$GYDQFHV LQ &URS 6FLHQFH DQG 7HFKQRORJ\

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5HVHDUFK $UWLFOH 20,&6 ,QWHU

Development of Species-Specific Microsatellite Markers for Broomcorn Millet (*Panicum miliaceum* L.) via High-Throughput Sequencing

Min-Xuan Liu[1], Yue Xu[2], Tian-Yu Yang[3], Zhi-Jun Qiao[4], Rui-Yun Wang[5], Yin-Yue Wang[6] and Ping Lu[1*]

[1]*The National Key Facility for Crop Gene Resources and Genetic Improvement/Institute of Crop Science, Chinese Academy of Agricultural Science, Beijing 100081, China*

[2]*School of Life Science, Jilin University, Changchun 130012, China*

[3]*Institute of Crop, Gansu Academy of Agricultural Sciences, Lanzhou 030000, Gansu, China*

[4]*Institute of Crop Genetic Resources, Shanxi Academy of Agricultural Sciences, Taiyuan 030031, Shanxi, China*

[5]*Shanxi Agricultural University, Taiyuan 030031, Shanxi, China*

[6]*Faculty of Life Science, Jilin Agricultural University, Changchun 130118, Jilin, China*

[*]**Corresponding author:** Ping Lu, The National Key Facility for Crop Gene Resources and Genetic Improvement/Institute of Crop Science, Chinese Academy of Agricultural Science, Beijing 100081, China, Tel: +86-010-6215-9962; E-mail: lupingcaas@163.com

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Abstract

Objectives: To discover and develop large-scale SSR markers of the *P. miliaceum* genome, which can be used in future genetic studies effectively.

Result: 223,894 putative SSR sequences were identified by next-generation sequencing. A total of 56,694 primer pairs were successfully designed and 240 primer pairs were randomly selected for effectiveness validation. The expected heterozygosity and observed heterozygosity varied from 0.0447 to 0.7713 and from 0 to 0.9545, respectively and the mean of Shannon information index (I) was 0.7254. A UPGMA dendrogram indicated the high quality and effectiveness of these novel genomic SSR markers developed via next-generation sequencing technology.

Conclusion: A large repertoire of SSR markers were successfully developed by next-generation sequencing of the *P. miliaceum* genome which will be useful for the construction of genetic linkage maps, the identification of QTLs, and marker-assisted selection breeding.

Keywords: 454 FLX titanium pyrosequencing; Marker development; Microsatellite; Panicum miliaceum L

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Broomcorn millet (*Panicum miliaceum* L, 2n=4x=36), an important member of the genus Panicum [1] was domesticated in China more than 10,000 year [2,3] and it is an outstanding cropconsumption in China [10].

Over 8,600 accessions (varieties and landraces) of *Panicum miliaceum* have been deposited in the National Gene Bank located at the Chinese Academy of Agricultural Sciences (Beijing, China). Although abundant morphological variation exist among broomcorn millet landraces, the characterization and LGHQWLILFDWLRQ of this variation at molecular level is limited. LWV limitation is primarily due to the tetraploid genome (2n=4x=36) of *P. miliaceum* and the paucity of sequencing data, which has limited molecular marker development [11]. To date, no more than seventy characterized SSR loci are available for broomcorn millet [12,13], and these loci have been validated in relatively few genetic backgrounds. Initially, Hu et al. used 46 simple sequence repeat (SSR) markers from rice, wheat, barley and oat to study the genetic diversity of 118 broomcorn millet landraces collected from various ecological areas in China [12]. URX the construction of a SSR-enriched library from broomcorn millet genomic DNA, Cho et al. developed and LGHQWLILHG 25 polymorphic microsatellite markers to analyze the genetic diversity of 50 *P. miliaceum* accessions from Mongolia, India, the Republic of Korea, Russia, Italy, and Uzbekistan [13].

