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# Genetic Diversity of Broomcorn Millet (Panicum miliaceum L) Cultivars and Landraces Based on Microsatellite

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## Abstract

Broomcorn millet (Panicum miliaceum L.), one of the first domesticated crops, has been grown in Northern China for at least 10,000 years. The species is presently a minor crop, and evaluation of its genetic diversity has been very limited. In this study, we analyzed the genetic diversity of 88 accessions of broomcorn millet collected from various provinces of China. Amplification with 67 simple sequence repeat (SSR) primers revealed moderate levels of diversity in the investigated accessions. A total of 179 alleles were detected, with an average of 2.7 alleles per locus. Polymorphism information content and expected heterozygosity ranged from 0.043 to 0.729 (mean = 0.376) and 0.045 to 0.771 (mean = 0.445), respectively. Cluster analysis based on the unweighted pair group method of mathematical averages separated the 88 accessions into four groups at a genetic similarity level of 0.633. A genetic structure assay indicated a close correlation between geographical regions and genetic diversity. The uncovered information will be valuable for defining gene pools and developing breeding programs for broomcorn millet. Furthermore, the millet-specific SSR markers developed in this study should serve as useful tools for assessment of genetic diversity and elucidation of population structure in broomcorn millet.

**Keywords:** Genetic; Diversity; Population structure; SSR markers; Panicum miliaceum L; Varieties

## Introduction

Broomcorn millet (Panicum miliaceum L. (Poaceae); 2n = 4x = 36) is an annual warm season crop also known as proso, hog, white, yellow, or common millet. One of the most ancient grain crops, its agricultural use in North China pushed back to the Pleistocene–Holocene boundary. Broomcorn millet is cultivated widely across China; the main production area is along the Great Wall, where it serves as an important staple food. The species is also planted for human and avian consumption in central Europe, Russia, India, Pakistan, Korea, Japan, and other parts of Eurasia, and has emerged as one of the most aggressive grass weeds in North America and Canada. Broomcorn millet has the shortest growing cycle of any cereal, reaching maturity 60–90 days after sowing. The crop also has low water and nutrient requirements, allowing it to be cultivated at a wide range of altitudes, even on marginal agricultural land where other cereals do not succeed Broomcorn millet is also a health food because of its unique nutritional benefits: it features protein contents, especially those of alkaline ones, which are higher than levels in crops such as wheat, rice, and oats, an abundance of easily absorbed amino acids, and a relatively balanced array of trace elements and vitamin precursors [1]. For these reasons, broomcorn millet continues to be an important component of the Chinese diet.

The collection, evaluation, conservation, and utilization of crop germplasm have become one of the top agricultural research priorities in China. Interest in the genetic diversity and structure of natural populations has increased because of the need to broaden knowledge of genetic variation in cultivated species. A detailed understanding of genetic relationships among germplasm resources is vital for future breeding process like yield, quality, and resistance (including pest and disease). In addition, a thorough dig and research of germplasm conserved in gene bank can facilitate the introgression of useful gene into the existing commercial crop genetic base According to the differences in morphological traits, isozymes, DNA markers, as well as pedigree information and geographic origins, crop genetic diversity and relationship can be evaluated [1]. Compared with restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), and random amplification of polymorphic DNA (RAPD) markers, simple sequence repeats (SSRs) have been shown to produce higher levels of polymorphisms and to have much greater ability to identify unique alleles in crop germplasm. SSRs constitute a superior molecular marker system, offering the advantages of being codominant, abundant, highly reproducible, highly polymorphic, and easy to assay. SSRs have been used to study genetic diversity in various crop species, including maize, soybean, sorghum, cowpea, and foxtail millet. SSRs have also been used to construct linkage maps, assess phylogenetic and population genetic relationships, and identify molecular markers for marker-assisted selection [2].

More than 8700 accessions (landraces and varieties) of P. miliaceum (Panicum miliaceum) are conserved in the National Gene Bank of the Institute of Crop Science, Chinese Academy of Agricultural Sciences (Beijing, China). Although abundant morphological variation exists within the broomcorn millet accessions, assessment of their genetic diversity using DNA markers has been inadequate. Previous analyses of genetic variation in P. miliaceum have employed isozymes, RAPDs, AFLPs, and SSRs transferred from other cereal species, as well as markers developed in broomcorn millet by de novo methods. The resulting data are limited, however, and cannot fully reveal genetic relationships among accessions [3]. Furthermore, no research has been performed on the genetic diversity and inter-relationships of cultivated varieties of broomcorn millet in China.

