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Short Communication

Development of EST-SSRs in Finger Millet (*Eleusine coracana* ssp *coracana*) and their Transferability to Pearl Millet (*Pennisetum glaucum*)

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EST-SSR markers were developed using sequence information from 1740 expressed sequence tags (ESTs) of finger millet available in the public domain. A set of 31 SSR markers were synthesized based on di, tri, tetra and penta-nucleotide repeat sequences. These were used for PCR analysis of 11 elite germplasm lines of finger millet of Indian and African origin. Out of 31 SSR markers, amplification products were obtained for 17 primer pairs. Of these nine were found polymorphic with two alleles per locus. These 17 SSR primer pairs were also tested for amplification in three varieties of pearl millet (*Pennisetum glaucum*) and 11 could be transferred to pearl millet. The informative EST-SSR markers developed, can be used in finger millet as well as pearl millet genetic improvement projects.

***Key words*:** ESTs, SSRs, alleles, finger millet, pearl millet.

Finger millet or ragi [*Eleusine coracana* (L) Gaertn ssp *coracana*], belonging to the family Poaceae, is a crop of considerable potential. Ragi has protein, fat and minerals higher than rice, corn or sorghum. Finger millet is an allotetraploid (2n = 4x = 36 chromosomes) with AABB genomic constitution. Ribosomal DNA variation and isozyme data showed the allopolyploid nature of this species and molecular information has shown *E. indica* to be one of the genomic donors (1, 2). The second genomic donor is yet to be identified. The closest wild relative of finger millet is *E. coracana* ssp *africana*, which is native to Africa. Finger millet is cultivated in Eastern and Southern Africa and in Southern Asia. In India, finger millet is cultivated in Karnataka, Tamil Nadu, Andhra Pradesh, Orissa, Maharastra, Uttar Pradesh, Uttaranchal, Bihar and Gujarat.

DNA-based molecular markers have the obvious advantage of sampling the genome directly, and can be used for crop improvement and germplasm conservation. There are only few reports available on molecular characterization of finger millet (3-5). Compared to other DNA markers, simple sequence repeats (SSRs) are markers of choice as these are highly polymorphic and can be analyzed by a rapid, technically simple and inexpensive PCR-based assay that requires only small quantities of DNA. A set of 82 SSR markers were developed by Dida *et al* (6) in finger millet. The development of additional SSRs in finger millet through data mining from EST sequences will greatly aid in diversity and genome analysis efforts. ESTs are valuable markers representing transcribed regions and often have putative functions. Though less polymorphic as compared to genomic-SSRs, EST-SSRs by-pass laborious and costly development and sequencing of SSR-enriched (or random) genomic libraries and are more likely to function in distantly related species than genomic-SSRs. The objective of this study was to develop EST-SSRs from finger millet and study their polymorphism and transferability to pearl millet.

A total of 1740 finger millet EST sequences obtained from dbEST NCBI database were used to develop the SSR markers. The SSR identification and primer designing was done using one pipeline tool "SSR Primer", which is an integrated program of SPUTNIK (SSR repeat finder) and Primer3 software (7). DNA was extracted by cetyl trimethyl ammonium bromide (CTAB) method (8) from 11 elite germplasm lines of finger millet [1: GE 68 (Uttaranchal); 2: GE 292 (West Bengal); 3: GE 314 (Andhra Pradesh); 4: GE 469 (Kerala); 5: GE 1028 (Karnataka); 6: GE 4752 (Kenya); 7: GE 4833 (Malawi); 8: GE 4951 (Zambia); 9: GE 4990

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Abbreviations: ESTs - Expressed sequence tags; SSRs - Simple sequence repeats.