Microsatellite is a type of DNA marker that is frequently used in many areas of research [14]. However, for the species which no genomic resources are available, the HvHFWLYH development and de novo isolation of SSR markers are limited [15]. H application of next-generation sequencing (NGS) technology has brought about a revolution in biological and agricultural applications because they can sequence DNA at unprecedented speed [16,17]. In conjunction with selective hybridization, NGS technologies can be used in high-throughput applications to develop and identify sequences that ~DQ simple sequence repeat (SSR) regions. 6SHFLHV VSHFLILF 66Rs in mung beans [18], endangered dwarf bulrushes [19], fava beans [20], and grass peas [21] have been LGHQWLILHG using this method and can be

used as ORFXV VSHFL}F markers to promote the study of downstream genotyping.

In this study, we used next-generation sequencing technology to inexpensively and HyFLHQWO\ develop genomic SSR loci of broomcorn millet. Furthermore, 240 primer pairs were selected and DPSOLHG in 40 broomcorn millet genotypes aim to identify novel PLOOHW VSHFL}F SSR markers for future study.

5HVXOWV

4XDOLW\ HYDOXDWLRQ RI WKH 665 HQULFKHG '1$ OLEUDUL DQG UHDG FKDUDFWHUL]DWLRQ RI VHTXHQFLQJ GDWD

H quality of the SSR enriched broomcorn millet library was tested by sequencing 192 randomly selected clones. H result showed that the recombination rate within the constructed *P. miliaceum* library was 86.5%, and 30.7% of the cloned sequence contained SSR motifs with an insert that varied between 200 and 1000 bp in size.

A total of 1,087,428 reads were generated using the Roche 454 GS FLX Titanium platform, and 904,311 reads were selected for next study DV H removal of adaptor. H most abundant nucleotide in the reads was adenine, accounting for 32.98% of the sequences, followed by cytosine (24.95%), guanine (23.71%), and thymine (18.34%). H average GC content was 48.66%. H most of read lengths were between 350 and 500 bp with a mean length of 370.4 bp and a maximum length of 565 bp (Figure 1).

