture. Furthermore, Devos et al. (1998, 2000) did comparative genomic research among foxtail millet, rice and pearl millet. They found that some homologous chromosomal fragments existing among these genomes, indicating close relationships of the gramineous crops. Due to the limiting number of RFLP markers and inconvenience of operation, other marker systems are needed. SSRs, or simple sequence repeats, which are distributed widely and throughout plant genomes, are valuable genetic markers because of their high polymorphism and simple operation procedures (Tautz and Renz 1984; Matsuoka et al. 2002). However, until now none of SSR markers are available for foxtail millet although some expressed sequence tag-based SSR can be used (Jia et al. 2007). Therefore, the objectives of this study were to develop SSR markers and to construct SSR-based genetic linkage map in foxtail millet.

Materials and methods

Plant materials

Two foxtail millet varieties, i.e. "Shengtiebang" which is a landrace in Shanxi Province, and "Tiegu 10" which is a variety widely grown in northeastern China, were selected for microsatellite library construction. Forty foxtail millet accessions from different regions of China were randomly selected to test the usefulness of the new developed SSR markers (Table 1). The two mapping parents (*S. italica* acc. B100 and *S. viridis* acc. A10) and 124 F_2 progenies for linkage map construction were obtained from John Innes Center, kindly provided by Dr M. Gale. All the plant materials above were grown in the experimental field of the Institute of Crop Science, Chinese Academy of Agricultural Sciences. The fresh leaves were collected at the five-leaf stage for DNA isolation.

Isolation of microsatellite fragments and primer design

Two microsatellite enriched libraries for (GA)n and (CA)n were constructed. The first library (library I) was constructed by using "Shengtiebang" whose genomic DNA was fragmentized by restricted enzyme *Rsa*I (NEB, Beijing, China) while the second library (library II) was constructed by using "Tiegu 10" whose genomic DNA was fragmentized by ultrasonic method. The procedures used were similar to that described by Edwards et al. (1996) with some modifications. Briefly, the genomic DNAs digested by *Rsa*I were ligated directly to 5′-end phosphorylated adaptors being constituted by Rsa21 (CTCTTGCTTACGCGTGGACTA) and Rsa25 (p-TAGTCCACGCGTAAGCAAGAGCACA) (Edwards et al. 1996). The genomic DNAs fragmentized by the ultrasonic method was firstly filled by Klenow fragments of DNA polymerase to obtain blunt-end fragments, then phosphorylated by T4 polynucleotide kinase and ligated to the adaptors mentioned above. The ligated fragments were amplified with the Rsa21 primer. Following hybridization with two microsatellite probes [$(GA)_{15}$ and $(CA)_{15}$] fixed on Hybond-N + nylon membranes (Amersham, IL, USA), the DNA fragments hybridized to Hybond-N + nylon membranes were washed down by boiling in water for 5 min. The fragments obtained were then amplified with the Rsa21 primer. The enriched libraries were constructed by ligating the PCR products into pMD-18 vector (Takara, Dalian, China). Top10 competent cells (Tiangen, Beijing, China) were used for transformation of cloned DNA fragments.

An anchored PCR method was used in library screening according to Hayden and Sharp (2001). The anchored PCR used for positive clones filtering was performed with a 10-μl volume: 1 μl overnight-cultured bacterium as template, 5 pmol each of pMD-18 forward (GAGCGGATA ACAATTTCACACAGG) and reverse (CGCCAGGGTT TTCCCAGTCACGAC) primer in combination with SSR anchored primer PGA6 and PCA6 (Fisher et al. 1996), 1 μl 10 × PCR buffer, 200 μM dNTPs, 0.75 U *Taq* DNA polymerase, and ddH_2O added to 10 μl. A touchdown program described by Hayden and Sharp(2001)was used for amplification: for the first 12 cycles, 60 s at 92°C, 60 s for annealing and 30 s at 72°C; for later cycles, 30 s at 92°C, 30 s for annealing and 30 s at 72°C. The annealing temperature for the first cycle was 65°C, and was reduced by 1°C/cycle for the next six cycles. The annealing temperature of 59°C was for another five cycles. For the remaining 25 cycles, the annealing temperature was assigned to 57°C. The positive clones were sent to Sunbiotech Company (Beijing, China) for sequencing.

