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RESEARCH ARTICLE

Development and characterization of twenty-five new polymorphic microsatellite markers in proso millet (*Panicum miliaceum L.*)

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Abstract
Millets such as proso millet have excellent nutritional properties and could become a basic resource for crop breeding programs and food diversification. In this study, 25 polymorphic microsatellite markers were developed and characterized through construction of an SSR-enriched library from genomic DNA of proso millet (*Panicum miliaceum* L.). In total,110 alleles were detected, with an average of 4.4 alleles per locus. Values of major allele frequency (M_{AF}) and expected heterozygosity (H_E) ranged from 0.36 to 0.98 (mean = 0.73) and from 0.04 to 0.74 (mean = 0.37), respectively. The mean genetic similarity coefficient was 0.3711, indicating that among 50 accessions of proso millet there was wide genetic variation. The newly developed microsatellite markers should be useful tools for assessing genetic diversity, understanding population structure, and breeding of proso millet.

Keywords Microsatellite markers; *Panicum miliaceum* L.; Proso millet; SSR-enriched library

Introduction

Panicum miliaceum L., an annual warm season grass generally known as proso, hog, broomcorn, white, hershey, yellow, or common millet, has a short growing period of about 10–11 weeks, requires little water, and is able to grow at a wide range of altitudes (Jana and Jan, 2006; Lágler et al., 2005). This crop is extensively cultivated in central Europe, Russia, China, India, and the Middle East (Martin et al., 1976). In the United States and Europe, grains of proso millet are used as bird and livestock feed, whereas they are used for human consumption in other countries (Jana and Jan, 2006). Proso millet is cultivated for its unique nutritional value, which is superior to that of the more common cereals, wheat, rice, and oats. Since it has a high alkaline content that counteracts acids, and a high protein content, it is also considered a health food (Chang, 1968; Geervani and Eggum, 1989).

Simple sequence repeats (SSRs) have recently provided simple but powerful markers for several studies in diverse plant taxa (Li et al., 2003). SSRs, as DNA markers, have advantages over many other markers because they are abundant and highly polymorphic, co-dominantly inherited, analytically simple, and readily transferable (Weber, 1990). SSR markers are reported to be more variable than other markers such as RFLP or RAPD, and have been widely utilized in plant genomic studies (He et al., 2003). However, widespread use of these markers is often limited by the time and cost involved in their development, which requires DNA library construction, sequencing, identification of SSR-containing clones, and the design of primer pairs from flanking sequences of SSR motifs. Recent developments in library enrichment techniques and automated sequencing have simplified and sped up the development of these markers in a cost-effective manner (Zane et al., 2002). The development and characterization of SSR markers in foxtail millet (Jia et al., 2009), garlic (Ma et al., 2009), mung bean (Gwag et al., 2006), and sesame (Dixit et al., 2005) have recently been reported. Although some studies have developed DNAmarkers such as AFLP (Karam et al., 2004), RAPD (M'Ribu and Hilu, 1994),

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and ISSR (Lágler et al., 2005) for Panicum millets, there is no information available regarding isolation of microsatellite sequences from the proso millet genome or the development of a microsatellite-based marker system for this crop.

The present study details the development and characterization of 25 new polymorphic microsatellite markers that should serve as useful tools for assessment of genetic diversity and understanding of population structure in proso millet.

Materials and Methods

Plant materials and DNA extraction

For the present study, a total of 50 accessions of proso millet (*Panicum miliaceum* L.) were obtained from the Rural Development Administration (RDA) gene bank of the Republic of Korea (http://genebank.rda.go.kr) (Table 1). These accessions were from six countries (15 origins): Mongolia, Republic of Korea, Russia, India, Italy, and Uzbekistan. The seeds of each accession were cultivated in a glass greenhouse, and DNA was extracted from fresh leaves of 15-day-old seedlings using the Qiagen DNA extraction kit (Qiagen, Hilden, Germany). The relative purity and concentration of extracted DNA was estimated with NanoDrop ND-1000 (NanoDrop Technologies Inc., Wilmington, DE, USA). The final concentration of each DNA sample was adjusted to 20ng/μl.

