Genetic Diversity and Classification of Chinese Elite Foxtail Millet [Setaria italica (L.) P. Beauv.] Revealed by Acid-PAGE Prolamin

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Abstract
Arid and semi-arid regions of China account for more than half of the country. Because of drought resistance and high nutritive value, elite foxtail millet (Setaria italica (L.) P. Beauv.) is one of the most important cereal crops in China. Evaluation of germplasm and genetic diversity of foxtail millet is still in its infancy, but prolamin could play an important role as a protein marker. To investigate the genetic diversity and population structure of foxtail millet from different ecological zones of China, 90 accessions of foxtail millet were collected from three major ecological areas: North, Northwest, and Northeast China. The prolamin contents were examined by acid polyacrylamide gel electrophoresis (acid-PAGE). Five to twenty-two prolamin bands appeared in tested varieties, of which were polymorphic, so prolamin patterns of foxtail millet varieties can be used in variety identification and evaluation. Structure analysis identified six groups, which matches their pedigree information but not their geographic origins. This indicated a high degree (87.78%) of consistency with a phylogenetic classification based on SSR. The results showed prolamin banding patterns were an effective method for analyzing foxtail millet genetic variability.

Keywords
Foxtail Millet [Setaria italica (L.) P. Beauv.], Seed Storage Protein, Protein Polymorphism, Prolamin

1. Introduction
Insufficient water supply is a major issue in the world for crop production. Chi-
na has serious water shortage problems, especially in northern regions. Foxtail millet (*Setaria italica* (L.) P. Beauv.) has drought-tolerant properties that render it an important cereal crop species in these regions. Foxtail millet originated in the Yellow River Valley of China at least 8700 years ago [1]; it has a short life cycle, high photosynthetic efficiency, and is enriched in various nutrients, and thus is an important crop used extensively for food, fodder, bioenergy in arid and semi-arid areas of Asia, North Africa, South and North America [2] [3].

For foxtail millet, some high yield types [4], disease resistance types [5], and other types of preferred varieties [6] have accumulated in long-term cultivation and domestication processes, yet basic research on foxtail millet lags major cereal crops such as wheat. Evaluation of germplasm and genetic diversity of foxtail millet is still in its infancy. The genetic diversity analysis of some foxtail millet pedigrees suggests potential new cultivars based on agronomic performance [7].

In agricultural studies have focused on morphology [7] [8], cytology, physiology, and biochemistry [9]. Morphological characters are affected by the environment, which leads to challenges in genetic identification. Biochemical markers, such as isoenzymes and proteins, are the product of gene expression, not all show the codominant inheritance, polymorphism of produce is limited, the close genetic relationship and genetic basis of complex material are difficult to identify [9]. With the development of molecular biology and genomics, the research and application of DNA molecular markers have rapidly developed. Molecular markers, such as amplified fragment length polymorphism (AFLP) [10], random amplified polymorphic DNA (RAPD) markers [11], and simple sequence repeat (SSR) markers [12] [13] have been used in genetic diversity research in foxtail millet. However, these techniques have drawbacks. The sensitivity of RFLP to DNA polymorphism detection is not high, it requires large quantities of DNA and a DNA fragment as a probe, and employs radioisotope and nucleic acid hybridization technology which are neither safe nor easy to automate. The stability and repeatability of RAPD experiments are poor and very sensitive to reaction conditions, such as template and Mg2+ concentrations. AFLP requires high genomic purity and reaction conditions, and SSR detection relies on a series of standard primers with a high polymorphism that covers all chromosomes in the genome, and the detection and analysis depends on a large number of samples.

Knowledge of genetic diversity is used for efficient germplasm management and utilization, genetic fingerprinting, and genotype selection [14]. prolamin, a heterogeneous group of alcohol-soluble storage proteins, is encoded by highly conserved multigenic families. An original methodology for their electrophoretic separation is acid polyacrylamide gel electrophoresis (acid-PAGE) [15] that can be used to detect the complex polymorphisms of prolamins. As prolamin is genotype-specific, the entire process includes protein extraction, electrophoresis, and band analysis—simple, repeatable, relatively cheap, and independent of environmental variation [16] or stage of plant ontogenesis. Genetic polymorphisms
have been used to evaluate genetic diversity in many plants, such as wheat [17], barley [18] [19], Leymus [20], tall fescue cultivars [21], triticale [22], vetches [16] [23] and rice [24].

Clustering results based on prolamin banding patterns and SSR analysis are not always in agreement [17], but both methods produced similar total genetic diversity results for Chinese wheat landrace [25]. The application of prolamin in foxtail millet varieties is not as advanced as in other crops, because of a limited heterogeneity in the genetic background [26] [27] and protein content is lower than other crops. As the foxtail millet germline source is not clear, the breeding efficiency of high-yield foxtail millet is low. China has diverse ecological types with numerous foxtail millet varieties in different ecological regions. The homology of foxtail millet prolamin may reflect the evolution of different varieties and guide the selection of parents. However, there are few reports on prolamin of cultivated varieties in different ecological regions. In this work, the prolamin A-PAGE method was used to analyze genetic diversity among 90 elite foxtail millet parental lines collected from different ecological regions in China, to guide the breeding, identification, and evaluation of new foxtail millet varieties.

2. Materials and Methods

2.1. Plant Materials

A total of 100 foxtail millet accessions were collected in 2016 from all the breeding programs in the North China summer foxtail millet region and Northwest and Northeast China spring foxtail millet regions. Those materials were initially evaluated for agronomically and economically-important traits and high yield potential at the breeding observation nursery of the Institute of Foxtail Millet Crops, Shijiazhuang, Hebei, China. After initial screening, 90 accessions were selected as basic breeding materials based on their yield and adaptation performance. Most of these materials were from eight foxtail millet growing provinces: Hebei, Henan, Shandong, Liaoning, Jilin, Shanxi, Inner Mongolia, Shaanxi (Supplemental Table 1).

2.2. Protein Extraction and Electrophoresis

Electrophoresis of prolamin was performed based on Wrigley’s method [28] with some modifications. For the analysis, 20 healthy seeds were randomly selected in each accession. Prolamins were extracted from the individually milled seed by adding 200 μL sample extract solution (70% isopropanol, 15% sucrose) into 1.5 mL tubes (Eppendorf, Germany) that were then incubated at 220 rpm (60°C) for 60 min. The extract was then centrifuged at 12000 r/min (4°C) for 10 min. The supernatant was transferred into a new tube and 100 μL of methylene green solution (80% glycerin, 0.02% methyl green) was added for pre-staining. The solution was heated in an oven at 60°C for 30 min, during which it was taken out and shaken every 10 min, and then put in a thermostat at 4°C. The
A-PAGE gel formula was: 12% acrylamide, 0.4% methylene diacrylamide, 2% glacial acetic acid, 6% urea, 0.1% ascorbic acid, 0.075% glycine, and 0.004% ferrous sulfate; 2 μL hydrogen peroxide catalyst was used to prepare the gel with a thickness of 1 mm. A glycolic polymorphism was detected in a 15 μL reaction system with 0.4% glacial acetic acid and 0.04% glycine as the electrode solution at 500 V constant pressure and 15°C for 80 min. After electrophoresis, acid-PAGE gel was stained with a 0.1% Coomassie Bright Blue R-250, 40% isopropyl alcohol, and 10% glacial acetic acid solution for 25 min. It was rinsed and decolorized with running water then photographs were taken with camera (Nikon, WJHH).

2.3. Statistical Analysis

To detect population genetic structure and assign individuals to subpopulations, the data obtained from acid-PAGE was scored based on the results of electrophoretic band spectra (Supplemental Table 2) for the presence or absence of the bands and entered as a binary data matrix. Population structure was determined by STRUCTURE software v2.3.4 [29] [30], which uses a Bayesian approach to identify clusters based on a fit to the Hardy-Weinberg equilibrium model and linkage equilibrium. Ten independent runs for each number of subpopulations value (k), which ranged from 3 to 13, were performed after the admixture model with 100,000 replicates for burn-in and 100,000 replicates during analysis. The optimal subgroup (k) value was determined based on 1) likelihood plots of these models, 2) stability of grouping patterns across the ten runs, and 3) information about the materials used in the study. The output was exported into Structure Harvester [31] to determine the most likely number of K clusters (K = 6 was optimum for this analysis, Figure 1A using Evanno’s ΔK method [31]. Results from 10 independent STRUCTURE runs for the most likely K were assessed with the software CLUMPP [32] and plotted using DISTRUCT [32].