Sequences obtained were analyzed and duplicated sequences were removed using BLAST software (http://www.ncbi.nlm.nih.gov/). The primers flanking simple sequence repeats were designed using DNAMAN software

Table 1 List of 40 foxtail millet accessions used in analysis of genetic diversity

Serial no.	CNG no.[a]	Name	Origin	Serial no.	CNG no.	Name	Origin
1	0003573	"Zuan Zi Huang"	Gansu	21	0024169	Yugu 1	Henan
2	0003659	"Jiu Gen Qing"	Gansu	22	0025657	"Bai Zhua Gu"	Qinghai
3	0003726	"Gao Gan Hong"	Gansu	23	0025666	"She Pi Gu"	Qinghai
4	0003732	"18"	Gansu	24	0025667	"Da Jin Gu"	Qinghai
5	0003902	"Bai Liu Sha"	Shaanxi	25	0026839	K-3645	Russia
6	0005556	"Huang Mao Gu"	Shanxi	26	0026840	K-3680	Russia
7	0005859	"Ya Ta Che"	Shanxi	27	0026939	"Ba Di"	Hebei
8	0005965	"Hong Miao Hui"	Shanxi	28	0026948	9120-3-1	CAAS
9	0006036	"Da Mao Gu"	Shanxi	29	0027023	1123 F-3-1-1	CAAS
10	0006164	"Ruan Gu"	Shanxi	30	0027155	Zheng 111-(1)	Henan
11	0006442	"Bian Qu 1"	Shanxi	31	0027173	Zheng Fu 47-(1)	Henan
12	0006484	"Wa Hui Bai"	Shanxi	32	0027201	Jingu 17	Shanxi
13	0010461	"Zi Gan Gu"	Henan	33	0027212	Jian 19	Shanxi
14	0014845	ISE-89	India	34	0027215	Jian 38	Shanxi
15	0021960	"Ai Ri60-1"	Hebei	35	0027383	SR3522	CAAS
16	0022206	"Ba-910934"	Hebei	36		Yugu 5	Henan
17	0022815	"Shui Li Hong"	Hebei	37	0027144	Yugu 8	Henan
18	0022816	"Xi Sui Huang"	Hebei	38	0027261	Tiegu 12	Liaoning
19	0022825	"Bai Gu"	Hebei	39	0019600	Lugu 7	Shandong
20	0022826	"Xiao Bai Gu"	Hebei	40	0027184	Lugu 10	Shandong

The accessions with quotation marks are landraces of Chinese origin and most of the others are varieties developed

[a] CNG No. refers to the serial number of the accession preserved in China National Genebank (CNG) (similar to PI No. of germplasm in the U.S.A.)

(version 6.0). The main criteria for primer design were 17–24 nucleotides long, GC content ranging from 35 to 70% and annealing temperature ranging from 45 to 60°C.

SSR assays

The primers designed were first used to amplify 4 of 40 foxtail millet accessions. PCR reactions were performed on PTC-100 Thermal cycler (MJ Research Inc., USA) in 10 μl volumes containing 50 ng of genomic DNA, 200 μM dNTPs, 1 μl 10 × PCR buffer, 0.5 μM each of forward and reverse primer, 0.75 U *Taq* DNA polymerase (Tiangen, Beijing, China). The PCR profile included an initial denaturation step at 94°C for 4 min, followed by 35 cycles of 40 s at 94°C, 40 s at annealing temperature (45–60°C) and 1 min at 72°C. Finally, an extension of 72°C for 5 min was followed. Afterwards, 3 μl of loading buffer (98% deionized formamide, 10 mM EDTA, bromophenol blue and xylene cyanol) was added to each reaction. Samples were denatured at 94°C for 5 min, and cooled for 5 min on ice. A total of 5 μl of each sample was subjected to electrophoresis at 80 w on 5% denaturing polyacrylamide gels for 40 min. After electrophoresis, gels were silver-stained as described in Bassam et al. (1991). The SSRs with clear and scorable amplification were selected for further diversity study and linkage map construction.

