

Heterotic Classes and Utilization Patterns in Chinese Foxtail Millet [*Setaria italica* (L.) P. Beauv]

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Abstract

Utilization of heterosis to develop hybrid cultivars can significantly increase yield of most crops including foxtail millet. However, previous foxtail millet hybrid cultivars have been largely developed from crosses between sterile lines and conventional varieties or between sterile lines and varieties that are geographically distant from the sterile lines. The research on classification of heterotic classes and determination of heterotic patterns has not been reported, which results in uncertainty in selection of parents for crosses and delays progress in utilization of high yielding hybrids in large-scale commercial production. In this study, a core collection of 128 accessions from China was grouped into six classes using combined analyses of population structure, pedigree, and clustering. The classification was conducted based mainly on molecular clustering of genotypic data, also considered the population structure and mathematical clustering using phenotypic data, and was finally validated through pedigree analysis. According to the transgressive and superstandard heterosis for grain yield, plant height, panicle length, panicle diameter, single panicle weight, grain weight per panicle, and 1000-grain weight collected from an incomplete-diallel-cross experiment, we identified six superior heterosis patterns (C2/C1, C2/C4, C2/C5, C2/C6, C1/C5 and C4/C5) and four inferior heterosis patterns (C1/C3, C1/C4, C1/C6 and C4/C6), and explored their potential applications in millet hybrid breeding. This study laid a foundation for effective use of foxtail millet heterosis in improving millet hybrid yield.

Keywords

Foxtail Millet, Heterotic Classes, Heterotic Pattern, Molecular Clustering, Population Structure

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Analysis, Pedigree Analysis, Mathematic Clustering

1. Introduction

Breeding hybrid cultivars using heterosis has been a critical strategy to increase grain yield in maize (*Zea mays* L.) [1] [2], sorghum (*Sorghum bicolor* L.) [3] [4], rice [5] and many other crop species. Utilization of heterosis in foxtail millet [*Setaria italica* (L.) P. Beauv.] also significantly improves millet yield [6].

The degree of heterosis in a hybrid depends on the genetic relationship of its parents. Due to lack of knowledge on heterotic classes and the genetic relationship among these classes, foxtail millet hybrid cultivars have been largely developed by crossing sterile lines to conventional cultivars or sterile lines to cultivars that are geographically distant from the sterile lines [7]. Geographically distant parents, however, are not necessary to be genetically distant from each other due to extensive exchange of germplasm among different ecological regions and intensive use of a few common parents such as Yugu 1, Qingdaolao, Zhaogu 1, Qinyuanmujizui and Moligu [8]-[11] in almost all Chinese hybrid breeding programs, which might be the major reason for current hybrid cultivars far from reaching its yield potential. Investigating heterotic classes and determining best heterotic class combinations, or heterotic patterns, can provide a guideline for effective use of heterosis in hybrid breeding, therefore, to further improve the level of hybrids' heterosis.

Foxtail millet is a minor crop. It is mainly cultivated in developing countries such as China and India. Basic research on the heterosis utilization in foxtail millet is far behind other cereal crops. There are only limited studies on classification and utilization of heterotic classes to predict heterotic hybrids in breeding. Study on pedigree evolution of foxtail millet cultivars revealed dynamic changes in pedigrees of mainstay cultivars from the northern China summer millet region released at different historic periods [8] [10]-[12]. However, some cultivars do not have pedigrees available or have incorrect pedigrees, and even when the accurate pedigree is available, different lines selected from the same cross may have different genetic makeups. Therefore, pedigree analysis alone cannot accurately classify current breeding materials into reasonable heterosis classes.

DNA marker provides the most accurate method for exploring genetic relationship among genotypes. Markers including amplified fragment length polymorphism, randomly amplified polymorphic DNA and simple sequence repeat (SSR) have been used in many studies on millet genetic diversity [13]-[17]. Only a few studies on millets reported use of SSR markers in cluster analysis [14] [16] [17] and population structure analysis [9]. Reports on heterotic classes and heterotic patterns that can guide effective use of heterotic millet resources have not been documented.

Studies on heterotic classes and patterns were reported in maize [18]-[22], rice [23]-[28] and *Triticeae* crops [29]-[32]. These studies classified heterotic classes or established heterotic patterns using molecular clustering, mathematical clustering, or pedigree analysis. However, using only a single analytic method in each of these studies might not be able to reflect the actual heterotic classes. Among different analytic methods used for heterotic classification, marker data are usually used to construct molecular clusters, but it is difficult to set an objective genetic distance threshold for class separation in a clustering tree. Population structure analysis that calculates the distribution probability of tested materials in each group based on the degree of gene contribution is more objective than other methods, but it classifies those tested materials in the groups with the highest distribution probabilities based on only the absolute values of distribution probabilities of the materials in each group, thus the resulting groups may not be correct for the tested materials that have similar distribution probabilities in the two groups with the highest probability. Mathematical clustering is based solely on the phenotypic values of multiple traits, and results from the analysis alone may also deviate from actual genetic relationship among the groups due to significant environmental effects and genotype by environment interactions. Thus a more comprehensive analytic approach is needed for effective classification of heterotic classes.

Previously, 128 Chinese foxtail millet accessions were analyzed using SSR markers, their molecular clusters and population structure were determined, and their pedigrees were analyzed to confirm the grouping results [9]. This study was conducted to classify 128 Chinese foxtail millet accessions into heterotic groups using mathematical clustering of the phenotypic data from an incomplete diallel cross experiment and to determine their heterotic patterns.