3. Results

3.1. Genetic Diversity in Foxtail Millet

The prolamin contents were examined by acid-PAGE. Analysis of variance showed that 5 to 22 prolamin bands (Supplemental Table 2, Figure 2) appeared in tested varieties, of which were polymorphic. The results indicated that the genetic diversity of the breeding materials used in this study was high and should be valuable for breeding application.

3.2. Population Structure

STRUCTURE analysis of the population structure of the 90 foxtail millet accessions showed that the most appropriate grouping was six subpopulations with a ΔK peak of 6 (Figure 1A. Supplemental Table 1). The group of foxtail millet accessions was divided into six subpopulations by the prolamin method (Figure
Among the six subpopulations, the level of genetic diversity within pG6 (group 6 by the prolamin method) was the highest (26.67%), followed by pG1 (24.44%), pG3 (24.44%), pG2 (11.11%), pG4 (7.78%), and pG5 (5.56%) (Table 1, Supplemental Table 2).

**Figure 1.** Population structure analysis for 90 accessions of foxtail millet. (A) Delta K values for different numbers of populations assumed (K) in the structure analysis. (B) Classification of 90 accessions into four subpopulations according to preset K value using STRUCTURE program. The distribution of the accessions to different subpopulations is indicated by color (G1: red; G2: blue; G3: Dark green; G4: purple; G5: jade-green; G6: green).
Figure 2. Prolamin patterns of some cultivars or lines. 1. V41 (Zheng9188), 2. V61 (Jingu16), 3. V9 (K523), 4. V8 (Jigu24-1), 5. V2 (Cang156), 6. V43 (C164), 7. V13 (C208), 8. V47 (C138), 9. V42 (Cang344), 10. V64 (Datong28), 11. V61 (Jingu16), 13. V62 (Datong14), 12. V35 (Dungu1), 13. V63 (Datong30), 14. V65 (Jigu28), 15. V66 (Datong27), 16. V34 (Y61), 17. V17 (Shi207286).

Table 1. Common parents, their major ancestors, geographic distribution, number of accessions, and ecotypes of six subpopulations derived from structure analysis.

| Group       | pG1 (22) | pG2 (10) | pG3 (22) | pG4 (7) | pG5 (5) | pG6 (24) |
|-------------|----------|----------|----------|---------|---------|----------|
| Accessions  | 22 (24.44%) | 10 (11.11%) | 22 (24.44%) | 7 (7.78%) | 5 (5.56%) | 6 (26.67%) |
| Ancestors % |          |          |          |         |         |          |
| Riben60ri   | 9 (40.91%) | 5 (50%)  | 14 (63.64%) | 3 (42.86%) | 2 (40%)  | 4 (16.67%) |
| 60rihuancang| 5 (22.73%) | 4 (40%)  | 14 (63.64%) | 2 (28.57%) | 3 (42.86%) | 4 (16.67%) |
| Mihuangu    | 4 (18.18%) | 2 (20%)  | 7 (31.82%)  | 2 (28.57%) | 2 (40%)  | 3 (12.5%)  |
| Tulong      | 3 (13.64%) | 1 (10%)  | 7 (31.82%)  | 1 (14.29%) | 1 (20%)  | 3 (12.5%)  |
| Maichagu    | 1 (4.55%)  | 1 (10%)  | 5 (22.73%)  | 1 (14.29%) | 1 (20%)  | 1 (4.17%)  |
| Xiaoliugen  | 1 (4.55%)  | 1 (10%)  | 3 (13.64%)  | 1 (14.29%) | 1 (20%)  | 1 (4.17%)  |
| Qingshouweicao| 1 (10%) | 1 (4.55%)| 1 (4.55%) | 1 (14.29%) | 1 (20%)  | 1 (4.17%)  |
| Kenniya     | 2 (9.09%)  | 1 (14.29%) | 1 (14.29%) | 1 (14.29%) | 1 (20%)  | 1 (4.17%) |
| Lvxiugui    | 1 (4.55%)  | 1 (4.55%) | 1 (4.55%)  | 1 (14.29%) | 1 (20%)  | 1 (4.17%) |
| Yapche     | 1 (4.55%)  | 1 (4.55%) | 1 (4.55%)  | 1 (14.29%) | 1 (20%)  | 1 (4.17%) |
| Changxiuwan | 1 (4.55%)  |          |          |          |         |          |
| Pedigree unclear | 13 (59.09%) | 5 (50%) | 4 (18.18%) | 1 (14.29%) | 3 (60%) | 9 (37.5%) |
PG1 was collected from the summer foxtail millet region of central and southern Hebei Province. Riben60ri and 60rihuancang can be found in the pedigrees of most of these accessions. Nine accessions have definite ancestries, thirteen accessions did not. PG2 consisted of most accessions from central and southern Hebei Province and Liaoning Province. Riben60ri and Tulong can be found in the pedigrees of most of these accessions. Five accessions had definite ancestries and five accessions did not. PG3 was collected from Hebei Province. Most of these were the derivatives Riben60ri and Tulong, such as Yugu1 and its derivatives. Riben60ri is a Japanese landrace. Eighteen accessions had definite ancestries and four accessions did not. Riben60ri and Tulong can be found in the pedigrees of most of these accessions. PG4 was from Shanxi Province and was spring foxtail millet. Three can be traced back to founder-Moligu, two can be traced back to founder-Qitouhuang, one can be traced back to founder-Hainangu and Qinyuanmujizui. Six accessions had definite ancestries and one accession did not. The phylogeny of accessions was ambiguous. PG5 was collected from Shanxi Province, and two can be traced back to founder-Shanxidabaigu and two breeding materials that did not have clear pedigree information. One was breeding material from Hebei Province without clear pedigree information. Two accessions had common ancestors and three accessions did not. PG6 had diverse pedigrees and geographic origins. They include 8 accessions from Liaoning, 6 from Hebei, 3 from Shanxi, 3 from Neimenggu, 2 from Jilin, 1 from Henan, and 1 from America. Fifteen accessions had definite ancestries and nine accessions were unclear.

3.3. Population Structure, Pedigree, and Geographic and Ecological Distributions

There was no tight association between structure and ecological group (summer or spring foxtail millet) (Supplemental Table 1) based on grouping results from structure analysis. No relationships among genetic diversity, geographic origin, ecological group (summer or spring foxtail millet) (Supplemental Table 1), and the genotypes were observed based on grouping results from structure analysis. In each structure group, both summer and spring foxtail millet types were identified. However, the majority of accessions in pG1, pG2, pG3 and pG6 were the summer type and pG4, pG5 and pG6 were spring type. PG1 had the highest proportion of summer type (90.91%) and pG4 had the highest proportion of spring type (85.71%). All six groups consisted of accessions from different ecological regions, with pG1 and pG3 having the most accessions (81.82% and 77.24%, respectively) from the central and south Hebei Province, and accessions in pG4 and pG5 (84.6% and 80%) were mainly from central and south Shanxi Province. Accessions in pG2 consisted of most accessions from the central and south Hebei Province and Liaoning Province. Accessions in pG6 were from seven different regions with diverse ecological conditions. Only a small proportion of accessions (<35%) can be traced to each region.
Basic germplasm, parent-of-origin analysis (Supplemental Table 1), and pedigree analysis (Figure 3, Supplemental Table 3) indicated the following. Thirty-three accessions were derived from Riben60ri, and these accessions belonged to pG1 (proportion, 40.91%), pG2 (50%), pG3 (63.64%), pG4 (14.29%), pG6 (16.67%). Twenty-three accessions were derived from Tulong, and these accessions belonged to pG1 (13.64%), pG2 (40%), pG3 (63.64%), pG4 (14.29%), pG6 (4.17%). Thirteen accessions were derived from 60rihuancang, and these accessions belonged to pG1 (22.73%), pG2 (10%), pG3 (31.82%). Nine accessions were derived from Qinggouweicao, and these accessions belonged to pG1 (4.55%), pG2 (10%), pG3 (31.82%). Nine accessions were derived from Mihuangu, and these accessions belonged to pG1 (22.73%), pG2 (10%), and pG3 (13.64%). Six accessions were derived from Xiaoliugen, and these accessions belonged to pG1 (4.55%), pG2 (10%), and pG3 (18.18%). Five accessions were derived from Shuangguayin, and these accessions belonged to pG4 (14.29%) and pG6 (16.67%). Nineteen accessions were derived from Yugu1, and these accessions belonged to pG1 (22.73%), pG2 (30%), pG3 (40.91%), pG4 (14.29%), and pG6 (4.17%). Nine accessions were derived from WR1, and these accessions belonged to pG1 (4.55%), pG2 (10%), and pG3 (13.64%). Eight accessions were derived from shi181-5, and these accessions belonged to pG1 (4.55%), pG2 (10%), and pG3 (27.27%). These data indicated that Riben60ri and Tulong were the major germplasm of three ecological areas, pG1, pG2, and pG3, mainly derived from Yugu1 which is a derivative of Riben60ri (Supplemental Table 1). They also showed that pG1, pG2, and pG3 were close affinities, pG4 and G6 were close affinities, and Riben60ri was the source of these five groups; pG5 is relatively independent of the other groups.

