Genet Resour Crop Evol (2007) 54:233–236
DOI 10.1007/s10722-006-9139-8

SHORT COMMUNICATION

Development of EST-SSR in foxtail millet (*Setaria italica*)

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Received: 11 June 2006 / Accepted: 1 November 2006 / Published online: 2 February 2007

Abstract The development of EST-SSR in foxtail millet (*Setaria italica*) for polymorphism and transferability study was reported here. From 1213 EST sequences, 30 SSRs were obtained and primers were designed for 26 SSRs. Among them, four pairs of SSR primers amplified polymorphic products in 12 foxtail millet cultivars and one accession of *Setaria viridis*, a wild relative of foxtail millet, with 10 alleles detected for the four loci and 2.5 alleles per locus. In addition, ten SSR markers could be transferred to other nine Gramineae species. The putative functions of 11 ESTs containing polymorphic and transferable SSRs were also identified.

Keywords EST-SSR · Foxtail millet · Polymorphism · *Setaria italica* · Transferability

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Abbreviations

SSRs	Simple sequence repeats
RFLP	Restriction Fragment Length Polymorphism
CTAB	Cetyl Trimethyl Ammonium Bromide

Introduction

Foxtail millet (*Setaria italica* (L.) P. Beauv.) is an important minor cereal in China and other countries in Asia because it is drought tolerant and well adapted to arid and semiarid regions. Because of its smaller genome and diploid nature, foxtail millet is an ideal model plant for genetics and molecular biology research (Wang et al. 1998). However, in foxtail millet only restriction fragment length polymorphism (RFLP) markers are currently available (Wang et al. 1998) and their use is limited due to the relatively complicated operation procedure and the use of radioactive probes. SSRs (simple sequence repeats) are comprehensively distributed through plant genome, for abundant polymorphism, co-dominant inheritance, transferability and simple technology (PCR-based), it is very suitable for constructing high-density linkage map (Beckmann and Soller 1990). The aim of this study is to develop

EST-SSR from *Setaria italica* and examine its polymorphism and transferability, making preparation for further SSR linkage map construction and comparative genome research.

Materials and methods

Totally 1213 EST sequences derived from leaf and root tissues of drought-stressed subtractive cDNA libraries were obtained previously. The SSRs were identified by Simple Sequence Repeats Identified Tool (http://www.gramene.org/db/searches/ssrtool) (Temnykh et al. 2001). Primers were designed flanking SSR with a minimum five repeats using PRIMER 3.0 software (Rozen and Skaletsky 2000).

DNA was extracted by Cetyl trimethyl ammonium bromide (CTAB) method (Dellaporta et al. 1983) from 12 foxtail millet cultivars including six landraces (i.e. 'Bailiusa', 'Damaogu', 'Zimiaotie', 'Shuilihong', 'Shepigu' and 'Baigu') and six released varieties (i.e. 'Jin17', 'Yu8', 'Tie12', 'Lu10', 'Ba-91-0934' and 'Bianqu1'), one green foxtail (*Setaria viridis* (L.) P. Beauv.) (the presumed wild ancestor of foxtail millet) accession ('710199') and one entry for each of oats (*Avena sativa* L.) ('G4'), job's tears *(Coix lachryma-jobi* L.) ('YZ71'), common millet *(Panicum miliaceum* L.) ('Dabaishu'), sorghum *(Sorghum bicolor* L.) ('Miyunhong'), Japanese barnyard millet *(Echinochioa crus-galli* (L.) Beauv.) ('B123 Bai'), finger millet *(Eleusine coracana* Gaertn.) ('Jizhaogu'), pearl millet *(Pennisetum glaucum* L.) ('Z68'), teff (*Eragrostis tef* (Zucc.) Trotter) ('T1') and rice *(Oryza sativa* L.) ('Aiqing'). All the species belong to the Gramineae family. They were selected because transferability of EST-SSRs among species from the same family should have higher possibility (Gutierrez et al. 2005).

Polymerase chain reactions (PCRs) were performed in a 20 μl reaction volume containing approximately 50 ng of template DNA, 2 μl of 10× PCR buffer containing 20 mM $MgCl_2$, 0.2 μM of forward and reverse primers, 0.2 mM of dNTPs, 1 U of *Taq* polymerase and sterile distilled water. The conditions for amplification were 4 min at 94°C followed by 24 cycles of 30 s at 94°C, 30 s at 62–51°C (each two cycles reduced 1°C) and 60 s at 72°C, then by 18 cycles of 30 s at 94°C, 30 s at 50°C and 60 s at 72°C, with a final extension time of 5 min at 72°C. The PCR products were separated on 6% polyacrylamide gel or 2% agarose gel.

Putative functions of the EST sequences containing polymorphic and transferable SSRs were identified by blastn and blastx (http://www.ncbi.nlm.nih.gov/).

Results and discussion

Of the 1213 EST sequences examined, 30 SSRs were found in 27 sequences (2.2% of all the EST sequences). Among the 30 SSRs, 14 (47%), 14(47%) and 2 (6%) had dinucleotide, trinucleotide and tetranucleotide repeats, respectively. The most common dinucleotide repeats motif was TC/AG (16%), which was accord with the reports in other crops (Cardle et al. 2000; Kantety et al. 2002; Thiel et al. 2003). CAG and TCT were the most common trinucleotide repeat motif in foxtail millet (10% for each). However, in other crops the most common trinucleotide repeat was CCG or AAC (Kantety et al. 2002). One possible reason for the difference is that the EST sequences used here derived from a drought-stressed subtractive cDNA library, which did not cover the whole genome of foxtail millet. Another probable reason is due to the difference of the genomes tested. Primers were successfully designed for 26 SSRs and 15 of them amplified single band with prospective size.