Construction of an SSR-enriched library

We constructed an SSR-enriched library from genomic DNA of proso millet using a modified biotin-streptavidin capture method (Dixit et al., 2005). Briefly, total DNA was digested with seven restriction enzymes, *EcoR*V, *Dra*I, *Sma*I, *Pvu*I, *Alu*I, *Hae*III, and *Rsa*I, in separate reactions. After being pooling together, the digested DNA was size-fractionated on a 1.4% agarose gel. Fragments ranging from 300 to 1500 bp were eluted from the gel, followed by purification using a gel extraction kit (Qiagen). DNA fragments were ligated to an adaptor (AP11-5'-CTCTTGCTTAGATCTGGACTA-3' and AP-12-5'-TAGTCCAGATCTAAGCAAGAGCACA-3'). The adaptor-ligated DNA was hybridized with a mixture of biotin-labeled SSR probes [$(GA)_{20}$, $(CA)_{20}$, $(AGC)_{15}$, $(GGC)_{15}$, $(AAG)_{15}$, $(AAC)_{15}$, $(AGG)_{15}$]. The hybridized DNA fragments were captured with streptavidin-coated magnetic beads. After stringent washings, the captured DNA fragments were eluted in 50 l distilled water. Final eluates were amplified with the AP11 primer and cloned into the pGEM-T easy vector (Promega, Madison, WI, USA).

DNA sequencing and design of SSR primers

White colonies were randomly picked from the primary transformation plates. Plasmid DNA was isolated using the QIAprep Spin Miniprep kit (Qiagen) and sequenced on an ABI 3100 DNA sequencer with a BigDye terminator kit (Applied Biosystems, Foster City, CA, USA). Identification of SSR clones, redundancy checking, and design of primer pairs was performed using the SSR Manager Program (Kim, 2004). For all types of microsatellites, a minimum length criterion of 12 bp was selected, and only perfect microsatellites were considered. Primer pairs were designed from the flanking sequences of SSRs and evaluated for polymorphisms in a panel of 10 accessions of Italian millet using a previously described procedure (Dixit et al., 2005). The size of polymorphic polymerase chain reaction (PCR) products was measured accurately following the M13 tail PCR method of Schuelke (2000). Amplification reactions were carried out in a total volume of 20 μl, containing 200 ng template DNA, 1×PCR buffer, 0.2 mM of each dNTP, 1U Taq DNA polymerase, 8 pmol of each reverse and fluorescent-labeled M13 (-21) primer, and 2 pmol of the forward primer with the M13 (-21) tail at its 5' end. Conditions of the PCR amplification were as follow: 94 ℃ for 3 min, then 30 cycles each at 94 ℃ for 30 sec, 55 ℃ for 45 sec, and 72 ℃ for 1 min, followed by 10 cycles at 94 ℃ for 30 sec, 53 ℃ for 45 sec, and 72 ℃ for 1 min, and a final extension at 72 ℃ for 10 min. SSR alleles were resolved on an ABI-PRISM 3100 DNA sequencer (Applied Biosystems) using GENESCAN 3.7 software, and sized precisely against 6-carboxy-X-rhodamine (ROX) molecular size standards using GENOTYPER 3.7 software (Applied Biosystems).

Data analysis

Basic statistics, including total number of alleles, allele frequency, accession-specific alleles, major allele frequency (M_{AF}), and polymorphic information content (PIC), were calculated from shared allele frequencies using PowerMarker V3.23 (Liu and Muse, 2005). The variability at each locus was measured in terms of number of alleles, observed heterozygosity (H_O), expected heterozygosity (H_E), and genetic distance between each pair of accessions using the genetic analysis package POPGENE version 1.31 (Yeh et al., 1999). The UPGMA algorithm was used to construct an unrooted phylogram from a distance matrix using MEGA4 software (Tamura et al., 2007).

Table 1. Details of the 50 accessions of proso millet (*Panicum miliaceum* L.) used in the present study.