2. Materials and Methods

2.1. Plant Materials, Phenotypic Data Analysis

The 128 foxtail millet accessions [9], DNA marker analysis, molecular clustering, and population structure analysis used in this study were described previously by Liu *et al.* (2011). All accessions were planted uniformly at the Experiment Station of Institute of Millet Crops, Hebei Academy of Agricultural and Forestry Sciences, Shijiazhuang, China in 2008 and 2009. The experiment used a lattice square design with four replications. A total of 16 agronomic traits were recorded during the growing period including plant height, panicle length, panicle diameter, panicle-neck internode length, the first leaf length and width, the second leaf length and width, the third leaf length and width, percentage of harvested panicles, single panicle weight, grain weight per panicle, 1000-grain weight, percentage of grain weight over panicle weight, and total growth days. All the traits were measured according to the description criteria of millet germplasm resources [33]. Mean phenotypic values of each trait over four measurements were used for mathematical clustering using NTSYS-pc ver. 2.1 [34].

2.2. Classification of Heterotic Classes

Heterotic classes were defined by combined analyses of molecular clustering, population structure, mathematical clustering and pedigree clustering. If an accession had two highest, but distribute probability difference less than 0.1 between the groups in population structure analysis, the result from NJ clustering analysis was evaluated. If the result was consistent between the two analyses, the accession was kept in the original group as determined by the structure analysis; otherwise, pedigree analysis or mathematical clustering was used to determine its class in the case of accurate pedigree was not available.

2.3. Establishment of Preponderance Heterotic Classes and Patterns

After grouping adjustment, we selected 8 representative accessions (sterile lines or restorer lines) from all six newly formed heterotic classes and made 256 incomplete diallel crosses between the lines from different heterotic classes in 2010. In 2011, F₁s from all the 256 crosses were evaluated along with their 51 male parents and a control cultivar, Jigu 19, for yield and yield-related traits, including plant height, panicle length, panicle diameter, single panicle weight, panicle grain weight and 1000-grain weight, in an experiment using a lattice square design with four replications. Transgressive heterosis and superstandard heterosis were calculated for all the traits, and used to determine heterotic patterns. If mean F₁s of cross combinations between two heterotic classes had yield transgressive heterosis $\geq 20\%$ and superstandard heterosis $\geq 8\%$, they were classified as a superior heterotic pattern; if mean F₁s of the combinations between two heterotic classes had yield transgressive heterosis between 5% to 20% and superstandard heterosis between 0% to 8%, they were classified as an inferior heterotic pattern based on the following formula:

$$\text{Transgressive heterosis} = \frac{(\text{Mean yield of hybrid combinations} - \text{mean yield of restorer}) \text{ in two classes}}{\text{Mean yield of restorer in the two classes}} \times 100\%$$

$$\text{Superstandard Heterosis} = \frac{\text{Mean yield of hybrid combinations of two classes} - \text{yield of control}}{\text{Yield of the control cultivar}} \times 100\%$$

3. Results

3.1. Classification of Heterotic Classes

Based on the marker data, the 128 accessions were previously classified into six branches “B1, B2, B3, B4, B5, B6” based on phylogenetic analysis [9] and further into six groups (G1, G2, G3, G4, G5, G6) based on their population structure [9]. In the population structure analysis, 13 accessions (V9, V15, V25, V32, V40, V42, V56, V68, V77, V90, V98, V111 and V120) showed no significant difference in probabilities between the two highest probability groups with distribution probability difference less than 0.1 between the groups (**Supplemental Table S1**), thus the NJ clustering data were used to adjust the groupings of these accessions. Six accessions (V15, V32, V42, V68, V111, and V120) had consistent grouping results between the structure analysis and the NJ clustering, therefore, those accessions were kept in the group originally assigned by the structure analysis;

whereas the NJ clustering gave different grouping results for other 7 accessions (V9, V25, V40, V56, V77, V90, and V98). Among the 7 accessions, only two (V98 and V25) had known pedigrees; V98 shared the same pedigree with V88 in G4 and V25 shared the same pedigree with G1 [9], thus V88 was moved to G4 and V25 remained in G1. The remaining five accessions had unknown pedigrees, thus were reclassified based on mathematical clustering results (Figure 1). Because V77 from G1 was more close to V52 in G1, V9 and V56 were more close to V91 also in G1 in mathematical cluster tree, these three accessions were assigned to G1; similarly, V90 remained in the G3 where V106 were, and V40 were reassigned to the G6 where V65 were. After the adjustment, three accessions (V25, V77 and V90) remained in their original groups, and four (V9, V40, V56 and V98) were reassigned to different groups based on their pedigree or phenotypic similarity. Thus, the newly adjusted six groups were redesignated as six classes (C1 to C6 in Table 1). This result suggested that the grouping results from population structure analysis are more close to adjusted new classes than clustering analysis, thus population structure analysis provides more accurate grouping information.

To verify the rationality of the new classification method developed in this study, four accessions (V9, V40, V56, and V98) that were reassigned to different classes were crossed to five accessions that were selected from their corresponding groups and classes before and after they were adjusted. The results showed that the combining abilities for these combinations between individuals within the new classes was lower than that within original groups, indicating the genetic relationship among accessions within the newly formed classes was much closer than among accessions within original groups. This result indicates that the new classification method in this study is more reasonable (Table 2).

3.2. Establishment of Heterotic Patterns

The mean hybrid yield results from incomplete diallel cross experiment showed that the transgressive heterosis and superstandard heterosis were 30.30% and 10.26%, respectively, for the patterns between accessions in C2 and C1, 33.16% and 9.83%, respectively, between C2 and C4, 38.04% and 8.55%, respectively, between C2 and C5, 27.50% and 8.97%, respectively, between C2 and C6, 21.05% and 8.12%, respectively, between C1 and 5, and 24.02% and 8.12%, respectively, between C4 and C5.