In this study, millet-specific SSR primers developed in our

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laboratory by high-throughput sequencing were used to identify polymorphisms and to analyze the genetic diversity and structure of 88 accessions comprising 56 main varieties cultivated in China and 32 parental lines.

## Results

### SSR Polymorphic variation

Using the 67 SSR primer pairs that produced clear polymorphic fragments among eight representatives during preliminary screening, we detected 179 alleles and 349 genotypes in the 88 studied accessions. Details of uncovered polymorphism levels and other parameters are given in Table 1. Observed number of alleles (Na), is one of the most important indexes of genetic differentiation associated with populations, types, and geographical sites [4]. Among the 88 accessions, Na per locus varied from 2 to 5, with a mean value of 2.7, and the number of amplified genotypes varied from 3 to 15, with an average of 5.2. The effective number of alleles (Ne) for each locus varied between 1.05 and 4.29, with an average of 1.995 per locus. Of 179 alleles, 10 (5.59%) were rare, with a frequency less than 0.05 in the entire set of samples. Approximately 50% and 32% of polymorphic SSR loci were associated with two and three alleles, respectively [5]. Values of Shannon's information index (I) varied from 0.1085 to 1.5194 per locus, with an average of 0.7254, while expected heterozygosity (He) and observed heterozygosity (Ho) ranged from 0.0447 to 0.7713 (mean = 0.4447) and 0 to 0.9545 (mean = 0.2348), respectively. Some loci, such as F786, F1036, F1067, F1071, F2185, BM306, and BM344, had a Ho of 0, suggesting universal outcrossing between individuals or perhaps between wild populations and nearby cultivated broomcorn millet. The value of genetic diversity which calculated according to Nei's 1973 (H) ranged from 0.0444 (for F1036) to 0.7669 (for F1380), with an average of 0.4419. With respect to FST, an index of genetic differentiation or the genetic distance between wild and cultivated accessions, values of the 67 applied markers ranged widely: from 0.0434 (BM114) to 0.8342 (F1071), with a mean of 0.2988. Polymorphism information content (PIC) values for each SSR ranged from 0.0434 (F1036) to 0.7288 (F1380), with an average of 0.376, indicating a moderate level of genetic diversity in Chinese broomcorn millet. In the analyzed samples, values of Na and Ne per locus were most strongly correlated with PIC ($r = 0.966–0.993$, $p < 0.05$), followed by I, He, and H [6].

### Genetic Relationships Based on Cluster Analysis

Unweighted pair-group method with arithmetic (UPGMA) cluster analysis based on genetic similarity values among the 88 broomcorn millet accessions yielded the dendrogram shown in Figure 1. As seen in the dendrogram, the most genetically similar accessions were two samples from Inner Mongolia bearing the same name: Dongsheng Erhuangmi [7]. The two most divergent accessions were Longshu3 from Heilongjiang and Ningmi15 from Ningxia, China. The cluster analysis divided the 88 accessions into four discrete groups at a genetic similarity value of 0.633 (Figure 1). Each group included accessions from at least one province, with each province represented in one to three groups Group A contained 25 accessions, including a series of Longshu varieties and their parents from Heilongjiang Province, China and 11 accessions from Inner Mongolia, China. This group was further subdivided in subgroups A1 (Heilongjiang), A2 (Inner Mongolia), and A3. Group B comprised 29 accessions: 16 of the 23 varieties collected from Inner Mongolia, four from Shanxi, China (Jinshu2, TianzhenShuzi, Jinshu9, and Ziluodai), three from Ningxia, China (Ningmi10, Ziganhong, and HaiyuanZiganhong), and two each from Heilongjiang (Nianfeng2 and Longshu3), Gansu, China, (Ganmi1 and Longmi3), and Shaanxi, China (Shenmuhongmizi and Yumi2). This group was further divided in three subgroups, of which B1 mainly included varieties and parents from Inner Mongolia [8]. Group C consisted of 33 accessions: 11 of the 15 samples from Shanxi, seven from Ningxia, five from Inner Mongolia, four from Gansu, and two each from Jilin, Shaanxi, and Heilongjiang. Group C was separated into four subgroups, with C2 and C4 mainly comprising varieties from Shanxi and Inner Mongolia, respectively. Group D consisted of only one accession, Ningmi15 from Ningxia. This grouping of accessions based on polymorphic SSR loci is consistent with the geographic source and genetic background of the analyzed samples.