Accession No.	IT. No.[a] (Temp. No.)	Variety name (or place of collection)	Origin[b]	History	Source of introduction	Donor Country[b]
PM-01	(803157)		MNG		research center	MNG
PM-02	(803684)	Kharikovskoe 65	UKR		research center	MNG
PM-03	(803693)		HUN		research center	MNG
PM-04	(803701)		KAZ		research center	MNG
PM-05	(803702)	Unikum	CSK		research center	MNG
PM-06	103463	GangwonMyeongju	KOR	landrace	farm	KOR
PM-07	103301	GangwonSamcheok	KOR	landrace	farm	KOR
PM-08	(K015760)		PRK		research center	RUS
PM-09	(K015761)		PRK		research center	RUS
PM-10	(K029746)	KURYNKIZAN	PRK	bred	research center	RUS
PM-11	(K011708)	GangwonYanggu	KOR	landrace	farm	KOR
PM-12	(K011642)	GangwonYangyang	KOR	landrace	farm	KOR
PM-13	185516	GangwonWonju	KOR	landrace	farm	KOR
PM-14	185523	GangwonInje	KOR	landrace	farm	KOR
PM-15	033463	GangwonJeongseon	KOR	landrace	farm	KOR
PM-16	(K016560)	GangwonCheorwon	KOR	landrace	farm	KOR
PM-17	185519	GangwonChunseong	KOR	landrace	farm	KOR
PM-18	123924	GangwonPyeongchang	KOR	landrace	farm	KOR
PM-19	100298	GyeonggiGapyeong	KOR	landrace	farm	KOR
PM-20	033474	GyeonggiHwaseong	KOR	landrace	farm	KOR
PM-21	180613	GyeongnamGeochang	KOR	landrace	farm	KOR
PM-22	033449	GyeongbukMungyeong	KOR	landrace	farm	KOR
PM-23	100312	GyeongbukBonghwa	KOR	landrace	farm	KOR
PM-24	100283	GyeongbukAndong	KOR	landrace	farm	KOR
PM-25	185538	GyeongbukYeongdek	KOR	landrace	fram	KOR
PM-26	123964	GyeongbukUljin	KOR	landrace	farm	KOR
PM-27	123992		IND			IND
PM-28	123996		IND			IND
PM-29	124012		IND			IND
PM-30	124027		IND			IND
PM-31	137670		THA		research center	ITA
PM-32	137674		THA		research center	ITA
PM-33	153512		RUS		research center	IND
PM-34	153518		RUS		research center	IND
PM-35	153528		RUS		research center	IND
PM-36	153532		RUS		research center	IND
PM-37	197005	Local	MNG		research center	MNG
PM-38	197019	Conuerhoe	UKR		research center	MNG
PM-39	197022	Kormovoe-2	UKR		research center	MNG
PM-40	197026	Typracinau	MNG		research center	MNG
PM-41	199330	Local	CHN		research center	RUS
PM-42	199331	Local	CHN		research center	RUS
PM-43	199332	Local	CHN		research center	RUS
PM-44	199344	Veselopodel lanskoe 403	UKR		research center	RUS
PM-45	199346	Tonkoplenchatoe	FRA		research center	RUS
PM-46	199347	Local	AZE		research center	RUS
PM-47	199348	Local	UZB		research center	RUS
PM-48	199349	Local	TJK		research center	RUS
PM-49	204184		MNG			MNG
PM-50	208540		UZB	landrace	market	UZB

[a] Number from the Rural Development Administration (RDA) gene bank of the Republic of Korea.

[b] MNG, Mongolia; UKR, Ukraine; HUN, Hungary; KAZ, Kazakhstan; CSK, former Czechoslovakia; KOR, Republic of Korea; PRK, People's Republic of Korea; IND, India; THA, Thailand; RUS, Russia; CHN, China; FRA, France; AZE, Azerbaijan; USB, Uzbekistan; TJK, Tajikistan; ITA, Italy.