Analysis of data for the five yield related traits (single panicle weight, panicle grain weight, panicle length, panicle diameter, and total growth days) collected from the incomplete diallel experiment identified six classes combinations that showed both strong transgressive heterosis and superstandard heterosis, including C2/C1, C2/C4, C2/C5, C2/C6, C1/C5 and C4/C5, thus, were defined as superior heterosis patterns to predict heterotic performance of their hybrids. Similarly, class combinations that have yield transgressive heterosis from 5% to 20% and superstandard heterosis from 0% to 8% is generally considered as an inferior heterotic pattern, and C1/C3, C1/C4, C1/C6 and C4/C6 met the criteria (Table 3).

3.3. Genetic Distance of Preponderance Classes, Heterotic Patterns and Classes

Based on the above analysis, C1, C2, C3, C4, C5 and C6 correspond to G1, G2, G3, G4, G5 and G6, respectively, with only adjustment of four lines. Therefore, the genetic distances among the original groups (G1 to G6) basically represents the genetic distances among corresponding newly formed classes (C1 to C6). The genetic distances among the newly formed classes determined by the incomplete diallel cross tests were basically identical to that of Nei's minimum distances and pairwise F_{st} s (Table 3 and [9]). In general, a heterotic pattern with the genetic distance larger than 0.17 between two heterotic classes in a heterotic pattern belonged to a superior heterotic pattern, and a heterotic pattern with genetic distances from 0.13 to 0.17 belonged to an inferior heterotic pattern. Based on this result, transgressive heterosis from "24.2%" to "38.4%" and superstandard heterosis from "8.12%" to "10.26%" can be considered superior heterosis patterns and transgressive heterosis from "7.33%" to "14.08%" and superstandard heterosis from "0%" to "4.70%" can be considered inferior heterotic patterns in this study. Thus heterotic classes and heterotic patterns established in this study are useful guideline for hybrid breeding using these germplasm.

3.4. Heterosis Performance of Different Traits and Relationship between Transgressive Heterosis and Superstandard Heterosis among Traits

Among different traits, heterosis between classes was not significant for 1000-grain weight and plant height

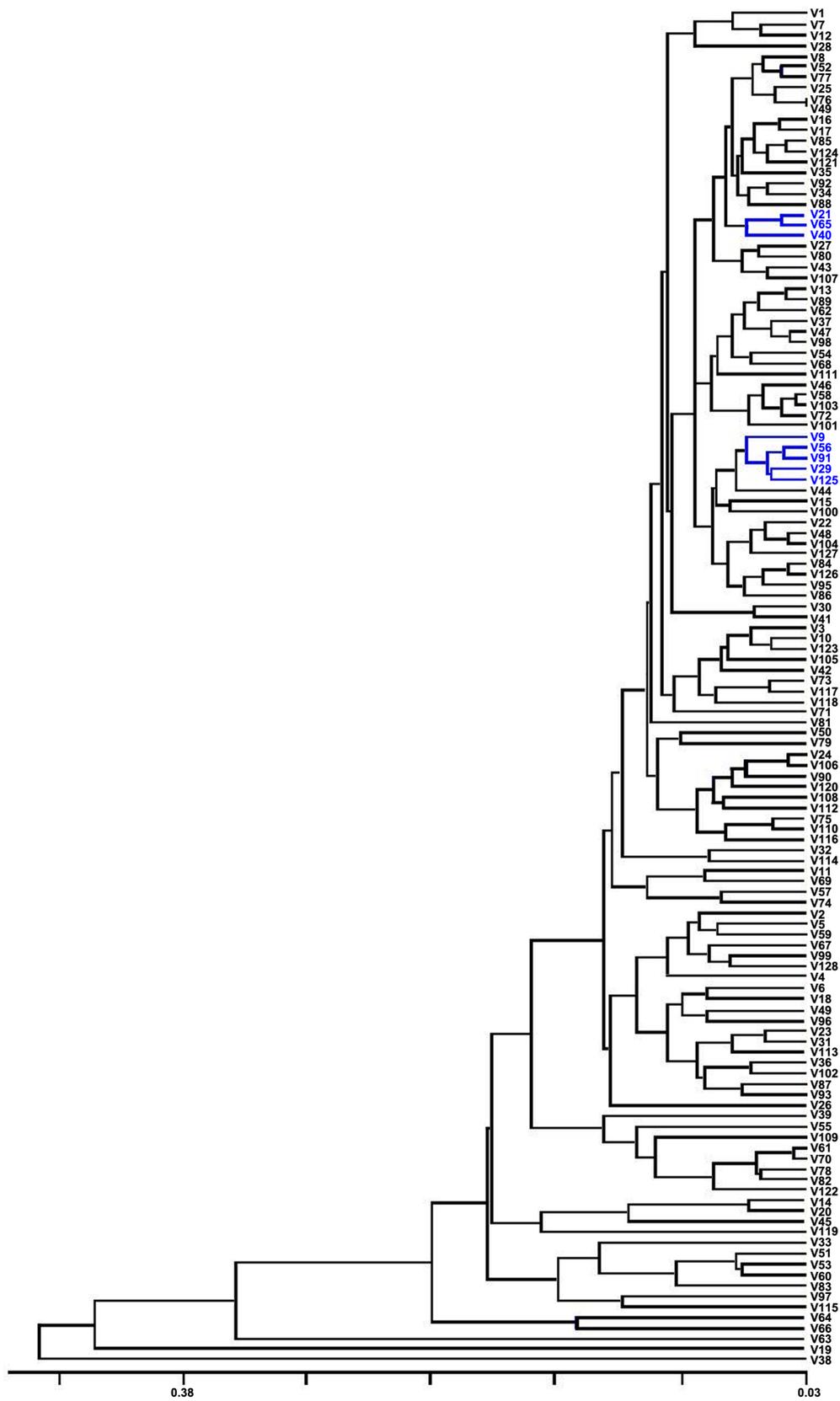


Figure 1. Mathematical cluster based on 128 accessions.