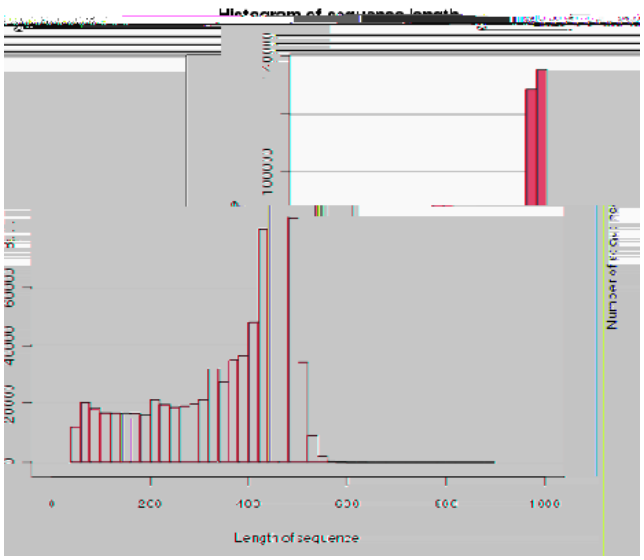
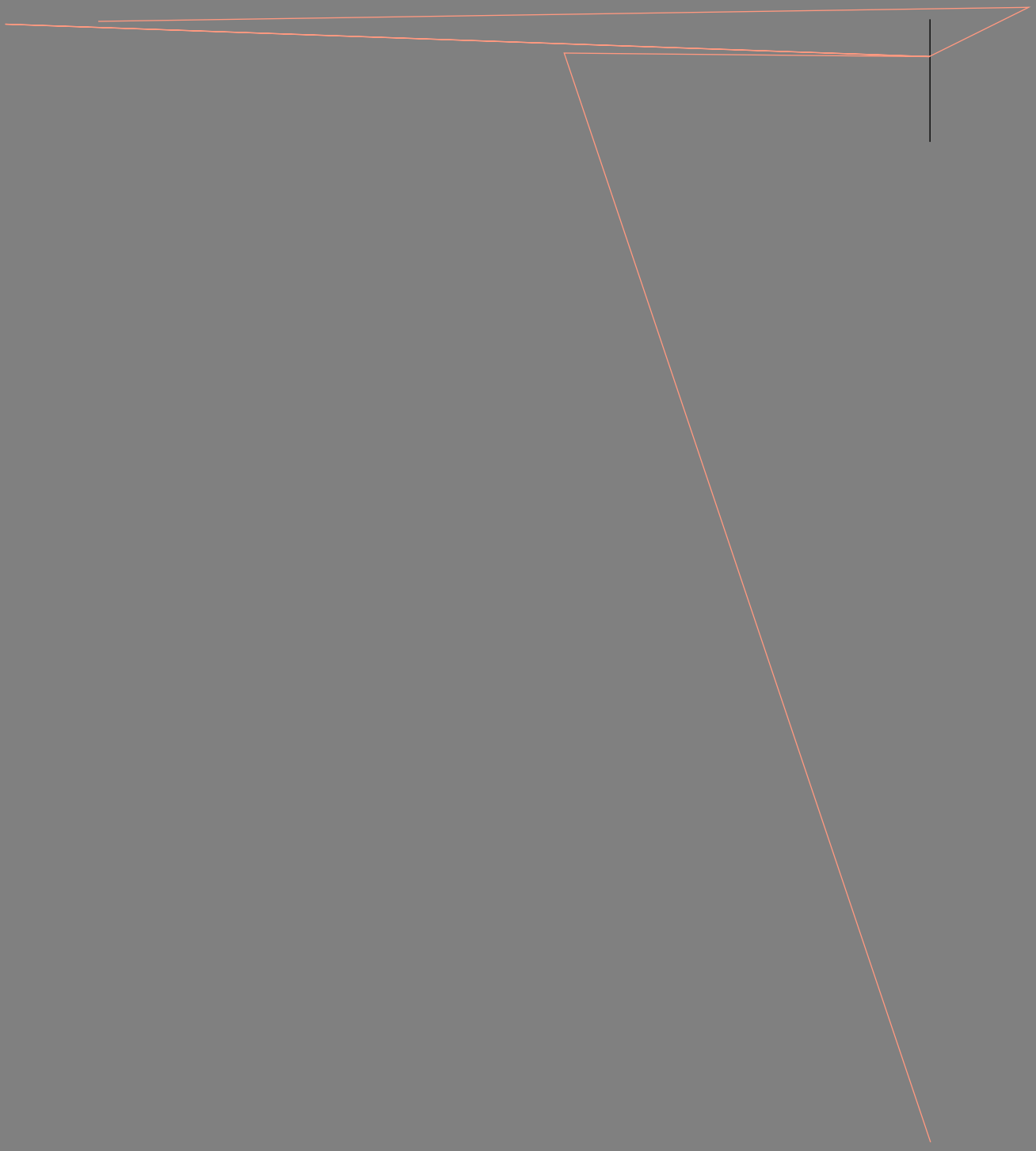


Figure 1: Frequencies length distribution of 454 clean read sequences.

H microsatellite L G H Q W L) L F D W L R Q tool (abbreviated to MISA, and can be downloaded from (http://pgrc.ipk-gatersleben.de/misa/) was used for SSR loci mining. A total of 223,894 reads contained one SSR loci, and 289,155 SSRs were distinguished. Furthermore, there are altogether 45,604 sequences containing more than one SSR loci, and 61,908 containing compound SSR loci (Table 1).

We analyzed the distribution of SSR loci start positions and found that a total of SSR motif reads length was 11,299,460 bp with an average valueof 199 bp. In the SSR motifs, most (78.6%) were situated within 320 bp of the 5'-terminus and middle regions of the cloned sequences. Few SSRs were located near the 3'-terminus (Figure 2). For later study of locus D P S O L } F D W L R Q 56,654 SSR primers were successfully designed by the Primer 3.0 public shareware to meet the criteria including size range of D P S O L } F D W L R Q products, optimal melting temperature, GC content, etc (Additional } O H Table 2).