### Plant Materials

A total of 88 broomcorn millet accessions (56 varieties and 32 parents) collected from seven main millet-producing Chinese provinces were provided by the institutions listed in Table S1.These accessions were divided into 11 populations according to sources. Populations 8–11 are four landrace populations, with all accessions from a given population having the same name. As indicated in Table S1, the 88 accessions belonged to five different ecotypes: Northeast (20 accessions), Loess Plateau (17), Mongolian Plateau (29), Northwest (16), and Alpine Region (6). Prior to experimental use, all plant materials were reproduced for three generations through strict self-crossing [9].

### DNA isolation

Seeds of each accession were sown in plastic pots (10 cm diameter) and grown under greenhouse conditions. Total genomic DNA was extracted from young leaves of 15–20-day old seedlings based on the modified cetyltrimethylammonium bromide method described by Edward et al. [35]. The relative purity and concentration of extracted DNA was evaluated on a Nano Drop ND-1000 instrument (Nanodrop, Wilmington, DE, USA). The final concentration of each DNA sample was adjusted to 30 ng•μL$^{-1}$.

### Primer screening and microsatellite amplification

We used 500 pairs of SSR primers developed in our laboratory by high-throughput sequencing to identify polymorphisms in eight representatives randomly selected from the 73 non-repeated accessions. All primers were synthesized by Dingguo Gene Co. (Beijing, China). A total of 162 primer pairs producing clear and reproducible polymorphic fragments among the eight accessions were used in further tests to assess the genetic diversity of all 88 accessions.

Polymerase chain reaction (PCR) amplifications were performed in 10 μL volumes containing 1.6 μL of 10× PCR buffer (containing 20 mM•Mg2+), 0.2 μL of each 10 mM dNTP, 0.1 μL of 5 U•μL$^{-1}$ Taq DNA polymerase, 0.5 μL of a 5 μM solution of each primer, 1 μL of 30 ng•μL$^{-1}$ genomic DNA, and 6.1 μL of ddH2O. Reactions were carried out in a PTC-100 Thermo-Cycler (ALT INC., East Lyme, CT, USA) using the program as follows:

(1) Initial denaturation at 94°C for 5 min;

(2) 39 cycles of denaturation at 94°C for 45 s

(3) Annealing at 55°C for 50 s;

(4) Extension at 72°C for 1 min;

(5) A final extension at 72°C for 10 min. The PCR-amplified products were resolved by 8% polyacrylamide gel electrophoresis, with DNA bands visualized by silver nitrate staining. Allele sizes were

determined using a 50-bp DNA ladder (Tiangen, Beijing, China).

### Data analysis

Allele presence and absence was scored for each SSR marker as 1 and 0, respectively. These scores were stored in an Excel file as a binary matrix and served as the basis of the genetic diversity analysis.

POPGENE 1.31 was used to calculate the following measures of genetic diversity: observed number of alleles (Na), effective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), Nei's gene diversity (H), and the Shannon–Weaver index (I). Geographical differentiation was evaluated by estimating F-statistic (FST) values among geographical regions using POPGENE. The Simpson diversity index for each SSR, also known as the polymorphism information content (PIC), was calculated using the program PIC-CALC 0.6. Using a similarity matrix generated from the proportion of shared fragments, genetic relationships among genotypes were determined by cluster analysis based on the unweighted pair group method of mathematical averages (UPGMA) as implemented in NTSYS2.1. We used STRUCTURE version 2.3.4 to identify genetic groups within the 88 broomcorn millet varieties and their parents. STRUCTURE analysis is a Bayesian approach that uses no a priori classification and divides samples into K populations according to the allele frequencies at each locus. The most likely number of genetic groups (K = 1 to 10) was estimated following the procedure of Evanno et al, who proposed the ad hoc statistic ΔK. Program settings included admixture ancestry and correlated marker frequency models, with α inferred from the data and lambda set to 1 [10]. Twenty independent Markov chain Monte Carlo runs, each consisting of 1,000,000 iterations with a burn-in of 500,000 iterations, were carried out for each K.

## Conclusion

In conclusion, our data indicates there have abundant genetic variation within different ecological growth areas and complex genetic relationships between various populations of broomcorn millet. On the other hand, the millet-specific SSR markers developed in this study can be served as effective molecular tools for the assessment of genetic diversity and the elucidation of population structure in broomcorn millet.

### Conflicts of interest

The authors declare no conflict of interest.

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