Table 1. Comparison of accessions in original group (G) classified by structure analysis and newly formed classes (C) assigned by multiple analyses of 128 accessions.

Newly formed class	Accessions	Original group
C1	V91, V48, V29, V27, V76, V84, V80, V86, V34, V100, V44, V52, V125, V35, V47, V127, V70, V17, V115	G1
	V25, V77,	G1
	V56	G3
	V9	G4
C2	V73, V31, V36, V23, V114, V118, V117, V75, V110, V116, V32, V82, V50	G2
C3	V67, V59, V45, V26, V10, V2, V1, V12, V5, V51, V53, V33, V128, V7, V28, V3, V4, V6, V8, V24, V60, V64, V57, V120, V11, V54, V97, V20, V14, V83, V112, V16, V119, V18, V106, V108, V93, V74, V79	G3
	V90	G3
C4	V88, V102, V13, V104, V89, V96, V87, V113, V109, V122, V61, V15, V111	G4
	V98	G5
C5	V71, V101, V37, V81, V103, V58, V126, V92, V55	G5
C6	V99, V85, V43, V30, V38, V62, V94, V105, V107, V78, V72, V121, V69, V22, V63, V66, V19, V21, V124, V68, V95, V65, V41, V46, V49, V39, V42, V123	G6
	V40	G5

Table 2. Combining ability between accessions V9, V40, V56, and V98 and selected accessions from both original groups and corresponding newly formed classes in which these four accessions located.

Accessions	Original group (G)/newly formed class (C)	General combining ability (kg/4.5m ²)
V9	G4	2.34
	C1	2.13
V40	G5	2.18
	C6	2.09
V56	G3	2.29
	C1	2.15
V98	G5	2.56
	C4	2.01

Table 3. Heterotic classes and heterotic patterns assigned based on the integrated analysis.

Patterns	Transgressive heterosis (%)	Superstandard heterosis (%)	Type of pattern
C1, C2	30.30	10.26	Superior heterotic pattern
C2, C4	33.16	9.83	
C2, C5	38.04	8.55	
C2, C6	27.50	8.97	
C1, C5	21.05	8.12	
C4, C5	24.02	8.12	
C1, C3	14.08	0.43	Inferior heterotic pattern
C1, C4	7.33	0.00	
C1, C6	8.41	4.70	
C4, C6	9.09	2.56	
C2, C3	8.33	-16.67	Non-heterotic pattern
C4, C3	0.50	-14.1	
C3, C5	4.19	-15.00	
C3, C6	5.31	-6.84	

(Table 4). In each heterotic pattern, hybrid performance of the two traits did not follow a certain trend and was not predictable, ranging from similar to either parent and close to midparent. The other five traits including yield, single panicle weight, panicle grain weight, panicle length and panicle diameter showed hybrid heterosis. In the

Table 4. Heterotic performance of yield-related traits evaluated in an incomplete-diallel-cross experiment using selected accessions from different heterotic patterns.

Patterns	Type of Heterosis	Yield	1000-grain weight	Single panicle weight	Panicle grain weight	Panicle grain weight/panicle weight (%)	Plant height	Panicle length	Panicle diameter	Type of patterns
C1, C2	Transgressive (%)	30.30	Low parent	29.24	21.40	-5.95	Tall parents	53.2	49.54	Strong heterotic patterns
	Superstandard (%)	10.26		14.85	12.57	-1.25		37.52	41.06	
C2, C4	Transgressive (%)	33.16	Low parent	26.29	14.16	-10.23	Tall parent	66.29	31.81	
	Superstandard (%)	9.83		10.46	9.08	-1.25		43.22	35.90	
C2, C5	Transgressive (%)	38.04	Mid parents	32.73	29.89	-2.41	Short parent	62.1	46.92	
	Superstandard (%)	8.55		6.47	7.05	1.25		40.09	37.84	
C2, C6	Transgressive (%)	27.50	Mid parents	32.88	39.16	-1.22	Short parent	54.52	46.48	
	Superstandard (%)	8.97		14.53	15.33	1.25		41.32	38.96	
C1, C5	Transgressive (%)	21.05	Higher than both parents	38.65	39.47	-2.38	Short parent	60.62	47.19	
	Superstandard (%)	8.12		8.47	10.94	2.50		41.58	38.83	
C4, C5	Transgressive (%)	24.02	Higher than both parents	39.14	33.20	-3.57	Mid-parent	62.29	38.66	
	Superstandard (%)	8.12		9.28	9.70	1.25		37.15	42.96	
C1, C3	Transgressive (%)	14.08	High parents	19.97	18.30	-1.20	Short parent	31.08	22.13	
	Superstandard (%)	0.43		0.05	2.03	2.50		19.05	12.57	
C1, C4	Transgressive (%)	7.33	Higher than both parents	17.91	8.85	-7.95	Tall parent	30.82	15.49	Heterotic patterns
	Superstandard (%)	0.00		2.81	3.95	1.25		14.92	19.65	
C1, C6	Transgressive (%)	8.41	Higher than both parents	20.32	20.41	0.00	Short parent	27.46	25.37	
	Superstandard (%)	4.70		1.31	3.10	2.50		18.76	19.56	
C4, C6	Transgressive (%)	9.09	Higher than both parents	20.09	17.79	-2.38	Mid-parent	28.99	16.98	
	Superstandard (%)	2.56		1.49	4.11	2.50		15.51	21.82	
C2, C3	Transgressive (%)	8.33	Lower than both parents	8.38	6.33	-2.38	Shorter than both parent	17.81	15.13	
	Superstandard (%)	-16.67		-7.51	-5.24	2.50		4.97	5.55	
C4, C3	Transgressive (%)	0.50	Lower than both parents	9.15	3.56	-4.71	Mid-parents	17.4	4.14	No heterotic patterns
	Superstandard (%)	-14.10		-0.02	-0.05	1.25		2.36	5.13	
C3, C5	Transgressive (%)	4.19	Lower than both parents	1.09	-1.63	-3.66	Shorter than both parent	7.85	1.29	
	Superstandard (%)	-15.00		-24.34	-25.31	-1.25		-0.06	-0.07	
C3, C6	Transgressive (%)	5.31	Lower than both parents	-1.23	-5.91	-4.88	Shorter than both parent	6.15	0.00	
	Superstandard (%)	-6.84		-20.28	-22.89	-2.50		-0.02	-0.08	