Linkage map construction

The SSRs showing polymorphism between the two parents of the mapping population were used to genotype the 124 F_2 progenies. The segregation of each SSR marker was tested by chi-square test to determine if they were fit to expected Mendelian segregation ration. Then the segregation data of SSRs developed in this study were integrated with the 20 RFLP mapping data obtained previously and kindly provided by Dr K. Devos. The linkage map was constructed using MAPMAKER version 3.0 (Lander et al. 1987). A LOD score of 3.0 with a maximal map distance of 50 cM were set to identify linkage groups. The Kosambi mapping function was used to convert recombination frequencies into map distances (Kosambi 1944).

Analysis of genetic diversity

To verify the usefulness of the SSR markers developed, 37 markers as listed in Table 2 were used to analyze genetic diversity of the 40 foxtail millet accessions listed in

Table 2 Polymorphism for 37 SSRs in 40 foxtail millet accessions used in the present study

SSR marker	Repeat type	Number of alleles (A)	Polymorphism information content (PIC)
p2	(GT)33	7	0.790
p3	(GT)34	11	0.768
p5	(CA)27	4	0.570
p8	(AC)26	7	0.768
p12X	(AC)29	7	0.754
p16	(AC)16	9	0.815
p17X	(AC)10…(AC)9	7	0.745
p20	(CA)33(GA)11	5	0.681
p29	(GT)42	8	0.757
p44	(CA)22	4	0.645
p68	(CA)17…(CA)9	5	0.759
p74	(AC)14	4	0.554
p80	(CA)26(GA)17	5	0.734
p85	(CA)26	4	0.538
p87	(GA)21	4	0.475
p88	(AC)5(GT)22	8	0.738
p89	(GT)31	6	0.715
p92	(TG)12	4	0.691
p95X	(GA)29	7	0.808
b101	(CT)33	9	0.800
b102	(CT)31	9	0.816
b106	(CT)27	4	0.621
b115	(CA)18	4	0.413
b116	(GT)25	3	0.476
b122	(GT)31	6	0.789
b125	(CT)19(CA)13(CACGG)11	4	0.452
b127	(CA)24	7	0.793
b129	(CA)24	7	0.771
b142	(CA)29(TA)4	7	0.807
b147	(CT)35	6	0.678
b158	(GT)14	4	0.487
b161	(GA)21	4	0.609
b163	(CT)23	6	0.802
b165	(CT)36	6	0.752
b166	(CT)24	9	0.823
b194	(CT)24	12	0.847
b200	(GA)24	5	0.755
Average		6.16	0.697

Table 1. The amplified bands were scored based on 1/0 (presence/absence) system, but the missing bands were scored as 9. Genetic similarities (GS) were estimated using Nei and Li coefficient (Nei and Li 1979). Cluster analyses were carried out on the matrix of GS using the Unweighted Pair Group Method using Arithmetic Averages (UPGMA) clustering algorithm. The similarity matrix and dendrogram were constructed with the program NTSYS-pc 2.11 (Rohlf 1995). The parameters for SSR polymorphism evaluation included number of alleles per locus (A) and polymorphism information content (PIC) ($PIC_i = 1 - \sum P_{ij}^2$, P_{ij} represents the frequency of *j*th allele for loci *i*) (Anderson et al. 1993). Based on the amplification data of the 40 foxtail millet accessions, correlation analysis among PIC, number of repeat unit and number of alleles for SSR was performed by SAS software (version 8.0).

Results

Development of SSR markers

From the first library (library I), a total of 570 positive clones were sequenced. Among which, 169 (29.6%) were unique sequences containing a microsatellite suitable for primers' design, 134 (23.5%) were redundant, 62 were sequences that SSR were too close to one sequence end, and the remaining clones did not contain any SSR. From the second library (library II), a total of 430 positive clones were sequenced. Among which, 100 (23.2%) were unique sequences containing a microsatellite suitable for primers designed, 98 (22.7%) were redundant, 55 were sequences that SSR were too close to one sequence end, and the remaining clones did not contain any SSR. Finally, 269 (26.9%) primer pairs flanking microsatellite motifs were designed.