Results

SSR development

A library enriched for several di- and tri-nucleotide SSRs was constructed from the genomic DNA of proso millet. Developmental steps for the construction of the enriched library and its characteristic features are summarized in Table 2. A total of 504 putative recombinant clones (white colonies) were picked from the enriched library, sequenced, and analyzed for presence of SSRs. Sequence analysis revealed that 24 clones (4.76%) were redundant clones. Of the remaining 480 unique clones (95.24%), 157 (32.7% of the unique clones) were found to harbor SSR sequences. Sequence analysis of all the SSR-containing clones identified a total of 215 different repeat motifs. Tri-nucleotide SSRs were found to be more frequent (53.02%) than di-nucleotide SSRs (37.21%) (Table 3). Furthermore, a low number (9.75%) of tetra/penta/hexa nucleotide SSRs was also identified in the library. Among di-nucleotide SSRs, the AG/GAclass of repeat motif was the most frequent (52.5% of total di-nucleotide microsatellites), followed by the AC/CA class (42.50%). Among tri-nucleotide SSRs, the AGC/GCA/CAG class of repeat motifs was predominant (39.47%), followed by the AGG/GGA/GAG (18.42%) and CCG/CGC/GCC classes (14.04%). A total of 143 primer pairs could be designed from flanking sequences of different repeat motifs, and screened for polymorphism. Of these, 25 primer pairs produced very distinct and reproducible polymorphic bands and were therefore selected for further studies.

SSR polymorphism

The variability at each SSR locus was measured in terms of the number of alleles and expected heterozygosity. All 25 SSR loci were found to be polymorphic, and a total of 110 alleles were detected among 50 accessions. The number of detected alleles varied between two (GB-PMM-029, GB-PMM-031, GB-PMM-060, GB-PMM-117, and GB-PMM-145) and 11 (GB-PMM-134), with an average of 4.4 alleles per locus (Table 4). Of the 25 polymorphic loci, 20 were composed of perfect repeats of di- or tri-nucleotide SSRs and four included imperfect SSRs (interrupted with non-repeat sequence). One locus (GB-PMM-060) was found to be composed of a perfect tetra-nucleotide SSR. Four loci (GB-PMM-073, GB-PMM-094, GB-PMM-111, and GB-PMM-133) were composed of multiple repeats (more than one SSR), and three loci (GB-PMM-004, GB-PMM-121, and GB-PMM-126) possessed compound repeats (SSRs composed of different repeat motifs) (Table 4).

The values for expected heterozygosity (H_E) or gene diversity ranged between 0.04 (GB-PMM-029, GB-PMM-031, GB-PMM-60, GB-PMM-117, and GB-PMM-145) and 0.74 (GB-PMM-126 and GB-PMM-134), with an average of 0.37 for all the loci studied. Values of polymorphism information content (*PIC*) and major allele frequency ranged between 0.04 (GB-PMM-029, GB-PMM-031, GB-PMM-60, GB-PMM-117, and GB-PMM-145) and 0.70 (GB-PMM-134), with an average of 0.33, and between 0.36 (GB-PMM-126) and 0.98 (GB-PMM-029, GB-PMM-031, GB-PMM-60, GB-PMM-117, and GB-PMM-145) with an average of 0.73, respectively. All these loci deviated from Hardy–Weinberg equilibrium (*HWE*) at the significance threshold ($P < 0.05$). No clear correlation was evident between expected heterozygosity (H_E) values and number of alleles or repeat units (Table 4).

Phylogentic relationships

The proportion of shared alleles was used to calculate genetic distances between all pairwise combinations among 50 ac

Table 2. Screening steps in the construction and characteristic features of the microsatellite-enriched library for proso millet.

Screening step	Number and Percentage
Sequenced clones	504
Redundant clones	24 (4.76%)
Unique clones	480 (95.24%)
SSR clones	157 (32.71%)
Primer design	143
Polymorphic markers	25

Table 3. Identified microsatellite sequences in the enriched library for proso millet.

Repeat unit	Repeat class	Number	(%)
Di-nucleotide			
	AC/CA	34	42.50
	AG/GA	42	52.50
	AT/TA	2	2.50
	GC/CG	2	2.50
Total		80	37.21
Tri-nucleotide			
	AAC/ACA/CAA	6	5.26
	AAG/AGA/GAA	3	2.63
	AAT/ATA/TAA	1	0.88
	ACC/CCA/CAC	8	7.02
	ACG/CGA/GAC	8	7.02
	ACT/CTA/TAC	4	3.51
	AGC/GCA/CAG	45	39.47
	AGG/GGA/GAG	21	18.42
	ATC/TCA/CAT	2	1.75
	CCG/CGC/GCC	16	14.04
Total		114	53.02
Others (Tetra/Penta/Hexa)		21	9.76
Total repeat motifs		215	

Table 4. General characteristics of the 25 new polymorphic markers developed for proso millet.