3.4. The Consistency between the SSR and A-PAGE Prolamins Methods

The group of 90 foxtail millet accessions was both divided into six subpopulations by the SSR method and the A-PAGE prolamins method. Consistency analysis indicated that the accordant rate reached 87.8% (Table 2) between the two methods. The accordant rates of groups 1-6 were 87.5%, 100.0%, 71.9%, 100%, 100%, and 100%.

In sG1 (group 1 by the SSR method), Jigu25 and Gu10A were the accessions with inconsistent groupings. The parent-of-origin of Jigu25 is WR1 × Shi181-5, and the main germplasm base of Jigu25 are Riben60ri, 60rihuancang, Tulong, and Qinggouweicao. The main germplasm base rates (Table 1, Table 2, Figure 3) of sG1 are Riben60ri (68.75%), Tulong (68.75%), and Qinggouweicao (43.75%); Riben60ri (50%), Tulong (40%), and Qinggouweicao (10%) in pG2, therefore Jigu25 belonged to sG1. Data indicated that the SSR method is more robust. In sG3, the parent-of-origin of Heng968, Datong14, Datong28, and Datong27 are Lugu5 (7112 × (male-sterile lines × Riben60ri) × Lugu2) × 91101, Xiannong3 × Jingu9 (Shanxidabaigu), (Huangruangu × Zhangchunyi) F2 × Jingu9.
Figure 3. Pedigrees of major germplasm used in this study. G: group.
Table 2. The consistency analysis between SSR method and acid-PAGE prolamin method. G: Group, Note: pG1 = pG1a + pG1b, pG2 = pG2a + pG2b + pG2c, pG3 = pG3a + pG3b + pG3c, pG4 = pG4a, pG6 = pG6a + pG6b.

| G    | Number of varieties | Consistency | Consistency rate and number | Prolamins | Miss | Miss rate and number | Prolamin | The source of foxtail millet germplasm | The main germplasm base |
|------|---------------------|-------------|-----------------------------|-----------|------|----------------------|----------|---------------------------------------|------------------------|
| G1   |                    |             | All Pedigree clear Pedigree unclear | All Pedigree clear Pedigree unclear |       |                     |          |                                       |                        |
| sG1  | 16                  |             | 14 87.50% 1 | pG2a 2 1 150.0% |       |                     |          |                                       |                        |
|      | Riben60ri, Tulong, Shaungguanyin, Xiao huanggu, Haimuanggu, Qinyuanmujiuzui (1, 14.29%) |             | | | | | | | |
| sG2  | 7                   |             | 7 100.00% 1 | pG4a 0 0 0 0.00% |       |                     |          |                                       |                        |
|      | Riben60ri, Tulong, Shaungguanyin, Xiao huanggu, Haimuanggu, Qinyuanmujiuzui (1, 14.29%) |             | | | | | | | |
| sG3  | 32                  |             | 23 71.88% 8 | pG6a 9 3 28.12% |       |                     |          |                                       |                        |
|      | Riben60ri (5, 15.63%); Shaungguanyin (4, 12.5%); Dushaqi, Huangguzi (3, 9.38%); Shanzidabaigu (2, 6.25%); 60ruhuangcang, Tulong, Chaixianggu, Jinfen52, Qinhuanggu, Huainghuanggu, Yingxiugu, Jinxiangyu, Meiguodatou, Shanzhihezi, Xainnong3, Zhangchunyi (1, 3.13%) |             | | | | | | | |
| sG4  | 7                   |             | 7 100.00% 3 | pG5 0 0 0 0.00% |       |                     |          |                                       |                        |
|      | Riben60ri (4, 57.14%); Tulong (3, 42.86%); Mihuanggu (2, 28.57%); Chaixianggu, Xiao huanggu (1, 14.29%) |             | | | | | | | |
| sG5  | 7                   |             | 7 100.00% 2 | pG6b 0 0 0 0.00% |       |                     |          |                                       |                        |
|      | Riben60ri, Tulong (4, 57.14%); Mihuanggu (4, 32.86%); 60ruhuangcang, Qinggosweiacao, Yapoche, Lvsuigu (1, 14.29%) |             | | | | | | | |
| sG6  | 21                  |             | 21 100.00% 13 | pG6c 0 0 0 0.00% |       |                     |          |                                       |                        |
|      | Riben60ri (8, 38.10%); 60ruhuangcang, Mihuanggu (4, 19.05%); Tulong (3, 14.29%); Xiao huanggu, Chaixianggu, Maichagu (1, 4.76%) |             | | | | | | | |
| All  | 90                  |             | 79 87.78% 28 |              |       |                     |          |                                       |                        |

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(Shanxidabaigu) and (73-50 × Zao1) × Yi17, respectively. The main germplasm bases of Heng968, Datong14, and Datong28 are (Riben60ri, 60rihuancang), (Xiannong3, Shanxidabaigu) and (Huangruangu, Zhangchunyi, Shanxidabaigu), respectively. The main germplasm bases of pG5 are Shanxidabaigu (40%), Xainnong3 (20%), Huangruangu (20%) and Zhangchunyi (20%), whereas they were Riben60ri (15.63%); Shanxidabaigu (6.25%); 60rihuancang (3.13%), Huangruangu (3.13%), Xainnong3 (3.13%), and Zhangchunyi (3.13%) in sG3. They showed that Heng968 belonged to pG1, and Datong14, Datong28, and Datong27 belonged to pG5 by the main germplasm base identified by the two methods. These data indicated the A-PAGE prolamins method is closer to the pedigree analysis. Because the main germplasm bases of Gu10A, Tiedalihuang, Jinzhougugu14, shi207191, Datong27, Datong30, and Jigu28 are not distinct, it is difficult to judge which of the two methods is preferred.

4. Discussion

4.1. Prolamin and Genetic Diversity

Prolamin is the main storage protein of plant seeds, a gene expression product at a specific stage of seed development. The number and combination of its electrophoresis bands are controlled by genes, minimally affected by environmental factors, and thus can reflect the differences in gene coding sites of different crop varieties [33] [34]. Therefore, the analysis of plant varieties by prolamin can reveal specific genetic differences among varieties (Figure 2). The application of prolamin to the study of plant genetic resources has the advantages of simplicity, convenience, and accuracy. Lang et al. [35] found the glycolic homology degree in wheat generally reflects the distance of the genetic relationship among the main popularized wheat varieties in China and can be further used to guide the selection of parents.

In this research, a high level of polymorphism was identified for prolamin across the 90 accessions. It showed that 5 to 22 prolamin bands appeared in tested varieties. Structure analysis identified six groups, which matches with their pedigree information, but not with their geographic origins. The grouping consistency was 87.78% between the SSR method and the acid-PAGE prolamin method [13]. This might be due to highly diverse accessions which were collected from three major foxtail millet ecological regions.

4.2. Genetic Diversity and Population Structure of Chinese Foxtail Millet

Because it is genotype-specific, simple, repeatable, cheap, and independent of environmental variation nature, prolamin has frequently been used as a tool to examine the dynamics of genetic differentiation in a population. For example, structure analysis can estimate the number of subpopulations and the genetic relatedness among accessions. Cluster analysis can also group the assayed accessions into different groups. In this study, structure analysis identified six groups,
which matches with their germplasm information and SSR method grouping [13]. Basic germplasm and parent-of-origin analysis (Supplemental Table 1) indicated that there were not associated with a particular ecological environment, and the origins of accessions would differentiate among accessions if they are all landraces.