superior heterotic patterns, transgressive heterosis was strong and superstandard heterosis was obvious. In inferior heterotic patterns, transgressive heterosis existed but superstandard heterosis was weak or lacking. In those non-heterotic patterns, transgressive heterosis was weak or lacking, and superstandard heterosis generally showed disadvantages. In these superior heterotic patterns with strong heterosis in yield and yield-related traits, percentage of grain weight over panicle weight usually showed hybrid depression, which suggests that weaker heterosis for percentage of grain weight over panicle weight is associated with higher superior heterotic patterns for yield and yield-related components, thus increasing heterosis for percentage of grain weight over total panicle weight may improve yield potential.

Correlation analysis (Table 5) indicated that yield heterosis was significantly and positively correlated with single panicle weight, panicle grain weight, panicle length, and panicle diameter. These results indicate that transgressive heterosis and superstandard heterosis of yield are reliable parameters for classifying heterotic classes and establishing heterotic patterns.

4. Discussion

4.1. Heterotic Classes Classification

In the previous paper, population structure analysis was used to classify the groups based on the contribution of each accession to each group [9]. However, some accessions made similar contributions in the two highest probability groups with probability difference less than 0.1 between the groups. To validate the accuracy of classification based on structure analysis, the grouping result of NJ clustering analysis was compared. If NJ clustering groups the accessions in question to the same groups assigned by structure analysis, then we assume that the groups derived from the structure analysis are correct; otherwise, their groups need to be further adjusted according to either their pedigrees if available or mathematical clustering results if the pedigrees are not available. Using this integrated analysis method, some accessions in the six groups (G1 to G6) previously assigned by structure analysis were reassigned to different groups; thus the newly formed groups after adjustment were renamed as six heterotic classes (C1 to C6). Higher levels of heterosis were identified among the newly formed classes than within the classes in an incomplete diallel crosses experiment, which confirmed validity of newly formed classes and the new method used in this study. This work laid solid foundation for classification of heterotic classes and patterns for predicting the best cross combinations in heterotic hybrids breeding.

4.2. Criteria for Determining Different Levels of Heterosis

A new crop cultivar must be tested in regional trials at multiple locations and meet standard yield requirement before National Crop cultivar Evaluation and Approval Committee can approve it for release in China. The current national criterion is that a new foxtail millet hybrid cultivar has to increase yield for 8% over a conventional control. However, in research community, 15% of hybrid yield increase over a conventional control is considered as superior heterosis, thus becomes the breeding target for new hybrid cultivars. Because the conventional cultivar controls usually have better yield than the parents used in hybrid breeding programs, higher than 20% transgressive heterosis may be needed to reach required superstandard heterosis, which is equivalent to 8% or higher superstandard heterosis in superior heterotic patterns. Similarly, 5% to 20% of transgressive heterosis

Table 5. Correlation coefficients of transgressive heterosis and superstandard heterosis between traits.

Traits	Yield	Single panicle weight	Panicle grain weight	Percentage panicle grain weight over panicle weight	Panicle length	Panicle diameter
Yield	-----	0.883**	0.821**	-0.474	0.886**	0.724**
Single panicle weight	0.883**	-----	0.968**	-0.354	0.870**	0.684**
Panicle grain weight	0.821**	0.968**	-----	-0.132	0.829**	0.751**
Percentage panicle Grain weight over Panicle weight	-0.474	-0.354	-0.132	-----	-0.365	0.000
Panicle length	0.886**	0.870**	0.829**	-0.365	-----	0.878**
Panicle diameter	0.724**	0.684**	0.751**	0.000	0.878**	-----

**significant correlation.

should be the actual standards for inferior heterotic patterns, which will translated into 0% - 8% superstandard heterosis for inferior heterotic patterns.

4.3. Establishment of Heterotic Classes and Utilization Patterns

This study classified a core collection of breeding materials into different heterotic classes and heterotic patterns based on the combination of the genetic distance between classes, transgressive heterosis and superstandard heterosis of hybrids between the classes. The results suggested that the farther the genetic distance between two classes, the stronger the transgressive heterosis and superstandard heterosis of a hybrid, and the greater possibility to develop hybrids with superior heterosis when crosses are made between the two classes in a heterotic pattern.