Locus name	GenBank accession no.	Primer sequence		Repeated motif	T_A[a] (°C)	N_A[b]	Size range (bp)	H_O[c]	H_E[d]	M_{AF}[e]	PIC[f]
		Forward	fReverse								
GB-PMM-004	EF117724	GCAAGAGCGTGTGTGTGA	TGGTGTCAGGAACATTAAGGA	(TG)8-(GA)9	55	3	200 - 204	0.00	0.25	0.86	0.22
GB-PMM-013	EF117725	AGGGGTAGGGCTGATGAA	ATAGTGATCGCGACCGTG	(TCG)8	55	4	234 - 256	0.00	0.15	0.92	0.15
GB-PMM-014	EF117726	GGGAGACGCAGTGTGGTA	TACAGGTCCTGCGTGAGG	(CGT)3(CAT)(CGT)5	55	4	278 - 302	0.00	0.15	0.92	0.15
GB-PMM-023	EF117727	GCTAGCTTGTTGTTGCCG	GATGCGTACCGCTTGTGT	(GA)19	55	7	219 - 231	0.00	0.69	0.49	0.65
GB-PMM-025	EF117728	CTTGTGCTGGGCTAGGTG	AATGTGGTTGGCATTGGA	(CA)10	55	3	188 - 192	0.00	0.22	0.88	0.21
GB-PMM-029	EF117729	AGCTCCTGGCTCAGCTCT	CTTGCTGTCGTCGTCCTC	(CGA)5	53	2	190 - 214	0.00	0.04	0.98	0.04
GB-PMM-031	EF117730	AAACGGGATCGGTGTAGC	AATGAGGCACTGACAGCC	(TGC)3(TGA)(TGC)2	55	2	174 - 180	0.00	0.04	0.98	0.04
GB-PMM-060	EF117731	TCGTGACAGAGCGTCCTT	TCGATCTCGGTCTGCTGT	(AGTC)4	55	2	183 - 188	0.00	0.04	0.98	0.04
GB-PMM-061	EF117732	CTCCGCAGCAAGACATTT	TTTCGATCCGAGGAGGAT	(CTC)6	55	4	263 - 292	0.00	0.54	0.49	0.43
GB-PMM-066	EF117733	TAATGCCAAACCAAGCGT	GGTACAAGTACAAGCCCGC	(TGC)6	58	3	225 - 231	0.00	0.35	0.78	0.30
GB-PMM-073	EF117734	GCTCTCACCGTCTGATCG	CGCATTCTCTTCCCCTTT	(TC)21, (CGTG)4	55	9	275 - 311	0.00	0.66	0.53	0.62
GB-PMM-085	EF117735	CAGCCCATCACACTCGAT	CTTCTTCGTCGTCCCTCC	(AGG)3(AGC)(AGG)2	55	7	238 - 265	0.00	0.70	0.50	0.66
GB-PMM-094	EF117736	AAGAGCGAGGGCTAGCAT	CGGCAGCAACTCATCAAT	(AT)4, (GCG)4	55	3	106 - 228	0.00	0.52	0.54	0.40
GB-PMM-096	EF117737	GGCCTATGGGCTTTTTGT	GCGTTCGGAACAACTGAA	(GCC)6	55	3	117 - 188	0.00	0.30	0.82	0.27
GB-PMM-098	EF117738	TGCCAGGCACTACACCAT	CATCTCTTCGCTGTCCCA	(CTG)6	55	4	193 - 236	0.00	0.46	0.68	0.39
GB-PMM-106	EF117739	AGCGAGAGGAAACAGCGT	ATAGGCGTCGGAGATGGT	(TC)19	58	6	218 - 240	0.00	0.46	0.72	0.44
GB-PMM-107	EF117740	CTCAGCTCGCCCCTTATT	GCACCGGAATATGCAAGA	(GA)6	55	3	224 - 258	0.00	0.25	0.86	0.22
GB-PMM-111	EF117741	GTTCGAGGCTGATGCAAG	CGCATCACACGTCACATC	(AG)6, (AG)5	55	3	269 - 291	0.00	0.15	0.92	0.14
GB-PMM-115	EF117742	GCACGTCACACTCACACG	TGGGTGTATCAGGGCTTG	(AG)15	55	6	259 - 279	0.00	0.70	0.39	0.64
GB-PMM-117	EF117743	GTGAGGGTGATCACGAGG	CCACGCCAAACTCAAATC	(GA)6	58	2	292 - 316	0.00	0.04	0.98	0.04
GB-PMM-121	EF117744	GGACATACGCATGGTGGT	ACGATCGAATGAGCGAGA	(AT)7- (GTAT)9	55	6	212 - 282	0.00	0.65	0.46	0.58
GB-PMM-126	EF117745	CTTCCATAGGGTGCCTCC	CATCGCAATTGGGAAAGA	(GAA)5-(GA)20	55	8	128 - 272	0.00	0.74	0.36	0.69
GB-PMM-133	EF117746	TCTCAGTGCTTTACGCCG	AGGAACCGGAACCACCTA	(GAG)6, (AGG)6	55	4	224 - 299	0.00	0.22	0.88	0.21
GB-PMM-134	EF117747	CAGGCTCTGGCAAAGATG	CAAGGTCAGGGGAACCAT	(AG)22	55	11	241 - 267	0.00	0.74	0.47	0.70
GB-PMM-145	EF117748	TCCAAGAGCAGCACGG	AGACGTCGTGCCAAGAGA	(CAG)3(TAG)(CAG)2	55	2	194 - 197	0.00	0.04	0.98	0.04
Mean						4.4		0.00	0.37	0.73	0.33