The extensive exchange of genetic resources breaks barriers among the three major ecological areas in China. Following germplasm being introduced into a new ecological environment, it goes through continuous domestication and hybridization with local breeding lines and eventually results in new progenies. All the groups contain both summer and spring types. Riben60ri was introduced to the North China summer foxtail millet region and became major parent cultivars in this region. After that, Riben60ri and its derivatives were usually hybridized with a locally adapted parent. Breeders from different regions selected different genotypes according to their preferences. Thus, it is expected that many new cultivars are interrelated and some geographically distant accessions may also be genetically related.

Structure analysis mainly was based on the genetic relatedness among accessions. Thus, the grouping based on these two approaches may not necessarily match with their geographic origin and ecotype. In pG4, most of the accessions derived from Qitouhuang and Moligu, which originated from central and southern Shanxi Province. Most of the accessions in pG1, pG2, and pG3 were selected from crosses between Riben60ri and locally adapted materials. For example, Qingdaolao and Yugu1 were derived by crossing Riben60ri to the Chinese landrace Mihuangu derivative Xinnong724 and Japanese landrace Tulong. Further, Yugu1 was crossed to other locally adapted materials, and cultivars were selected for their adaptation to different growth areas, which generated pG1, pG2, and pG3. Qingdaolao derivatives mainly contributed to accessions in pG1, while accessions in pG3 were mainly transition types between Yugu1 derivatives and Qingdaolao derivatives. These included Yugu1 and Qingdaolao derivatives and progenies from the cross between the two cultivars. PG5 was mainly selected from locally adapted materials, including Shanxidabaigu, which originated from central and southern Shanxi Province. PG6 was scattered in several regions, but most accessions were from Northeast China and the nearby Northwest spring foxtail millet region.

4.3. The Implications of Genetic Improvement of Foxtail Millet

Insufficient water resources seriously affected agricultural production. Foxtail millet is a highly drought-tolerant crop. The research of genetic diversity and the population structure of foxtail millet germplasm resources will accelerate the effective utilization of the limited resources for breeding. The foxtail millet materials used in this study were derived from the main foxtail millet-producing area in China. Many accessions (38.89%) had no pedigree records; this study elucidated the genetic relationship between these unknown accessions and those with
a known pedigree to determine origins.

The study indicated that when breeding foxtail millet, prolamin analysis was simple, accurate, and efficient, which will improve breeding project design and selection accuracy. In past, male-sterile parents and geographical unrelated materials were used for hybrid breeding. The results from this study indicated that accessions separated by great geographical distance may not necessarily be genetically distant. Classification of 90 accessions into six groups matched with their genetic relatedness and thus provides a good reference for designing crosses to improve hybrid-breeding efficiency. Accessions in pG4 and pG5 have unique geographic origins and pedigrees that are different from other groups; therefore, cross accessions among pG4, pG5 and other groups are more likely to obtain expected recombination for developing both conventional and hybrid cultivars. Further research may be needed to evaluate the combining results among groups to determine the combinations of accessions from different groups with the best heterosis. Although accessions in pG6 had the Riben60ri consanguinity, they have the most diverse origins and the greatest variation within the group. Accessions in pG5 were an independent group with five other groups, and crosses between accessions within pG5 and the other five groups may generate useful heterosis. Thus, further research on accessions may facilitate effective the use of germplasm in this group.

The acid-PAGE prolamin method is reliable, and the grouping consistency was 87.78% between the SSR method and the acid-PAGE method. The classification is closer to the germline source of foxtail millet. Furthermore, the acid-PAGE prolamin method is not needed for designing a large number of specific PCR primers or high-quality genomic DNA, and it is a simpler operation with a lower cost. In sum, it is an effective method for the breeding, identification, and evaluation of new varieties of foxtail millet.

5. Conclusion

In general, the acid-PAGE prolamin method is reliable and advantageous in breeding, identification, and evaluation of new varieties of foxtail millet, which has highly consistent with SSR method in group classification and is closer to the germline source of foxtail millet. In addition, the acid-PAGE prolamin method is more convenient than SSR method, don’t have to design or select a large number of specific PCR primers or high-quality genomic DNA.

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Author Contributions

ZL and DL contributed to the study conception and design. SL and JZ collected
the foxtail millet materials. GM, YC, and QL genotyped the accessions, and GM and DL conducted the population structure analysis and other data analysis. The first draft of the manuscript was written by GM, and ZL revised the article. All authors read and approved the final manuscript.

**Conflicts of Interest**

The authors declare no conflicts of interest regarding the publication of this paper.

**References**

[1] Zohary, D., Hopf, M. and Weiss, E. (2012) Domestication of Plants in the Old World: The Origin and Spread of Domesticated Plants in Southwest Asia, Europe, and the Mediterranean Basin. Oxford University Press on Demand, Oxford. https://doi.org/10.1093/acprof:osobl/9780199549061.001.0001

[2] Lata, C., Gupta, S. and Prasad, M. (2013) Foxtail Millet: A Model Crop for Genetic and Genomic Studies in Bioenergy Grasses. *Critical Reviews in Biotechnology*, 33, 328-343. https://doi.org/10.3109/07388551.2012.716809

[3] Zhang, G., et al. (2012) Genome Sequence of Foxtail Millet (*Setaria italica*) Provides Insights into Grass Evolution and Biofuel Potential. *Nature Biotechnology*, 30, 549-554. https://doi.org/10.1038/nbt.2195

[4] Zhang, J., Zhang, G. and Liu, B. (2010) Demonstration and Analysis of Zhanggu in Chengde. *Hebei Agriculture*, 6, 13-14.

[5] Tong, X. and Jiang, X. (2000) Influence of Different Density and Row Ratios of Parents for Ningza No. 1 on the Seed Yield. *Journal of Nanjing Agricultural Technology College*, 16, 25-28.

[6] Shi, G., et al. (2012) Breeding and Seed Production Technology of New Millet Hybrid Variety jingu49. *Crops*, No. 5, 112-114.

[7] He, J. (2004) Research on Genetic Diversity of Elite Foxtail Millet Germplasm. *Gansu Agricultural Science and Technology*, No. 2, 12-14.

[8] Kawase, M. and Sakamoto, S. (1987) Geographical Distribution of Landrace Groups Classified by Hybrid Pollen Sterility in Foxtail Millet, *Setaria italica* (L.) P. Beauv. *Japanese Journal of Breeding*, 37, 1-9. https://doi.org/10.1270/jsbbs1951.37.1

[9] Liu, Y., Gao, P. and Wen, Q. (1989) A Study on Esterase Isoenzymes of Foxtail Millet Germplasms and Their Related Species. *Acta Agriculturae Boreali-Sinica*, 4, 36-41. (In Chinese)

[10] Liu, C., et al. (1994) An RFLP-Based Genetic Map of Pearl Millet (*Pennisetum glaucum*). *Theoretical and Applied Genetics*, 89, 481-487. https://doi.org/10.1007/BF00225384

[11] Schontz, D. and Rether, B. (1999) Genetic Variability in Foxtail Millet, *Setaria italica* (L.) P. Beauv.: Identification and Classification of Lines with RAPD Markers. *Plant Breeding*, 118, 190-192. https://doi.org/10.1046/j.1439-0523.1999.118002190.x

[12] Liu, Z., et al. (2014) Genetic Diversity and Classification of Cytoplasm of Chinese Elite Foxtail Millet [*Setaria italica* (L.) P. Beauv.] Germplasm. *Crop Science*, 54, 659-666. https://doi.org/10.2135/cropsci2012.11.0646

[13] Liu, Z., et al. (2011) Genetic Diversity and Population Structure of Elite Foxtail Millet [*Setaria italica* (L.) P. Beauv.] Germplasm in China. *Crop Science*, 51, 1655-1663.
[14] Aliyeva, A., Ojaghi, J. and Mehdiyeva, S. (2012) Electrophoretic Profiles of Gliadin Subunits to Evaluate Genetic Diversity of Azerbaijani Synthetic Branched Spike Wheat Accessions. *American-Eurasian Journal of Agricultural & Environmental Sciences*, **12**, 1343-1349.