(Tanzania); 10: GE 4991 (Tanzania) and 11: GE 5029 (Zimbabwe)] procured from All India Coordinated Small Millet Improvement Project (AICSMIP), Bangalore. For cross-species amplification. DNA was extracted from three varieties of pearl millet *viz.* ICMV 155, HC 10 and ICTP 8203. For both finger millet and pearl millet, same protocol was followed for PCR reaction conditions as well as thermal profiles. PCR was performed in a 25 µl volume containing 100 ng of DNA, 1.25 µM of $MgCl_2$ (Fermentas Life Sciences), 200 µM of dNTPs (Fermentas Life Sciences), 0.8 U of *Taq* DNA polymerase (Fermentas Life Sciences), 0.5 µM of forward and reverse primers (SBS Genetech Co Ltd), 1x buffer (Fermentas Life Sciences) and sterile water. The amplification was done at 94°C for 30 sec, followed by 10 cycles of 30 sec at 94°C, 30 sec at 62°C (decreasing 0.7°C / cycle) and 1.0 min at 72°C, then by 35 cycles of 30 sec at 94°C, 30 sec at 55°C and 1.0 min at 72°C, with final extension of 4.0 min at 72°C. The PCR products were separated on 2%, 3% and 4% Metaphor agarose gel depending on the size of amplified products and photographed with Bio Imaging System (SynGene).

In the present study, four hundred and sixty three SSRs were found in 351 sequences of the 1740 finger millet EST sequences examined, which was a frequency (20.2%) higher than expected based on reports from other crops (9). All the types of motifs identified are presented in Table 1. Among the 463 SSRs, 53 (11.4%), 349 (75.4%), 23 (5.0%) and 38 (8.2%) had di, tri, tetra and penta-nucleotide repeats, respectively (Table 1). In many other crops such as wheat, rice, maize, sorghum and barley also, high frequency of trinucleotide repeats was reported (10). This may be attributed to the selection against frameshift mutations that limit the expansion of non-triplet microsatellites. In contrast, di-nucleotide repeats were reported as most abundant motifs, at 40% in pearl millet (9) and in case of foxtail millet, both di and tri-nucleotide repeat motifs were present at 47% (11). Among the di-nucleotide motifs, GA/CT was the most abundant (66%), which was in accordance with the reports in other crops (11-13). The GA/CT motif represent codons GAG, AGA, UCU, CUC in mRNA which translate into the amino acids Arg, Glu, Ala and Leu respectively. In proteins, Ala and Leu are present in high frequencies that results in the abundance of GA/CT motifs in EST sequences. As for tri-nucleotide type, CGG/GGC/GCG (40.4%) was the most common repeat motif. Kantety *et al* (12) also reported CCG as the most common trinucleotide repeat motif in other

Table 1. Repeat type, repeat motif and number of SSRs present in ESTs of finger millet

Repeat type	Motif	Number
Dinucleotide	TG/GT/CA/AC	13
	GC	5
	GA/AG/TC/CT	35
Total		53
Trinucleotide	TAA	3
	TTG/ACA/CAA	10
	TGA/GAT/ATG	15
	TCT/CTT/TTC/GAA/AAG/AGA	23
	TGC/GCT/CTG/CGA/GAC	25
	GTA	1
	GTG/TGG/GGT/CCA/CAC	16
	GCA/CAG/AGC/CGT/GTC	33
	CCT/CTC/TCC/GAG/AGG/GGA	82
	CGC/GCC/CCG/CGG/GGC/GCG	141
Total		349
Tetranucleotide	AAAG	1
	ATAA	1
	ATGT/TACA	2
	ATCG/CGAT	2
	ACCC/CCAC	2
	TTTG	1
	TCCC	1
	TTCG/TCGT	2
	GCTC/CTCG	2
	CATC	1
	CCTG	1
	CGTG	1
	CCAG/GTCG	2
	CCGT/CGTC	2
	CTTC/TTCC	2
Total		23
Pentanucleotide	AGCAG	1
	ATCGA	1
	AAGAG/AGAGA/TTCTC	11
	TTTTC	1
	TGCTC	1
	TGATG	1
	TCGCT/CTTCG	4
	TCTCC/GAGGA	5
	GATTG	1
	GCGGC	1
	GGAGC	1
	GTGTC	1
	GAAGG/CTTCC	2
	CAAGA	1
	CAGAC	1
	CATGT	1
	CGCAG	1
	CGGTG	1
	CGGTT	2
Total		38
Grand Total		**463**

crops, while CAG and TCT were the most abundant trinucleotide repeat motif in foxtail millet (11).