The results show that the genetic distance between classes was consistent with the performance of transgressive heterosis and superstandard heterosis of hybrids in general, indicating that the methods for classifying heterotic classes and heterotic patterns are reliable. According to the established heterotic patterns in this study, we crossed sterile lines “Gu 572A” from C4 to restorer lines “JK6-9” from C2 and obtained a strong heterotic hybrid “57269” (Z-L, Liu, unpublished data). Multiple yield trials showed that “57269” increased yield by 24.54% in an average over a standard control used in the yield trails. Successful development of strong heterotic hybrid “57269” based on predicted superior heterotic pattern suggests that the heterotic patterns established in this study is useful for predicting hybrid performance of these germplasm.

4.4. Utilization of Heterotic Patterns in Hybrid Breeding

Heterosis usually refers to both transgressive heterosis and superstandard heterosis [7] [35]-[37]. Transgressive heterosis represents the yield potential of a hybrid, where superstandard heterosis evaluates potential for a hybrid to be grown in farmer fields. Superior transgressive heterosis does not necessarily mean a superior superstandard heterosis. For example, transgressive heterosis and superstandard heterosis of C2/C4 combinations were 33.16% and 9.83%, respectively; transgressive heterosis of C2/C5 combination was 38.04%, but superstandard heterosis was 8.55% only. This may be due to different yield levels of the parents. If the parents of a hybrid have similar yields to a control cultivar and their transgressive heterosis is high, and its superstandard heterosis should also be high. Therefore, for these parents that have strong transgressive heterosis but not strong superstandard heterosis, parental yield potential should be improved before they can be used in developing strong heterotic hybrids.

To quickly develop strong heterotic hybrids, attention should be paid to these heterotic patterns that have strong transgressive heterosis and superstandard heterosis. For example, C2/C4 pattern had transgressive heterosis up to 33.16% and superstandard heterosis up to 9.83%, and crosses among the accessions between the two classes will have a greater probability to develop hybrids with superstandard heterosis > 15%. The hybrid “57269” was a good example. In the long run, we should use a combination of traditional cross breeding and recurrent selection to improve the parental yields of all heterotic classes, especially the patterns whose transgressive heterosis is high but superstandard heterosis is low, such as in C2/C5 pattern.

5. Conclusion

In this study, a core collection of 128 accessions from China was grouped into six classes using combined analyses of population structure, pedigree, and clustering. The classification was conducted based mainly on molecular clustering of genotypic data, also considered the population structure and mathematical clustering using phenotypic data, and was finally validated through pedigree analysis. According to the transgressive and superstandard heterosis for grain yield, plant height, panicle length, panicle diameter, single panicle weight, grain weight per panicle, and 1000-grain weight collected from an incomplete-diallel-cross experiment, we identified six superior heterosis patterns (C2/C1, C2/C4, C2/C5, C2/C6, C1/C5 and C4/C5) and four inferior heterosis patterns (C1/C3, C1/C4, C1/C6 and C4/C6), and explored their potential applications in millet hybrid breeding. This study laid a foundation for effective use of foxtail millet heterosis in improving millet hybrid yield.

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Competing Interests

The authors declare that they have no competing interests.