The 269 primer pairs were pre-screened on one accession of *S. viridis* and four foxtail millet accessions, i.e. Bai Liu Sha, Jiu Gen Qing, Lugu 10 and Yugu 8. Totally 193 of the 269 primer pairs amplified clear and scorable profiles. In addition, 14 of the 193 primer pairs produced monomorphic profiles and the remaining primer pairs produced 2 to 4 alleles for each locus.

Construction of SSR Linkage map

The 193 primer pairs which have been pre-screened in four foxtail millet cultivars were tested for amplification in the two parents of the mapping population (see Supplementary material). A total of 104 (53.8%) SSR primers detected polymorphism. However, after amplification of the 124 F_2 progenies, four of the 104 primer pairs gave monomorphic amplification (segregated in only one of the parents). Thus, the remaining 100 SSRs that segregated in both of the parents were used for linkage map construction. Among the 100 SSRs, 86 (86%) were derived from dinucleotide motifs. The number of SSRs containing CA/GT motif was 46 while the number of SSRs containing GA/CT motif was 40. The remaining 14 SSRs were compounds.

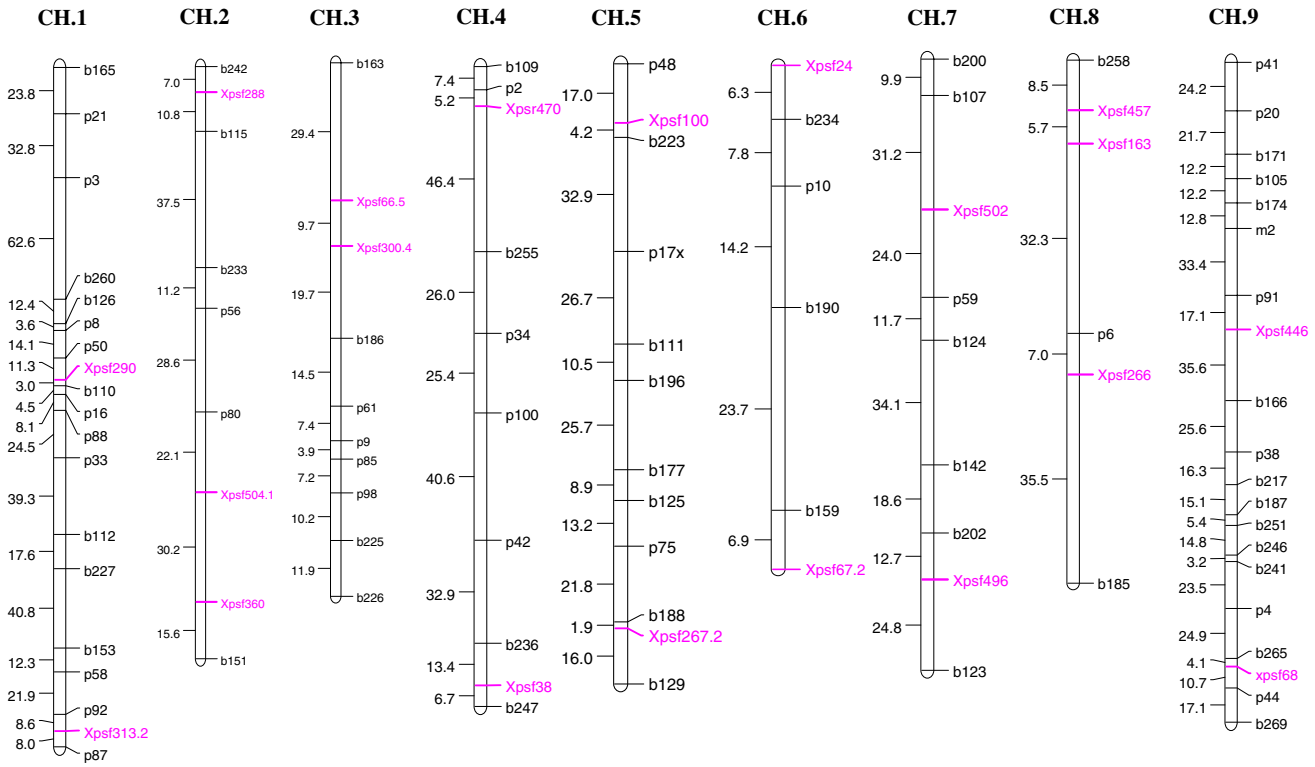

Fig. 1 SSR linkage map of foxtail millet. Markers with letters of "Xpsl" are anchored RFLP markers