An initial subset of 31 SSRs belonging to di, tri, tetra and penta-nucleotide repeats was selected for primer synthesis and tested for amplification in 11 elite germplasm lines of finger millet. Out of 31 primer pairs, 17 (54.8%) showed clear amplification products. Earlier also, Varshney *et al* (14) reported that 53-71% of the total microsatellites in the ESTs of cereal species had primer designing potential. Rest of the 14 primer pairs resulted in either no amplifications or very faint multiple bands and hence were not studied further. This may be due to the fact that ESTs are single-pass sequences that need further validation to give higher quality sequences. Out of 17 primer pairs, nine amplified polymorphic alleles in the lines studied. All the polymorphic loci showed 2 alleles per locus. The number of polymorphic primer pairs was highest for dinucleotide repeats (5), followed by three for trinucleotide repeats and one for pentanucleotide repeats. Four of the primer sets (FM 3, FM 4, FM 16 and FM 18) amplified alleles in the higher size range as compared to the expected size indicating the existence of introns (Table 2). Representative profiles with two primer pairs (FM 23 and FM 10) showing allelic polymorphism are depicted in Fig. 1. In the elite germplasm lines GE 4833 (Malawi), GE 4991 (Tanzania) and GE 5029 (Zimbabwe) 146 bp allele was amplified, while in rest of the lines 126 bp allele was generated using primer pair FM 23 (Fig. 1a). With primer pair FM 10 (Fig. 1b) elite germplasm line GE 314 (Andhra Pradesh) showed two alleles of size 220 bp and 236 bp, but in rest of the lines only 220 bp allele was present.

Table 2. Characteristics of EST-SSR primer pairs developed in finger millet and their transferability to pearl millet

Locus name	Accession number	Repeat motif	Forward and reverse primer sequence (5′-3′)	Allele Size (Finger millet) Expected (bp)	Allele Size (Finger millet) Observed (bp)	Number of alleles (Finger millet)	Transferable species (Pearl millet) Allele size (bp)
FM 2	CX265472	(GA)12G	CGAGATTAGTTAGCTGGTGG CGCCATTATTGCTATCTCTC	101	96-102	2	0
FM 3	CX265472	(AG)8A	GCGAGTGAGAGAGAGAGAGA ACGACGAGTCGTACTTGAAC	205	400*	1	380-410*
FM 4	CX265472	(TG)6T	CGACGTCCTAGTGTTCAAGT GGATCGATACAATACATCATCA	369	430-440*	2	0
FM 6	CX265357	(GC)7G	TGTAGAAGAAGCAGAGGAGG GCAAGAACTTCCAAACAGAC	237	236	1	250
FM 9	CX265329	(TCCC)5	GTCGATCAGTCAGTCATGC GCGAGGTATATATAGAGGCG	127	127	1	125
FM 10	CX265194	(CA)7	GCGGACCAAAGTGTAAATAG ATTCACAATTTCATTTCCCA	220	220-236	2	225
FM 12	CX265194	(GCG)7G	AGAACTACATGCAGACGGAG ATTCACAATTTCATTTCCCA	348	343-349	2	375
FM 13	CX265020	(AGA)18	CACTACACCGCATCATCTC GTAGTGGAGTAGGCGATGG	268	233-269	2	0
FM 16	CX264978	(AG)16	AGTGAGAGAGGGAGCTTAGAG TGCTGCAGATGAAGTAATTG	395	506-518*	2	600*
FM 17	CX264718	(GA)7	ACTCTCCTGTGAGTGAGTGA AGGGTGGAGATGAACTCAG	148	148	1	150
FM 18	CX264962	(GCG)7	TTGTCCATCTCGTCAGTTCT CTCCGACCTAAACATCAAGA	107	175*	1	175*
FM 23	CX264765	(AAGAG)7	CACCTGCTCCATCTACATCT CACAAGGACGATCGCAAC	151	126-146	2	130-150
FM 27	EB086245	(TC)15	GGACTCTAGTTTCCGCTTTC GGCGAGATGGTTAATGTAGA	378	380	1	400
FM 28	EB086162	(TC)9T	AAGTGATGATGATCGTTTCC TACTACATCTATGACCGCCC	174	170-176	2	0
FM 31	EB086242	(GGC)6	GCGGCTAAGGTAGTGAGTAG AGGGACTCGAGAGAATAAGC	374	380	1	0
FM 32	DY625749	(CGG)6	CGGATAAGAGTATCGATTGG CATATCCACTAGTACCCGCT	180	176-182	2	180
FM 34	EB187410	(TTC)8TT	TGTTCACTTTCCAGAGAGGT TGACTGCAATATGCACAACT	205	205	1	0