References

- [1] Hallauer, A.R. and Miranda, J.H. (1981) Quantitative Genetics in Maize Breeding. Iowa State University Press, Ames, 124-126.
- [2] Zhang, T.Z. (2003) Crop Breeding. China Agricultural Science Press, Beijing.
- [3] Wang, F. and Zhang, F.C. (1982) Study on Heterosis of Different Groups in Sorghum. *Journal of Jilin Agricultural Sciences*, **3**, 6-12.
- [4] Lu, Q.S., Sun, Y. and Hua, Z.T. (2001) Crop Heterosis. China Agricultural Science Press, Beijing.
- [5] Fu, X.Q. and Gong, S.W. (1994) The Great Achievements of Hybrid Rice in Thirty Years and Its Further Developing Strategies in China. *Hybrid Rice*, **3-4**, 17-21.
- [6] Li, Y.M. (1997) Foxtail Millet Breeding. China Agricultural Science Press, Beijing.
- [7] Liu, Z.L. (2010) Establish Method of Heterotic Group in Foxtail Millet and Its Research Progress. *Journal of Hebei Agricultural Sciences*, **14**, 102-104.
- [8] Liu, Z.L., Cheng, R.H., Zhang, F.L., Xia, X.Y., Shi, Z.H. and Hou, S.L. (2006) Analysis on Genetic Foundation and Study on Millet Variety and Its Pedigree in Boreali-Sinica Summer Millets Region. *Acta Agriculturae Boreali-Sinica*, 103-109.
- [9] Liu, Z.L., Bai, G.H., Zhang, D.D., Zhu, C.S., Xia, X.Y., Cheng, R.H. and Shi, Z.G. (2011) Genetic Diversity and Population Structure of Elite Foxtail Millet [*Setaria italica* (L.) P. Beauv.] Germplasm in China. *Crop Science*, **51**, 1655-1663. <http://dx.doi.org/10.2135/cropsci2010.11.0643>
- [10] Jiang, Z.K., Liu, J.R., Wang, S.Y., Lu, Z.G., Yan, H.S., Liu, H.P., Liu, R.F. and Li, D.H. (2008) Analysis on Genetic Bases and Pedigree Evolution of Foxtail Millet Variety in Henan Province. *Anhui Agriculture*, **5**, 124-127.
- [11] Zhang, H.J. (2007) The Main Millet Variety Pedigree Analysis in Liaoning Province. *Rain Fed Crops*, **5**, 343-344.
- [12] Liu, Z.L., Cheng, R.H. and Li, X.Y. (1996) The Pedigree Analysis and Evaluation of North China Summer Millets. *Crops*, **5**, 24.
- [13] Li, Y., Jia, J.Z., Wang, Y.R. and Wu, S.Z. (1998) Intraspecific and Interspecific Variation in *Setaria* Revealed by RAPD Analysis. *Genetic Resources and Crop Evolution*, **45**, 279-285. <http://dx.doi.org/10.1023/A:1008600123509>
- [14] d'Ennequin, M.L.T., Panaud, O., Toupan, B. and Sarr, A. (2000) Assessment of Genetic Relationships between *Setaria italica* and Its Wild Relative *S. Viridis* Using AFLP Markers. *Theoretical and Applied Genetics*, **100**, 1061-1066. <http://dx.doi.org/10.1007/s001220051387>
- [15] Wang, J.Z., Hao, X.F., Wang, Y.Q., Wang, L.Y. and Sun, M.R. (2006) Study of Genetic Diversity of Millet Germplasm Resources by Molecular Markers. *Biotechnology*, **16**, 10-14.
- [16] Jia, X.P., Tan, X.J., Li, Y.X., Wang, T.Y. and Li, Y. (2009) A Study on the Genetic Diversity of Foxtail Millet Cultivars by SSR Markers. *Acta Agriculturae Universitatis Jiangxiensis*, **31**, 633-638.
- [17] Jia, X.P., Zhang, Z.B., Liu, Y.H., Zhang, C.W., Shi, Y.S., Song, Y.C., Wang, T.Y. and Li, Y. (2009b) Development and Genetic Mapping of SSR Markers in Foxtail Millet [*Setaria italica* (L.) P. Beauv.]. *Theoretical and Applied Genetics*, **118**, 821-829. <http://dx.doi.org/10.1007/s00122-008-0942-9>
- [18] Chen, Y.H., Wang, L.M. and Dai, J.R. (2000) Studies on the Heterotic Patterns between Tropical Subtropical and Chinese Temperate Germplasms in Maize. *Acta Agronomica Sinica*, **5**, 557-564.
- [19] Li, J.K., Zhang, L., Huang, Z.P., Zhang, L.Y. and Dai, O.H. (2002) Study on Making Soybean Cross Combination with M Type Male Steril Lines Heteros of F1 and F2 Generation Yield of Hybrid Soybean and Its Utilization. *Soybean Science*, **21**, 241-244.
- [20] Li, X.H., Yuan, L.X., Li, X.H., Zhang, S.H., Li, M.S. and Li, W.H. (2003) Heterotic Grouping of 70 Maize Inbred Lines by SSR Markers. *Scientia Agricultura Sinica*, **6**, 622-627.
- [21] Liu, X.Z., Peng, Z.B., Fu, H., Li, L.C. and Huang, C.L. (1997) Heterotic Grouping of 15 Maize Inbreds with RAPD Markers. *Scientia Agricultura Sinica*, **3**, 44-51.
- [22] Yuan, L.X., Fu, J.H., Zhang, S.H., Liu, X.Z., Peng, Z.B., Li, X.H., Warburton, M. and Khairallah, M. (2001) Heterotic Grouping of Maize Inbred Lines Using RFLP and SSR Markers. *Acta Agronomica Sinica*, **2**, 149-156.
- [23] Yu, S.B., Li, J.X. and Xu, C.G. (1997) Importance of Epistasis as the Genetic Basis of Heterosis in an Elite Rice Hy-

- brid. *Proceedings of the National Academy of Sciences of the United States of America*, **94**, 9226-9231. <http://dx.doi.org/10.1073/pnas.94.17.9226>
- [24] Zhang, Q.F., Gao, Y.J., Saghui Marrof, M.A., Yang, S.H. and Li, J.X. (1995) Molecular Marker Heterozygosity and Hybrid Performance in Rice. *Molecular Breeding*, **1**, 133-142. <http://dx.doi.org/10.1007/BF01249698>
- [25] Zhang, Q.F., Zhang, Z.Q. and Yang, G.P. (1996) Molecular Marker Heterozygosity and Hybrid Performance in Indica and Japonica Rice. *Theoretical and Applied Genetics*, **93**, 1218-1224. <http://dx.doi.org/10.1007/BF00223453>
- [26] Yang, X. and Ouiros, C. (1993) Identification and Classification of Celery Cultivars with RAPD Marker. *Theoretical and Applied Genetics*, **85**, 205-212.
- [27] Xiao, J.H., Li, J.M. and Yuan, L.P. (1995) Dominance Is the Major Genetic Basis of Heterosis in Rice (*Oryza sativa* L.) as Revealed by QTLs Using Molecular Markers. *Genetics*, **140**, 745-754.
- [28] Luo, X.J., He, H.H., Fu, J.R., Ceng, X.R., Sun, J.L., Zhang, H.L. and Li, Z.C. (2006) Studies on Dividing Heterotic Groups in Indica Rice with SSR Molecular Markers. *Hybrid Rice*, **1**, 61-64.
- [29] Sun, Q.X., Huang, T.C. and Ni, Z.F. (1996) Study on Wheat Heterotic Group I. Genetic Diversity Revealed by Random Amplified Polymorphic DNA (RAPD) in Elite Wheat Cultivars. *Journal of Agricultural Biotechnology*, **2**, 103-110.
- [30] Zeven, A.C. and Van Hiattam, T.J.L. (1992) Classification of Landraces and Improved Cultivars of Hexaploid Wheats (*Triticum aestivum aestivum*, *T. compactum* and *T. spelta*) Grown in USA and Described in 1992. *Euphytica*, **59**, 33-47. <http://dx.doi.org/10.1007/BF00025359>
- [31] Tinker, N.A., Fortin, M.G. and Mather, D.E. (1993) Random Amplified Polymorphic DNA and Pedigree Relationships in Spring Barley. *Theoretical and Applied Genetics*, **85**, 976-984. <http://dx.doi.org/10.1007/BF00215037>
- [32] Heun, M., Murphy, J.P. and Phillips, T.D. (1994) A Comparison of RAPD and Isozyme Analysis for Determining the Genetic Relationships among *Avena sterilis* Laccensions. *Theoretical and Applied Genetics*, **87**, 689-696. <http://dx.doi.org/10.1007/BF00222894>
- [33] Lu, P. (2006) Descriptors and Data Standard for Foxtail Millet [*Setaria italica* (L.) Beauv.]. China Agricultural Science and Technology Press, Beijing.
- [34] Rohlf, F.J. (2000) NTSYS-PC. Numerical Taxonomy and Multivariate Analysis System. Version 2.10e. Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook.
- [35] Wei, Z.P., Zai, Y.J., Liu, S.R., Hong, X. and Jin, Z.J. (1985) Studies on the Heterosis and the Relationship between Parents and Offsprings of Hybrid Wheat with *T. timopheevi* Cytoplasm. *Acta Agriculturae Universitatis Pekinensis*, **11**, 81-88.
- [36] Li, M.S., Zhang, S.H., Li, X.H., Pan, G.T., Bai, L. and Peng, Z.B. (2002) Study on Heterotic Groups among Maize Inbred Lines Based on SCA. *Scientia Agricultura Sinica*, **6**, 600-605.
- [37] Yang, C.Q., Dang, Z.H., Zhang, J.P., Wang, L.M., Zhao, L., Xie, R.X. and Wang, B. (2009) Heterosis of Yield and Yield-Related Traits in the Oil Flax. *Journal of Gansu Agricultural University*, **44**, 73-76.