[15] Bushuk, W. and Zillman, R. (1978) Wheat Cultivar Identification by Gliadin Electrophoregrams. I. Apparatus, Method and Nomenclature. *Canadian Journal of Plant Science*, **58**, 505-515. [https://doi.org/10.4141/cjps78-076](https://doi.org/10.4141/cjps78-076)

[16] Lee, J. and Ronalds, J. (1967) Effect of Environment on Wheat Gliadin. *Nature*, **213**, 844-846. [https://doi.org/10.1038/213844b0](https://doi.org/10.1038/213844b0)

[17] Desheva, G.N., Kyosev, B. and Deshev, M. (2020) Assessment Genetic Diversity of Einkorn Genotypes (*Triticum monococcum* L.) by Gliadin Electrophoresis. *Acta Agriculturae Slovenica*, **116**, 327-336. [https://doi.org/10.14720/aas.2020.116.2.1430](https://doi.org/10.14720/aas.2020.116.2.1430)

[18] Vyháněk, T., et al. (2003) Use of Prolamin Polymorphism to Describe Genetic Variation in a Collection of Barley Genetic Resources. *Czech Journal of Genetics and Plant Breeding*, **39**, 45-50.

[19] Eshghi, R. and Akhundova, E. (2009) Genetic Diversity of the Monomeric Prolamins and Hordein in Hulless Barley Genotypes and Their Relation with Agronomical Traits. *African Journal of Biotechnology*, **8**, 1819-1826.

[20] Ruïwu, Y., et al. (2004) Genetic Polymorphism of Gliadin in Leymus. *Acta Botanica Yunnanica*, **26**, 103-110.

[21] Abernethy, R., et al. (1989) Classification and Pedigree Verification of Tall Fescue Cultivars Utilizing the Prolamin Seed Protein Fraction. *Crop Science*, **29**, 791-797. [https://doi.org/10.1038/213844b0](https://doi.org/10.1038/213844b0)

[22] Vyhnalék, T. and Bednar, J. (2003) Study of Genetic Diversity of Triticale Varieties by Prolamine Proteins. In Biotechnologicke metody v slachteni rastlin BIOS 2003, Nitra (Slovak Republic), 18 Sep. 2003. Slovenska polnohospodarska univ. v Nitre.

[23] Bechkri, S., Medoukali, I. and Khelifi, D. (2017) Ecogeographic Variability and Genetic Diversity Associated with Seed Albumins, Globulins and Prolamins Patterns in Vicia Taxa from Algeria. *Botanical Studies*, **58**, Article No. 27. [https://doi.org/10.1186/s40529-017-0177-7](https://doi.org/10.1186/s40529-017-0177-7)

[24] Aung, P., Kumamaru, T. and Satoh, H. (2002) Genetic Diversity of Prolamin Polypeptides in Myanmar Local Rice Cultivars.

[25] Tao, X.-P. (2006) Genetic Diversity of Chinese Wheat Landraces as Revealed by Gliadin and SSR Analyses. Chinese Academy of Agricultural Sciences, Beijing.

[26] Kumar, K. and Parameswaran, K.P. (1998) Characterisation of Storage Protein from Selected Varieties of Foxtail Millet (*Setaria italica* (L) Beauv). *Journal of the Science of Food and Agriculture*, **77**, 535-542. [https://doi.org/10.1002/(SICI)1097-0010(199808)77:4<535::AID-JSFA77>3.0.CO;2-G](https://doi.org/10.1002/(SICI)1097-0010(199808)77:4<535::AID-JSFA77>3.0.CO;2-G)

[27] Monteiro, P.V., Virupaksha, T.K. and Rao, D.R. (1982) Proteins of Italian Millet: Amino Acid Composition, Solubility Fractionation and Electrophoresis of Protein Fractions. *Journal of the Science of Food and Agriculture*, **33**, 1072-1079. [https://doi.org/10.1002/jsfa.2740331104](https://doi.org/10.1002/jsfa.2740331104)

[28] Wrigley, C. (1992) Identification of Cereal Varieties by Gel Electrophoresis of the Grain Proteins. In: *Seed Analysis*, Springer, Berlin, 17-41. [https://doi.org/10.1007/978-3-662-01639-8_2](https://doi.org/10.1007/978-3-662-01639-8_2)

[29] Falush, D., Stephens, M. and Pritchard, J.K. (2003) Inference of Population Struc-
ture Using Multilocus Genotype Data: Linked Loci and Correlated Allele Frequencies. *Genetics*, **164**, 1567-1587. [https://doi.org/10.1093/genetics/164.4.1567](https://doi.org/10.1093/genetics/164.4.1567)

[30] Pritchard, J.K., Stephens, M. and Donnelly, P. (2000) Inference of Population Structure Using Multilocus Genotype Data. *Genetics*, **155**, 945-959. [https://doi.org/10.1093/genetics/155.2.945](https://doi.org/10.1093/genetics/155.2.945)

[31] Earl, D.A. (2012) Structure Harvester: A Website and Program for Visualizing Structure Output and Implementing the Evanno Method. *Conservation Genetics Resources*, **4**, 359-361. [https://doi.org/10.1007/s12686-011-9548-7](https://doi.org/10.1007/s12686-011-9548-7)

[32] Jakobsson, M. and Rosenberg, N.A. (2007) CLUMPP: A Cluster Matching and Permutation Program for Dealing with Label Switching and Multimodality in Analysis of Population Structure. *Bioinformatics*, **23**, 1801-1806. [https://doi.org/10.1093/bioinformatics/btm233](https://doi.org/10.1093/bioinformatics/btm233)

[33] Kawaura, K., Mochida, K. and Ogihara, Y. (2005) Expression Profile of Two Storage-Protein Gene Families in Hexaploid Wheat Revealed by Large-Scale Analysis of Expressed Sequence Tags. *Plant Physiology*, **139**, 1870-1880. [https://doi.org/10.1104/pp.105.070722](https://doi.org/10.1104/pp.105.070722)

[34] Metakovsky, E., Novoselskaya, A.Y. and Sozinov, A. (1984) Genetic Analysis of Gliadin Components in Winter Wheat Using Two-Dimensional Polyacrylamide Gel Electrophoresis. *Theoretical and Applied Genetics*, **69**, 31-37. [https://doi.org/10.1007/BF00262533](https://doi.org/10.1007/BF00262533)

[35] Lang, M., Lu, S. and Zhang, R. (2001) Analysis of the Genetic Evolution of Gliadin Composition in the Major Wheat Cultivars Grown in North China. *Crops*, No. 6, 958-966.
### Supplementary Materials

#### Supplemental Table 1. The grouping of foxtail millet by acid-PAGE prolamins method.

| No. | Group % | Variety | Ecotypes (%) | Geographical position | No. accessions (%) | parent-of-origin | Basic germplasm |
|-----|---------|---------|--------------|-----------------------|-------------------|-----------------|-----------------|
| V1  | Shi202242 | summer | Hebei        |                       |                   |                 |                 |
| V2  | Cang156  | summer | Hebei        |                       |                   |                 |                 |
| V3  | Chaoga12 | spring | Liaoning     |                       |                   |                 |                 |
| V4  | Shi207393| summer | Hebei        |                       |                   |                 |                 |
| V5  | Shi207382| summer | Hebei        |                       |                   |                 |                 |
| V6  | Shi02521 | summer | Hebei        |                       |                   |                 |                 |
| V7  | Shi02399 | summer | Hebei        |                       |                   |                 |                 |
| V8  | Jigu24   | summer | Hebei        |                       |                   |                 |                 |
| V9  | K323     | summer | Hebei        |                       |                   |                 |                 |
| V10 | Shi206058| summer | Hebei        |                       |                   | Hebei           |                 |
| V11 | Shi207286| summer | Summer       | Hebei                 |                   | Hebei (18, 81.82%), Shandong (2, 9.09%), Liaoning (1, 4.55%) |                 |
| V12 | pG1 C445 | summer | Hebei        |                       |                   |                 |                 |
| V13 | C208     | summer | Hebei        |                       |                   |                 |                 |
| V14 | Ji9409   | summer | Hebei        |                       |                   |                 |                 |
| V15 | Jigu26   | summer | Hebei        |                       |                   |                 |                 |
| V16 | Jigu22   | summer | Hebei        |                       |                   |                 |                 |
| V17 | Shi98700 | summer | Hebei        |                       |                   |                 |                 |
| V18 | Jigu20   | summer | Hebei        |                       |                   |                 |                 |
| V19 | Ji9403   | summer | Shandong     |                       |                   |                 |                 |
| V20 | Richaogu | summer | Hebei        |                       |                   |                 |                 |
| V21 | Heng668  | summer | Hebei        |                       |                   |                 |                 |
| V22 | Jinfen1A | spring | Shanxi       |                       | 683A: 81-16       |                 |                 |
| V23 | An2491   | summer | Henan        |                       |                   |                 |                 |
| V24 | Jinzhoug12 | spring | Liaoning     |                       |                   |                 |                 |
| V25 | Shi6-439 | summer | Hebei        |                       |                   |                 |                 |
| V26 | pG2      | spring | Liaoning     |                       |                   |                 |                 |
| V27 | Lugu10   | summer | Shandong     |                       |                   |                 |                 |
| V28 | GuA3     | summer | Hebei        |                       |                   |                 |                 |
| V29 | Jigu25   | summer | Hebei        |                       |                   |                 |                 |
Continued