*Allele size is higher than the expected value

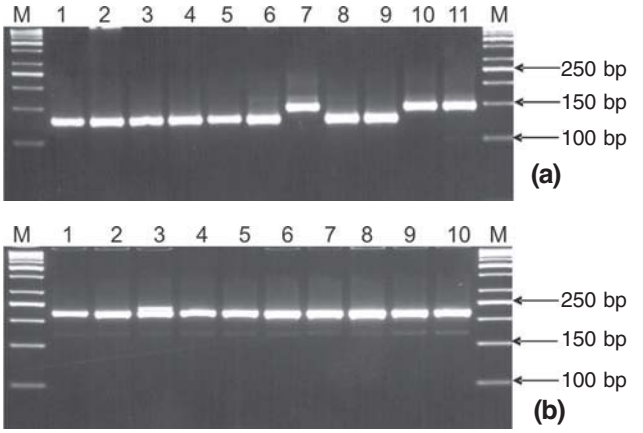

Fig. 1. Allelic polymorphism with finger millet EST-SSR markers FM 23 **(a)** and FM 10 **(b)**. M is 50 bp molecular weight marker and lanes 1-11 elite germplasm lines of finger millet as detailed in the text.

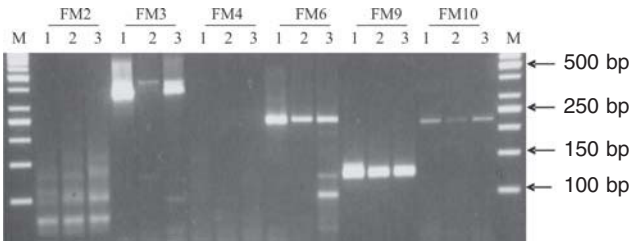

Fig. 2. Transferability of finger millet EST-SSRs (FM 2, FM 3, FM 4, FM 6, FM 9 and FM 10) in three varieties of pearl millet (1: ICMV 155, 2: HC 10, 3: ICTP 8203). M is 50 bp molecular weight marker.

Transferability of the EST-SSRs developed in this study was evaluated in pearl millet, and amplification was detected in 11 (64.7%) out of 17 SSRs tested, ranging from one to two alleles per locus. In this experiment a high percentage of transfer of EST-SSRs across two genera reaffirms the fact that EST-SSRs are more transferable as compared to genomic SSRs in the same family. The primer pairs FM 3, FM 16 and FM 18 showed alleles of higher size in pearl millet also (Table 2). A representative photograph illustrating the transfer of four (FM 2, FM 3, FM 4 & FM 6) out of six primer pairs tested in three varieties *viz.* ICMV 155, HC 10 and ICTP 8203 of pearl millet is depicted in Fig. 2. These EST-SSR markers that have been developed through data mining involving low cost and less time, will be valuable for studying genetic relationships, diversity analyses and marker-assisted selection. These can also be used for understanding of SSR distribution and frequency, development of EST-SSR genetic and physical maps in finger millet and can also be used for comparative genomics.

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