[a]T_A, Annealing temperature [b]N_A, Number of alleles; [c]H_O, Observed heterozygosity; [d]H_E , Expected heterozygosity; [e]M_{AF}, Major allele frequency; [f]PIC, Polymorphism information conten

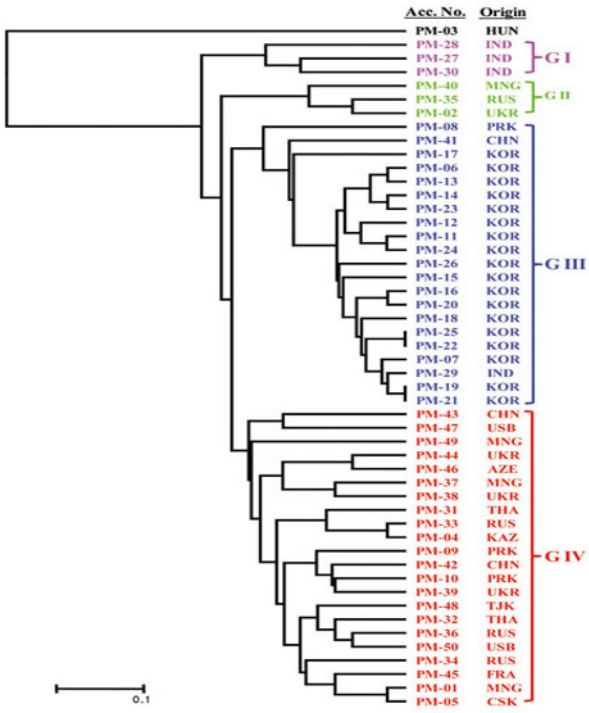

Figure 1. UPGMA dendrogram showing genetic relationships among 50 accessions of proso millet.

cessions used in this study. The genetic distance matrix generated by PowerMarker was utilized to construct a UPGMA tree using Mega4 software (Tamura et al., 2007).The similarity coefficients among the 50 proso millet accessions ranged from 0 to 1.0. The wide variation in genetic similarity among the different accessions revealed by SSRs reflected a high level of polymorphism at the DNA level (data not shown). The resulting dendrogram (Fig. 1) shows a complex distribution pattern of accessions at a genetic distance of 0.37. All 50 accessions could be clustered into four groups. Among four accessions collected from India, three were clustered into Group I, and the fourth clustered into Group III. Group II included three accessions of different origin (one each from Mongolia, Russia, and Ukraine). Group III included 18 accessions from the Republic of Korea and three of other origin (one each from the People's Republic of Korea, China, and India). All accessions from the Republic of Korea clustered into mixed subgroups in Group III. A total of 22 accessions of various origins clustered into Group IV.