V30 Gu3MA summer Hebei -
V31 Tiedalihuang spring Liaoning -
V32 Jinzhoug34 spring Liaoning Dungen
V33 Bagu214 spring Hebei -
V34 Y61 summer Hebei (Xang3 × Kenniya) × 433 Kenniya -
V35 Dungu1 spring Shanxi -
V36 K1011 summer Hebei 94355 × Jugu25 (WR1 × Shi181-5) Riben60ri, 60rihuancang, Tulong, Qinggouweicao
V37 K359 summer Hebei Gufeng2 (95307 × Lugu10 (Yugu1 (Riben60ri × Tulong)) × Bu5019 (Riben60ri × Xiaoilugren)) × Jugu25 (WR1 × Shi181-5) Riben60ri, 60rihuancang, Tulong, Qinggouweicao, Xiaoilugen
V38 K660 summer Hebei Y61 ((Kang3 × Kenniya) × 433) × S80 (433 × W82) Kenniya -
V39 An2367 summer Henan Jingu21 (Jinfen52 × Qitouhuang) × Ninghuang1 (Yugu1 (Riben60ri × Tulong)) Riben60ri, Tulong, Lvsuigu
V40 Zheng9188 summer Henan 8744 (Yugu2 × Zheng407 (Lugu2 × 7434B × Yugu1 (Riben60ri × Tulong))) × Lugu2 Riben60ri, 60rihuancang, Tulong, Xiaoilugen
V41 Cang344 summer Hebei Jugu25 (WR1 × Shi181-5) × Shi181-5 Riben60ri, 60rihuancang, Tulong, Qinggouweicao
V42 Ci164 spring Hebei Jugu25 (WR1 × Shi181-5) × Shi181-5 Riben60ri, 60rihuancang, Tulong, Qinggouweicao
V43 Shi97672 summer Hebei Y61 ((Kang3 × Kenniya) × 433) × 560 (433 × W82) Kenniya -
V44 Jigu21 summer Hebei - -
V45 Chengg2u2 summer Hebei Cao46-10 × Chengg2u2 Yapoche
V46 Tai22uan2 spring Shanxi Jingu21 (Jinfen52 × Qitouhuang) × Ninghuang1 (Yugu1 (Riben60ri × Tulong)) Riben60ri, Tulong, Mihuanggu
V47 Ci138 summer Hebei Shi181-5 × Jugu25 (Jugu25 (81407 (Shuli2uihun × Yugu1 (Riben60ri × Tulong)) × 81474 (Zhengg2u2 (Qingdaolao)) × Shi181-5)) × (Zhengai2 (Qingdaolao (Riben60ri × Xinnong724 (Mihuanggu)))) Riben60ri, Tulong, Xiaoilugen, Mihuanggu
V48 06-766 summer Hebei - -
V49 Jinangu1 summer Shandong Yugu2 × Zheng5 (81402 (Shuli2uihun × Yugu1 (Riben60ri × Tulong)) × 81474 (Zhengai2 (Qingdaolao (Riben60ri × Xinnong724 (Mihuanggu)))) Riben60ri, Tulong, Xiaoilugen, Mihuanggu
V50 Bao182 summer Hebei Zheng801607 × Bao849 (Bao842090 × Yugu1 (Riben60ri × Tulong)) Riben60ri, Tulong, Xiaoilugen, Mihuanggu
V51 L70 summer Hebei WR1 × Shi181-5 Riben60ri, 60rihuancang, Tulong, Qinggouweicao
V52 Shi207191 summer Hebei - -
V53 Jigu29 summer Hebei WR1 × Yugu1 (Riben60ri × Tulong) Riben60ri, Tulong, Xiaoilugen
V54 Changnong35spring summer Hebei - -
V55 Changnong35spring summer Hebei (1, 14.29%) × (6, 85.71%) Jingu21 (Jinfen52 × Qitouhuang) × Ninghuang1 (Yugu1 (Riben60ri × Tulong)) Riben60ri, Tulong, Xiaoilugen, Jingu21, Qitouhuang

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| **V56** | Yangui2 | spring | Shanxi | **Lvgu2** (Jingu6 (Moligu)) × 79-421 | Moligu |
| **V57** | Taixuan4 | spring | Shanxi | **95pin10** × **Jingu20** (Jingu6 (Moligu)) | Moligu |
| **V58** | Taixuan5 | spring | Shanxi | **Chigu5** (Shuangguayin1 (Shuangguayin)) × **Jingu20** (Jingu6 (Moligu)) | Moligu, Shuangguayin |
| **V59** | Gu11A | summer | Hebei | - | - |
| **V60** | Jingu35 | spring | Shanxi | **Jingu14 × Jingu21** (Jingu6 (Moligu)) | Jinfen52, Qitouhuang |
| **V61** | Jingu16 | spring | Shanxi | **Changnuang1** (Qinyuanmujizui (Qinyuanmujizui)) × **Hainangu** | Qinyuanmujizui, Hainangu |
| **V62** | Datong14 | spring | Shanxi | **Xianang3** × **Jingu3** (Shanxidabaigu) | Xiamong3, Shanxidabaigu |
| **V63** | Datong30 | spring | Shanxi | - | - |
| **V64** | Datong28 | spring (1, 20%); Spring (4, 80%) | Shanxi | **Shanxi** (4, 80%), Hebei (1, 20%) | **Huangruang1 × Zhaogu1** (F2) × **Jingu2** (Shanxidabaigu) |
| **V65** | Datong27 | spring | Shanxi | (73-50 × Zao1) × **Yi1** | **Jingu2** |
| **V66** | Datong29u | spring | Shanxi | - | Shaanxibei01 |
| **V67** | Shuangguayin | spring | Shanxi | - | - |
| **V68** | Tiegu14 | spring | Liaoning | **Tiegu5** (Daobaqi × Riben60ri) × **Wayin**'79127' | Riben60ri, Daobaqi |
| **V69** | Bagui214 | spring | Hebei | **Bagui214** | - |
| **V70** | Chigu4 | spring | Neimenggu | **Zhaogu1** (Shuangguayin) | Shuangguayin |
| **V71** | Changnu13 | spring | Liaoning | **Zhaogu21** (Zhaogu1 (Shuangguayin1)) × **Tiegu1** | Shuangguayin |
| **V72** | Datong29u | spring | Shanxi | - | - |
| **V73** | Tie807 | spring | Liaoning | - | - |
| **V74** | Gonggu68 | spring | Jilin | **Gonggu62** × **80026** | - |
| **V75** | Tie8240 | spring | Liaoning | **23-4 × Huanggu1** | Huanggu1 |
| **V76** | Jingu30 | summer | Hebei | - | - |
| **V77** | Tiegu5 | spring | Summer (6, 25%); Spring (17, 70.83%) | Liaoning | **Daobaqi × Riben60ri** |
| **V78** | Tiegu5 | spring | Liaoning | (8, 33.33%), Hebei (6, 25%), Shanxi (3, 12.5%), Neimenggu (3, 12.5%), Hainan (2, 8.33%) | **8215** ((Tiegu5 (Daobaqi × Riben60ri) × Tiegu1) × **Tie8240** (23-4 × Huanggu1)) |
| **V79** | An9217 | summer | Henan | **Yugu1** (Riben60ri × Tulong) × **Nangu1** | Riben60ri, Tulong |
| **V80** | Tiegu6 | spring | Unclear (1, 4.2%) | **Jingzhanggu1 × 78-8** | Huanggu1, Jinxianyu |
| **V81** | Meiyoudatou | - | American | Meiyoudatou | Meiyoudatou |
| **V82** | Gonggu70 | spring | Jilin | - | yingsuigu |
| **V83** | Chigu10 | spring | Neimenggu | **Chigu1** (Chigu1 (Shuangguayin1)) × **80-943** | Shuangguayin |
| **V84** | Gu10A | summer | Hebei | - | - |
| **V85** | Chigu8 | spring | Neimenggu | **Chigu5** (Zhaoonong1 (Shuangguayin1)) × **80-943** | Shuangguayin |
| **V86** | Shi02530 | summer | Hebei | - | - |
| **V87** | Jingu27 | summer | Hebei | - | - |
| **V88** | Chaolv | summer | Hebei | **Chaoxiangu** | Chaoxiangu |
| **V89** | Tiegu7 | spring | Liaoning | **Tiegu × Xuanzhuang** | - |
| **V90** | Yangui3 | spring | Shanxi | **Qitouhuang × Jingu21** (Jinfen52 × Qitouhuang) | Jinfen52, Qitouhuang |
### Supplemental Table 2. Prolamin patterns of 90 accessions of foxtail millet.