Because 14 SSR markers with unclear amplification were discarded, genotyping data of the 86 SSR markers mentioned above were combined first with those of the 20 RFLP anchored markers which were already mapped on linkage map (Wang et al. 1998). Linkage analysis indicated that five markers were unlinked with any of the nine linkage groups. The other 81 of the 86 SSR markers were mapped on linkage map (Supplementary Table 1), making the linkage map consisting of 101 markers (Fig. 1).

The 81 SSR markers covered the nine chromosomes of foxtail millet, with Ch.8 harbouring the least (3 SSRs) and Ch.9 the most (18 SSRs). The number of SSRs mapped on other chromosomes was 17 on Ch.1, 6 on Ch.2, 8 on Ch.3, 8 on Ch.4, 10 on Ch.5, 4 on Ch.6 and 7 on Ch.7, respectively. The map spanned 1,654 cM, with an average distance of 16.4 cM. The longest chromosome was Ch.1, covering 349.3 cM while the shortest chromosome was Ch.6, covering 58.9 cM. One gap (>50 cM) existed between marker p3 and marker b260 on Ch.1.

Analysis of genetic diversity and relationships in forty foxtail millet accessions

To further detect polymorphism level of the new developed SSR markers, 37 primer pairs randomly selected were used to amplify DNA of 40 foxtail millet accessions. A total of 228 alleles were detected for the 37 SSRs, with an average of 6.16 alleles per locus (Table 2). Primer b116 produced the least alleles (3), while primer b194 produced the most (12). The PIC values of these 37 SSR loci ranged from 0.413 to 0.847, with an average PIC value of 0.697 per loci.

The correlation among PIC, number of repeat unit and number of alleles for the 37 SSRs were analyzed. Very significant positive correlations between PIC and number of repeat unit and between PIC and number of alleles per locus were found, with the correlation coefficients of 0.429 and 0.802 ($P < 0.01$), respectively. Moreover, significant correlation between number of repeat unit and number of alleles was also found, with the correlation coefficient of 0.394 ($P < 0.05$) (Table 3).

Table 3 Analysis of Pearson correlations among PIC, number of alleles per locus and number of repeat unit for the 37 SSR markers used in the study

	PIC	Number of alleles per locus	Number of repeat unit
PIC	1		
Number of alleles	0.802 (**)	1	
Number of repeat unit	0.429 (**)	0.394 (*)	1