Primer pair ID	Repeat	F (5'-3')	R (5'-3')	Size (bp)	Ta(°C)
ICSBM2	$(GA)_{13}$	GGCTTTGCTAGGGTTTCTCC	GGTGTGAAGTTGCCCAGATT	226	60
ICSBM3	$(GA)_{12}$	GTGTCTCTTTCGTCTTGCCC	GGGACACTTCCACCATCATC	204	60
ICSBM5	$(GT)_{13}$	TGTCTAGACCATCGCCATCA	CACTCACACACACATTTTCTTGG	218	60
ICSBM8	$(AC)_{14}$	GTGGTACAGCTGCTCGTTCA	AGGAGGAACCAGGAAGCAAT	254	60
ICSBM10	$(AC)_{15}$	GTGGTACAGCTGCTCGTTCA	GTGGTACAGCTGCTCGTTCA	15	GÕGTACAGCTGCTCG



ICSBM123	$(TAG)_{14}$	CGAGTCGGTGAAGAGAGACC	TTTGCAATGTTCACCCAACT	290	59
ICSBM126	$(TC)_{8}$	CAACAAGGTTGGTTGGCTTT	ATGCTGCTGCAGATGTTTTG	165	60
ICSBM127	$(AC)_{16}$	TATTCGAGCCCCATTTCTTG	GCGTTATCCGGATGATGAAG	184	60
ICSBM130	$(AC)_{17}$	CTGATCAAATCAATGCAGCAA	GTTTTTAGGTCCGTGGCGTAAAG	132	60
ICSBM132	$(CA)_{14}$	CACACAGATATTTGGCACCG	TGAGGATCCGAAAAGATTGG	216	60
ICSBM135	$(CA)_{7}$	GCCGGAGTATAGATCCGACA	GTCAGGCCGTGAACGTTATT	175	60
ICSBM139	$(CA)_{16}$	ATGCACGCACGAACACATA	TCTTGATCATCACCAGCACC	280	59
ICSBM144					

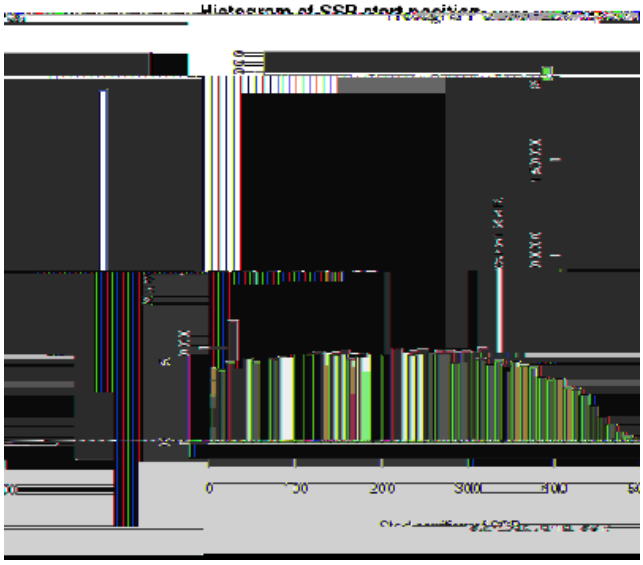


Figure 2: Number of the SSR motif start position from the

generate clear and reproducible polymorphic fragments [12]. LV numerous genomic SSR markers developed in the study will facilitate the evaluation of genetic structure and the construction of high-resolution maps in broomcorn millet.

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LVstudy provides a broad discovery and characterization of microsatellites loci in the broomcorn millet genome using 454 GS FLX Titanium sequencing technology. Moreover, massive SSR-enriched sequence data were }UVgenerated, facilitating the discovery and utilization of genomic SSR markers, further to accelerate the genomic and genetic research of broomcorn millet.

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