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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.

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SSR P c a a

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 di erentiation 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 ampli ed genotypes varied from 3 to 15, with an average of 5.2. e e ective 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. e

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

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Allele presence and absence was scored for each SSR marker as 1 and 0, respectively. ese scores were stored in an Excel le 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), e ective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), Nei's gene diversity (H), and the Shannon–Weaver index (I). Geographical di erentiation was evaluated by estimating F-statistic (FST) values among geographical regions using POPGENE. e 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 classi cation and divides samples into K populations according to the allele frequencies at each locus. e 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.

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

C c e e

e authors declare no con ict of interest.

1. Jana K, Jan M (2006)