Twelve foxtail millet accessions and one green foxtail (*Setaria viridis*) accession were selected for polymorphism analysis. The results indicated that four of the 15 SSR primer pairs could amplify polymorphic bands. A total of 10 alleles were observed, with 2.5 alleles per locus (Table 1).

To test the transferability of the EST-SSRs developed in this study in other Gramineae species, DNAs of oats, job's tears, common millet, sorghum, Japanese barnyard millet, finger millet, pearl millet, teff and rice were amplified using the 15 SSR primers. Ten of them could amplify in one to seven of these nine crops (Table 2).

Table 1 Polymorphic EST-SSR primers for foxtail millet

Primer name	Repeat motif	Primer sequences	Annealing temperature (°C)	Allele size range (bp)	No. of alleles
P2	(CT)5	F: CCAACACGCAATCGCAGAA R: AGGCAGTGGGTTTGAGCAT	58	120–127	3
P5	(CAT)5	F: TTGCCTTGAGCTCTTTGATG R: GCTGATACTGATATGTCTGATGAGGA	58	300–307	2
P13	(CA)6	F: GGAGAGATTCCGGGCTCTAGT R: ACGGTTCCGACATTTTAACG	58	166–170	3
P18	(CCAT)5	F: TTCTCTCGTTGGAATTTTTGTG R: GGAACAGATATCCTTTTCACTCTT	58	166–170	2

F: forward primer; R: reverse primer

Table 2 Transferability of EST-SSR markers developed from foxtail millet

Primer name	Repeat motif	Primer sequences	Annealing temperature (°C)	Transferable species	Allele size range (bp)
P1	(TG)6	F: CGGATACTAATTGGCGTTGG R: TTCTACGTCGCAGAGATGGA	59	*Sorghum bicolor*	240
P6	(GCA)5	F: TCAGACACACATCCACGAGC R: CGGCAACAGGTTCATCAAGAC	58	*Pennisetum glaucum*	212
P19	(TTC)5	F: GGCTGCCACACCAAATTATAC R: CTGCTCGTGGATGTGTGTCT	59	*Oryza sativa*	156
P3	(GCT)5	F: CAAGCAGAACCGAGACCAAC R: AAGCGGCGTTTGTGATTTAG	55	*Echinochloa crus-galli* and *Pennisetum glaucum*	100–109
P9	(GCA)6	F: GGCTTCTCTCCATCAACCAG R: TATTGCTGCTTGGGGATCAT	59	*Coix lachryma-jobi, Panicum miliaceum,* and *Sorghum bicolor*	201
P5	(TCA)5	F: TTGCCTTGAGCTCTTTGATG R:GCTGATACTGATATGTCTGATGAGGA	59	*Panicum miliaceum, Sorghum bicolor, Echinochloa crus-galli, Eleusine coracana* and *Pennisetum glaucum*	307–320
P10	(TA)5	F: AGCACATAAGAGACCACCACA R: GGAGGACCAGAAGCAGAATAGC	55	*Avena sativa, Panicum miliaceum, Echinochloa crus-galli, Sorghum bicolor, Eleusine coracana* and *Pennisetum glaucum*	230
P18	(TCCA)5	F: TTCTCTCGTTGGAATTTTTGTG R: GGAACAGATATCCTTTTCACTCTT	57		166–170
P13	(CA)6	F: GGAGAGATTCCGGGCTCTAGT R: ACGGTTCCGACATTTTAACG	59	*Avena sativa*, *Coix lachryma-jobi*, *Panicum miliaceum*, *Sorghum bicolor*, *Echinochloa crus-galli*, *Eleusine coracana* and *Pennisetum glaucum*	166–200
P24	(GAT)5	F: GCGATGCAATGCTTGGGACT R: TGGCTTCCTTCCTCCTTGTA	59		194

F: forward primer; R: reverse primer

Table 3 The putative proteins identified by blastn and blastx (http://www.ncbi.nlm.nih.gov/) of 11 EST sequences containing polymorphic and transferable SSRs

Primer	Putative protein	Organism	E-value	Accessible No.
P2	Transcriptional Regulator, *AraC* family	*Rhodospirillum rubrum* ATCC 11170	2.5	YP 427425
P5	OSJNBa0089N06.18	*Oryza sativa* (japonica cultivar-group)	1.00E-42	XP 473996
P13	Unknown protein	*Oryza sativa* (japonica cultivar-group)	1.2	XM 550459
P18	Dipeptidase	*Mesorhizobium loti* MAFF303099	5.5	BAB 54426
P1	Protein-glutamate *O*-methyltransferase	*Shewanella baltica* OS155	4.3	ZP 00582450
P6	*S*-adenosylmethionine synthetase 1	*Hordeum vulgare*	1.00E-31	P 50299
P19	*S*-adenosylmethionine synthetase 1	*Hordeum vulgare*	1.00E-28	P50299
P3	Hypothetical protein UM01200.1	*Ustilago maydis* 521	9.5	XP 757347
P9	Putative pol protein gene	*Zea mays*	4.8	AF 466202
P10	Hypothetical protein	*Oryza sativa* (japonica cultivar-group)	3.00E-04	XP 476015
P24	Major intrinsic protein 2	*Solanum tuberosum*	0.84	CAB 46351

Although some primers could be used in amplifying in most of the nine crops (e.g. P13 and P24), almost no polymorphism was found among the transferred species. One possible reason is that the corresponding genes containing these SSRs are highly conservative in these crops. The putative proteins of the 11 EST sequences containing polymorphic and transferable SSRs were given in Table 3.

Acknowledgements We are grateful to Prof. Guo-ying Wang for providing information of ESTs in foxtail millet. The work was supported by the grants No. 2005CCA02500 and 2005DIA4J019.

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