(*) and (**) indicate significance at 0.05 and 0.01 level, respectively

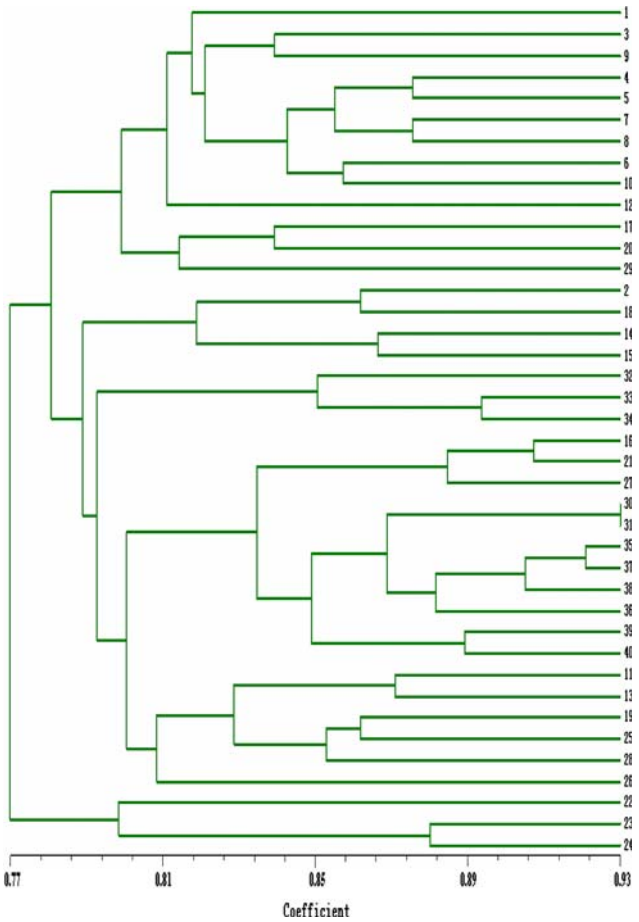

Fig. 2 Dendrogram of 40 foxtail millet accessions based on SSR markers

After UPGMA analysis, the dendrogram with 40 foxtail millet cultivars was constructed (Fig. 2). Two groups could be unambiguously formed at the genetic similarity coefficient of 0.77. One group included three cultivars "Bai Zua Gu", "She Pi Gu" and "Da Jin Gu", all of which originated from Qinghai Province, China. The other group included the remaining 34 foxtail millet accessions which originated from regions except Qinghai. When the genetic similarity coefficient was at about 0.8, five groups could be clustered. Group 1 included 13 accessions, most of which were the landraces from the Loess Plateau region and North China Plain region. Group 2 was a mixed group, including four accessions from different regions. Group 3 included three modern varieties from Shanxi Province. Group 4 included 17 accessions, which could be divided into two sub-groups, that is, one mixed sub-group including six accessions, the other sub-group including the remaining 11 modern varieties. Group 5 also included three landraces from Qinghai Province.

Discussion

Development of SSR markers in foxtail millet

Although SSRs have been used in diversity study, linkage map construction and marker-assisted selection for their easy transferability, high polymorphism and codominance, the process of SSR marker development is rather laborious and expensive. Previous studies showed that the microsatellite enrichment level ranged from 11 and 99% (Edwards et al. 1996; Ueno et al. 2003; Pandey et al. 2004). In our study, the enrichment levels for libraries I and II reached 87 and 80.5%, respectively, indicating that the anchored PCR program modified from Hayden and Sharp (2001) was effective. However, only 169 (33.8%) and 100 (28.9%) of the microsatellite-containing clones from library I and library II, respectively, were suitable for primer design and the others were mainly redundant sequences. These redundant sequences were likely to be caused by selective amplification of certain sequences during enrichment, or some SSRs belonged to certain repeat sequences families (Schmidt et al. 1991).

In plants, the most frequently occurring dinucleotide repeat is TA (Toth et al. 2000). However, this type of repeat is not suitable for hybridization because of its autocomplementary feature. Moreover, some studies supported that the GA/CT repeat type showed higher abundance than CA/GT repeat type in plants (Toth et al. 2000; Sargent et al. 2003; Merdinoglu et al. 2005) while other researchers obtained a contrary conclusion (Maguire et al. 2000; Marinoni et al. 2003). In this study, it was found that 138 (51%) of the 269 unique SSRs were CA/GT dinucleotide repeat type, 101 (37.7%) were GA/CT dinucleotide repeat type, and the remaining were compound type. The frequency of CA/GT repeat was higher than that of GA/CT repeat type in the foxtail millet genome. This may be due to the higher efficiency of hybridizing with CA/GT probe than GA/CT or the anchored PCR program was more effective for screening of CA/GT type repeat than GA/CT repeat type.

Construction of SSR linkage map in foxtail millet

Some SSR loci were clustered in certain genome regions, which has been observed in sorghum (Bhattramakki et al. 2000) and rice (McCouch et al. 2002), possibly due to non-uniform distribution of recombination events in mapping populations (Castiglioni et al. 1999). In the present study, the microsatellites were not also randomly or evenly distributed on the nine chromosomes of foxtail millet. For instance, Ch.1 and Ch.9 harboured 43% of the 81 SSR markers. The probable reasons included: (1) much higher polymorphism of the SSRs on these two chromosomes

existed between the two mapping parents; and (2) more SSRs on these two chromosomes were isolated than those on other chromosomes. It should be pointed out that only a few SSR markers were located on some chromosomes of foxtail millet. For example, only three SSRs were located on Ch.8 in this study. However, interestingly, a lot of RFLP markers were located on this chromosome by Wang et al. (1998). This is possibly due to that RFLP markers are mainly derived from single-copy or low-copy regions of plant genomes while most of SSRs are distributed in intergenic regions. Some researchers proposed that the use of BAC libraries for SSRs isolation rather than enriched libraries could map more SSRs in low-density regions (Cregan et al. 1999; Bhattramakki et al. 2000).