| V  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|----|---|---|---|---|---|---|---|---|---|----|
| V1 | − | + | + | + | − | + | − | − | + | + |
| V2 | + | − | + | + | + | − | + | + | + | + |
| V3 | − | + | + | + | − | + | + | + | + | + |
| V4 | − | + | + | + | + | + | + | + | + | + |
| V5 | + | − | + | + | + | + | + | + | + | + |
| V6 | − | + | + | + | + | + | + | + | + | + |
| V7 | + | − | + | + | + | + | + | + | + | + |
| V8 | − | + | − | − | + | + | − | + | + | + |
| V9 | + | − | + | + | + | + | + | + | + | + |
| V10| − | + | + | + | − | + | + | + | + | + |
| V11| + | − | + | + | + | − | + | + | + | + |
| V12| − | + | + | + | − | + | + | + | + | + |
| V13| + | − | + | + | + | + | + | + | + | + |
| V14| − | + | + | + | − | + | + | + | + | + |
| V15| − | + | + | + | + | + | + | + | + | + |
| V16| − | + | + | + | + | + | + | + | + | + |
| V17| − | + | + | + | − | + | + | + | + | + |
| V18| − | + | + | + | + | + | + | + | + | + |
| V19| − | + | + | + | + | + | + | + | + | + |
| V20| − | + | + | + | + | + | + | + | + | + |
| V21| − | + | + | + | + | + | + | + | + | + |
| V22| − | + | + | + | + | + | + | + | + | + |
| V23| − | + | + | + | + | + | + | + | + | + |
| V24| − | + | + | + | + | + | + | + | + | + |
| V25| − | + | + | + | + | + | + | + | + | + |
| V26| − | + | + | + | + | + | + | + | + | + |
| V27| − | + | + | + | + | + | + | + | + | + |
| V28| − | + | + | + | + | + | + | + | + | + |
| V29| − | + | + | + | + | + | + | + | + | + |
| V30| − | + | + | + | + | + | + | + | + | + |
| V31| − | + | + | + | + | + | + | + | + | + |
| V32| − | + | + | + | + | + | + | + | + | + |
| V33| − | + | + | + | + | + | + | + | + | + |
| V34| − | + | + | + | + | + | + | + | + | + |
| V35| − | + | + | + | + | + | + | + | + | + |
Continued

|   |   |   |
|---|---|---|
| V36 |   |   |
| V37 | + | + |
| V38 | + | + |
| V39 | + | + |
| V40 | + | + |
| V41 | + | + |
| V42 | + | + |
| V43 | + | + |
| V44 | + | + |
| V45 | + | + |
| V46 | + | + |
| V47 | + | + |
| V48 | + | + |
| V49 | + | + |
| V50 | + | + |
| V51 | + | + |
| V52 | + | + |
| V53 | + | + |
| V54 | + | + |
| V55 | + | + |
| V56 | + | + |
| V57 | + | + |
| V58 | + | + |
| V59 | + | + |
| V60 | + | + |
| V61 | + | + |
| V62 | + | + |
| V63 | + | + |
| V64 | + | + |
| V65 | + | + |
| V66 | + | + |
| V67 | + | + |
| V68 | + | + |
| V69 | + | + |
| V70 | + | + |
| V71 | + | + |
| V72 | + | + |
Continued

| Entry | Name | G1     | G2     | G3     | G4     | G5     | G6     |
|-------|------|--------|--------|--------|--------|--------|--------|
| V1    | 0.9759 | 0.0053 | 0.004  | 0.0037 | 0.0067 | 0.0044 | 0.9759 |
| V2    | 0.9658 | 0.0095 | 0.0056 | 0.004  | 0.0099 | 0.0052 | 0.9658 |
| V3    | 0.9642 | 0.0066 | 0.006  | 0.0092 | 0.0033 | 0.0107 | 0.9642 |
| V4    | 0.9619 | 0.0072 | 0.0116 | 0.0056 | 0.0074 | 0.0063 | 0.9619 |
| V5    | 0.9613 | 0.0071 | 0.0082 | 0.009  | 0.007  | 0.0074 | 0.9613 |
| V6    | 0.9601 | 0.005  | 0.0094 | 0.0083 | 0.0066 | 0.0106 | 0.9601 |
| V7    | 0.9601 | 0.0115 | 0.0093 | 0.0081 | 0.0041 | 0.0069 | 0.9601 |
| V8    | 0.9565 | 0.0092 | 0.005  | 0.003  | 0.0202 | 0.0061 | 0.9565 |
| V9    | 0.955  | 0.005  | 0.0067 | 0.0087 | 0.0206 | 0.004  | 0.955  |
| V10   | 0.945  | 0.0111 | 0.0105 | 0.0065 | 0.0081 | 0.0188 | 0.945  |
| V11   | 0.9333 | 0.0124 | 0.0071 | 0.0065 | 0.0286 | 0.0121 | 0.9333 |
| V12   | 0.927  | 0.0161 | 0.0315 | 0.0108 | 0.0048 | 0.0098 | 0.927  |
| V13   | 0.9153 | 0.0297 | 0.0049 | 0.004  | 0.0368 | 0.0093 | 0.9153 |
| V14   | 0.896  | 0.0283 | 0.0138 | 0.0355 | 0.0166 | 0.0098 | 0.896  |