Discussion

Proso millet as one of the most ancient cereal and drought-resistant crop with extremely short ripening time is the lowest water requirement of any grain crops (Lágler et al., 2005). This crop is not only as an alternative crop, or a fodder crop, but also cultivated for its unique nutritional value which is superior to main cereals such as wheat and rice. Also, it has high alkaline content which counteracts acids, and high protein content, it is utilized as health food (Chang,1968; Geervani and Eggum, 1989; Jana and Jan, 2006). Therefore, this crop as a source of crop breeding program has a high availability.

The work described in this paper is the first reported development of polymorphic SSR markers from an enriched library of genomic DNA of proso millet. Characterization of SSR-containing clones in the enriched library with reference to relative abundance of various repeat motifs revealed predominance of tri-nucleotide SSR (53.02%) over di-nucleotide SSR (37.21%). This is a feature common to other plant genomes, as several recent surveys on distribution patterns of various SSRs in genomic sequences of several plant species, including *Arabidopsis* (Morgante et al., 2002), foxtail millet (Jia et al., 2009), garlic (Ma et al., 2009), ginseng (Ma et al., 2007), Italian millet [our unpublished results], *Medicago, Lotus,* and *Glycine* (Mun et al., 2006), mung bean (Gwag et al., 2006), rice (Goff et al., 2005), and sesame (Dixit et al., 2005), have indicated that tri-nucleotide repeats are the most common type of SSR in plants. Occurrence of the AG/GA class of repeat motif among di-nucleotide repeats is consistent with reports from other plant species (Langercrantz et al., 1993; Morgante and Oliveiri, 1993; Powell et al., 1996, our unpublished results). Among tri-nucleotide repeats, the AGC/GCA/CAG class was the most frequent, followed by the AGG/GGA/GAG and CCG/CGC/GCC classes, in contrast to the general notion that the CCG/CGC/GCC class is predominant in cereal genomes (Rota et al.,2005; Varshney et al., 2002; Kantety et al., 2002). The designed 143 primer pairs from flanking sequences of different repeat motifs were screened for polymorphism. Of these, 25 polymorphic SSR primers produced very distinct and reproducible polymorphic bands. This result is higher polymorphic rate (17.5%) as compared with previous reports of other plants, including garlic (Ma et al., 2009) and sesame (Dixit et al., 2005).

The 25 SSRs developed in the present work were successfully utilized to develop phylogenetic relationships within a world germplasm collection of 50 landraces from various countries. The SSR markers were highly polymorphic, producing a total of 110 alleles with an average of 4.4 alleles per locus and a mean value of expected heterozygosity (H_E) or gene diversity of 0.37 for all loci. Thus, the SSR markers among the 50 proso millet accessions in the present study were effective for assessing genetic diversity and understanding population structure.

All 50 accessions collected from different countries (15 origins) could be clustered into four groups (Fig. 1). Each group was composed of accessionsfrom a variety of origins, excluding Group I. In Group III, 18 accessions from the Republic of Korea were

mixed into several subgroups with three Asian accessions, one each from the People's Republic of Korea, China, and India. A total of 22 accessions originating from several countries dominated in Group IV, possibly as a result of similar natural or human selection within regions or seed movement and gene flow. The Asian genotypes showed more genetic affinity with each other than with the European types (M'Ribu and Hilu, 1994). Only one Hungarian accession (HUN 6) appeared ungrouped, and may have been from a smaller sample or had a different domestication region than the others. The information provided in this paper will be valuable for developing breeding programs for proso millet.

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