In rice and maize, the segregation distortion ratio of SSR markers in mapping populations ranged from 10 to 23% (Xu et al. 1997). This may associate with mapping population type (the DH population and RIL population usually show higher distortion ratios), selection against closely linked lethal or sub-lethal genes, linkage with incompatibility alleles, and man-made errors etc. In addition, some studies reported that during hybridization between genetically distant parents (e.g. cultivated and wild relatives), the alleles of wild species could be frequently lost, which led to severe distorted segregation (Xu et al. 1997). In the present study quite a few SSRs mapped on the linkage map also segregated distortedly from the expected Mendelian ratio (28/81). The mapping parents of our study are the foxtail millet variety B100 and its wild relative species *S. viridis*. The results showed that about 90% of distortedly segregated SSRs deviated towards B100. Similar phenomenon was observed for RFLP markers by Devos et al. (1998) using the same population. It could be presumed that many of the alleles of wild parent *S. viridis* were lost in the F_2 mapping population, resulting in the considerable distorted segregation of SSR markers in this study. Intraspecific hybrid pollen sterility reported previously in foxtail millet (Kawase and Sakamoto 1987) may also be one of the factors to distort the segregation.

Although the first SSR linkage map was constructed in this study, there is still some work which is needed to be done in the future. Particularly, more SSR markers should be added to the linkage map which can be useful in QTL locating since only 81 SSRs were mapped here, leading to the average map distance of 16.4 cM and some big gaps between the markers (e.g. between marker b227 and b153 on Ch.1, marker b255 and Xpsr470, p100 and p42 on Ch.4).

Usefulness of SSR markers developed

In this study totally 193 SSR markers were developed and they may be used in analysis of genetic diversity. To verify the usefulness of these markers, 37 SSR markers were randomly chosen to detect genetic variation of 40 foxtail millet. The average number of alleles per locus was 6.16, which was comparable to other studies, e.g. 3.9 in lychee (Viruel and Hormaza 2004), 5.7 in barley (Russell et al. 1997) and 7.38 in wheat (Prasad et al. 2000). The average PIC value in our study was 0.697, higher than that reported in soybean (0.60, Powel et al. 1996), but lower than that reported in maize (0.72, Pejic et al. 1998). For other DNA markers such as RFLP and RAPD, the PIC value ranged from 0.3 to 0.4 (Powel et al. 1996). Therefore, the SSR markers developed in the present study were informative and suitable for diversity study of foxtail millet germplasm resources. However, it should be pointed out that the foxtail millet accessions used here are only from limited geographical origins (most from China) and thus the parameters mentioned above can not reflect the general profile of diversity in this crop.

Although no correlation was found between number of repeat unit and the polymorphism level for SSRs in some plants such as *Arabidopsis* (Bell and Ecker 1994) and rice (Panaud et al. 1996), in our study a positive correlation between polymorphism level and number of repeat unit was existent for the SSRs in foxtail millet. This phenomenon was also supported by evidence from grapevine (Thomas and Scott 1993) and tomato (Smulders et al. 1997).

Based on the SSR data obtained, the 40 foxtail millet accessions were divided into five clusters. Basically, for the landraces of foxtail millet, the clustering groups based on SSR markers were largely consistent with the ecotypes (cluster 1 and cluster 5). However, for the modern varieties of foxtail millet, no clear corresponding relationship between clustering groups and ecotypes was found, and most of those varieties from different regions tended to be grouped together (one sub-cluster in cluster 4) except three from Shanxi forming an independent cluster. Interestingly, the foxtail millet accessions from Qinghai, one province on the Qingzang Plateau, showed distinct difference from those from other provinces in China, suggesting the particularity of those germplasm. Additionally, it was observed that cluster 2 and cluster 4 included some accessions with different geographical origin. The reasons are not clear, possibly including the germplasm exchange, which are needed to be investigated further.

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