**Note:** V: varietie, B: band, +: showed protein band, -: no protein bands.

**Supplemental Table 3.** 90 foxtail millet accessions used in this study.
|     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|
| V15 | 0.8867 | 0.0148 | 0.0187 | 0.011 | 0.0303 | 0.0385 | 0.8867 |
| V16 | 0.8798 | 0.0132 | 0.051 | 0.0354 | 0.0044 | 0.0162 | 0.8798 |
| V17 | 0.8651 | 0.0314 | 0.0382 | 0.0154 | 0.0044 | 0.0455 | 0.8651 |
| V18 | 0.8072 | 0.0103 | 0.0084 | 0.1599 | 0.005 | 0.0092 | 0.8072 |
| V19 | 0.711 | 0.1712 | 0.0478 | 0.037 | 0.0132 | 0.0197 | 0.711 |
| V20 | 0.6037 | 0.0751 | 0.0353 | 0.0496 | 0.0085 | 0.2278 | 0.6037 |
| V21 | 0.5799 | 0.2184 | 0.079 | 0.0386 | 0.0632 | 0.021 | 0.5799 |
| V22 | 0.5107 | 0.0767 | 0.055 | 0.1023 | 0.0062 | 0.2492 | 0.5107 |
| V23 | 0.0042 | 0.9603 | 0.005 | 0.005 | 0.0042 | 0.0213 | 0.0042 |
| V24 | 0.0052 | 0.9515 | 0.0049 | 0.004 | 0.003 | 0.0314 | 0.0052 |
| V25 | 0.0067 | 0.9425 | 0.0073 | 0.024 | 0.014 | 0.0055 | 0.0067 |
| V26 | 0.017 | 0.9206 | 0.016 | 0.0304 | 0.0079 | 0.0081 | 0.017 |
| V27 | 0.0168 | 0.9095 | 0.0076 | 0.0325 | 0.0245 | 0.0091 | 0.0168 |
| V28 | 0.0126 | 0.8815 | 0.0107 | 0.0047 | 0.0079 | 0.0826 | 0.0126 |
| V29 | 0.0104 | 0.7698 | 0.1525 | 0.0257 | 0.0035 | 0.038 | 0.0104 |
| V30 | 0.0335 | 0.6717 | 0.0608 | 0.0847 | 0.0404 | 0.1089 | 0.0335 |
| V31 | 0.0074 | 0.661 | 0.1602 | 0.0245 | 0.0076 | 0.1393 | 0.0074 |
| V32 | 0.0083 | 0.6299 | 0.0133 | 0.011 | 0.0068 | 0.3307 | 0.0083 |
| V33 | 0.0073 | 0.0071 | 0.9696 | 0.005 | 0.0039 | 0.0071 | 0.0073 |
| V34 | 0.0036 | 0.0059 | 0.9666 | 0.0133 | 0.0039 | 0.0067 | 0.0036 |
| V35 | 0.0114 | 0.0078 | 0.9546 | 0.011 | 0.0101 | 0.0051 | 0.0114 |
| V36 | 0.0102 | 0.0049 | 0.9526 | 0.012 | 0.0124 | 0.0079 | 0.0102 |
| V37 | 0.0055 | 0.0125 | 0.9342 | 0.0089 | 0.0061 | 0.0328 | 0.0055 |
| V38 | 0.008 | 0.0074 | 0.933 | 0.0083 | 0.0032 | 0.0401 | 0.008 |
| V39 | 0.0259 | 0.0092 | 0.9325 | 0.0077 | 0.005 | 0.0197 | 0.0259 |
| V40 | 0.0068 | 0.0099 | 0.9307 | 0.0192 | 0.005 | 0.0284 | 0.0068 |
| V41 | 0.004 | 0.0056 | 0.9302 | 0.0334 | 0.0143 | 0.0125 | 0.004 |
| V42 | 0.009 | 0.0209 | 0.9224 | 0.0184 | 0.0166 | 0.0127 | 0.009 |
| V43 | 0.005 | 0.0473 | 0.9119 | 0.0163 | 0.0048 | 0.0147 | 0.005 |
| V44 | 0.0151 | 0.0065 | 0.9094 | 0.0243 | 0.0216 | 0.0231 | 0.0151 |
| V45 | 0.0751 | 0.0075 | 0.8892 | 0.0203 | 0.0022 | 0.0057 | 0.0751 |
| V46 | 0.0235 | 0.0504 | 0.8413 | 0.0154 | 0.0065 | 0.0629 | 0.0235 |
| V47 | 0.0279 | 0.0144 | 0.8338 | 0.008 | 0.1048 | 0.0111 | 0.0279 |
| V48 | 0.071 | 0.0212 | 0.8303 | 0.0262 | 0.0394 | 0.0119 | 0.071 |
|   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|
| V49 | 0.0261 | 0.0132 | 0.7773 | 0.1744 | 0.005 | 0.004 | 0.0261 |
| V50 | 0.0185 | 0.0412 | 0.7403 | 0.015 | 0.0118 | 0.1732 | 0.0185 |
| V51 | 0.0156 | 0.0462 | 0.7295 | 0.0102 | 0.0067 | 0.1918 | 0.0156 |
| V52 | 0.0551 | 0.2294 | 0.5172 | 0.0217 | 0.007 | 0.1696 | 0.0551 |
| V53 | 0.0135 | 0.1717 | 0.4603 | 0.0089 | 0.0155 | 0.3302 | 0.0135 |
| V54 | 0.0946 | 0.3411 | 0.4015 | 0.121 | 0.0116 | 0.0302 | 0.0946 |
| V55 | 0.0043 | 0.013 | 0.0129 | 0.9571 | 0.004 | 0.0087 | 0.0043 |
| V56 | 0.0104 | 0.0125 | 0.0074 | 0.9559 | 0.0058 | 0.008 | 0.0104 |
| V57 | 0.008 | 0.0179 | 0.0039 | 0.9555 | 0.0097 | 0.005 | 0.008 |
| V58 | 0.009 | 0.0077 | 0.0123 | 0.9553 | 0.0087 | 0.007 | 0.009 |
| V59 | 0.0056 | 0.0072 | 0.0176 | 0.9538 | 0.0065 | 0.0093 | 0.0056 |
| V60 | 0.0069 | 0.0138 | 0.0131 | 0.9335 | 0.0058 | 0.0269 | 0.0069 |
| V61 | 0.003 | 0.0083 | 0.0447 | 0.9292 | 0.0087 | 0.0061 | 0.003 |
| V62 | 0.0022 | 0.0026 | 0.003 | 0.004 | 0.9852 | 0.003 | 0.0022 |
| V63 | 0.0037 | 0.003 | 0.003 | 0.003 | 0.9843 | 0.003 | 0.0037 |
| V64 | 0.0155 | 0.0095 | 0.0136 | 0.016 | 0.9295 | 0.0159 | 0.0155 |
| V65 | 0.1099 | 0.0905 | 0.0051 | 0.0054 | 0.7723 | 0.0168 | 0.1099 |
| V66 | 0.4112 | 0.0105 | 0.0039 | 0.0059 | 0.5383 | 0.0302 | 0.4112 |
| V67 | 0.003 | 0.0078 | 0.0044 | 0.003 | 0.002 | 0.9798 | 0.003 |
| V68 | 0.0079 | 0.0121 | 0.004 | 0.004 | 0.0041 | 0.9679 | 0.0079 |
| V69 | 0.0076 | 0.0067 | 0.0085 | 0.0056 | 0.0042 | 0.9674 | 0.0076 |
| V70 | 0.006 | 0.0161 | 0.0077 | 0.005 | 0.0072 | 0.958 | 0.006 |
| V71 | 0.0125 | 0.0046 | 0.0106 | 0.0079 | 0.0125 | 0.9519 | 0.0125 |
| V72 | 0.0068 | 0.0159 | 0.0098 | 0.004 | 0.0154 | 0.9481 | 0.0068 |
| V73 | 0.0061 | 0.0151 | 0.0203 | 0.0049 | 0.007 | 0.9466 | 0.0061 |
| V74 | 0.004 | 0.0474 | 0.007 | 0.004 | 0.0038 | 0.9338 | 0.004 |
| V75 | 0.0123 | 0.0318 | 0.012 | 0.0129 | 0.0075 | 0.9235 | 0.0123 |
| V76 | 0.0242 | 0.0172 | 0.0323 | 0.006 | 0.0063 | 0.9139 | 0.0242 |
| V77 | 0.0265 | 0.0143 | 0.0255 | 0.0096 | 0.0115 | 0.9126 | 0.0265 |
| V78 | 0.0098 | 0.0223 | 0.0058 | 0.01 | 0.0463 | 0.9058 | 0.0098 |
| V79 | 0.006 | 0.0335 | 0.0303 | 0.0061 | 0.0276 | 0.8965 | 0.006 |
| V80 | 0.0175 | 0.0447 | 0.0101 | 0.0208 | 0.0112 | 0.8957 | 0.0175 |
| V81 | 0.035 | 0.02 | 0.0088 | 0.0211 | 0.0462 | 0.8689 | 0.035 |
| V82 | 0.036 | 0.011 | 0.0664 | 0.0163 | 0.0129 | 0.8598 | 0.0336 |
|    |    |    |    |    |    |    |
|----|----|----|----|----|----|----|
| V83 | 0.0237 | 0.112 | 0.0188 | 0.0068 | 0.0033 | 0.8354 | 0.0237 |
| V84 | 0.0419 | 0.075 | 0.0374 | 0.0092 | 0.0176 | 0.819 | 0.0419 |
| V85 | 0.0144 | 0.0197 | 0.0087 | 0.1111 | 0.0309 | 0.8152 | 0.0144 |
| V86 | 0.0647 | 0.0486 | 0.0211 | 0.0186 | 0.043 | 0.8039 | 0.0647 |
| V87 | 0.017 | 0.046 | 0.0852 | 0.0265 | 0.0328 | 0.7925 | 0.017 |
| V88 | 0.0099 | 0.0619 | 0.0334 | 0.039 | 0.0762 | 0.7796 | 0.0099 |
| V89 | 0.0073 | 0.3449 | 0.0104 | 0.008 | 0.004 | 0.6254 | 0.0073 |
| V90 | 0.0127 | 0.0626 | 0.3313 | 0.0186 | 0.0639 | 0.5109 | 0.0127 |