Abbreviations

G, group;
C, class;
P, probability;
SSR, simple sequence repeat.

Supplemental

Table S1. 128 foxtail millet accessions used in this study (Liu *et al.*, 2011).

Entry	Name	G1	G2	G3	G4	G5	G6
1	Tiegu8	0.006	0.006	0.852	0.049	0.033	0.054
2	Tiegu6	0.004	0.012	0.544	0.005	0.234	0.202
3	Tie487	0.007	0.011	0.937	0.008	0.01	0.027
4	Gonggu68	0.005	0.004	0.561	0.017	0.406	0.007
5	Tiegu14	0.006	0.006	0.87	0.008	0.041	0.07
6	Chao438	0.201	0.005	0.474	0.27	0.044	0.006
7	Jinzhougu 14	0.005	0.004	0.815	0.024	0.092	0.06
8	Tiegu7	0.007	0.008	0.581	0.31	0.07	0.025
9	Jinzhougu12	0.44	0.003	0.009	0.535	0.005	0.009
10	Tie8240	0.013	0.012	0.933	0.008	0.014	0.02
11	Xinggu88	0.025	0.222	0.527	0.013	0.167	0.046
12	Tiegu5	0.017	0.009	0.844	0.012	0.027	0.091
13	An2491	0.034	0.022	0.19	0.443	0.279	0.032
14	Datong29lv	0.005	0.005	0.968	0.01	0.006	0.006
15	Shi206065	0.109	0.122	0.362	0.365	0.019	0.023
16	An9217	0.004	0.005	0.87	0.066	0.009	0.046
17	Bagu214	0.765	0.01	0.032	0.038	0.081	0.074
18	Jigu30	0.016	0.037	0.682	0.041	0.041	0.183
19	Jigu26	0.034	0.008	0.199	0.006	0.007	0.747
20	Datong29zi	0.008	0.006	0.971	0.005	0.005	0.006
21	Shi206058	0.065	0.11	0.072	0.007	0.011	0.734
22	Shi207226	0.017	0.018	0.034	0.051	0.022	0.859
23	Changgao146A	0.005	0.858	0.009	0.011	0.005	0.113
24	Shi207191	0.068	0.008	0.472	0.331	0.054	0.067
25	Jigu25	0.343	0.043	0.312	0.177	0.035	0.09
26	Chaogu13	0.014	0.024	0.909	0.009	0.013	0.031
27	K359	0.585	0.005	0.004	0.004	0.395	0.006
28	Tiedalihuang	0.007	0.01	0.795	0.006	0.053	0.129
29	L70	0.954	0.003	0.01	0.004	0.007	0.022
30	K523	0.347	0.032	0.095	0.004	0.03	0.493

Continued

31	Changgao117A	0.004	0.908	0.056	0.018	0.004	0.011
32	Yangu No.12	0.009	0.526	0.437	0.01	0.014	0.005
33	Gonggu70	0.011	0.007	0.881	0.023	0.018	0.06
34	Jigu29	0.761	0.004	0.015	0.018	0.181	0.021
35	S80	0.505	0.005	0.006	0.005	0.305	0.174
36	Changgao229A	0.007	0.875	0.019	0.024	0.006	0.07
37	Jinangu13	0.067	0.009	0.007	0.003	0.784	0.129
38	Cang156	0.044	0.004	0.005	0.02	0.01	0.918
39	Jinfen1A	0.007	0.339	0.052	0.007	0.004	0.59
40	Fu532	0.013	0.005	0.125	0.31	0.407	0.14
41	C445	0.013	0.004	0.004	0.023	0.015	0.941
42	Chaogu12	0.013	0.426	0.054	0.006	0.004	0.498
43	C208	0.219	0.004	0.021	0.009	0.071	0.676
44	C138	0.556	0.005	0.01	0.023	0.008	0.398
45	Chigu No.4	0.01	0.023	0.948	0.004	0.005	0.01
46	Shi02399	0.056	0.007	0.141	0.004	0.229	0.562
47	Y61	0.564	0.025	0.024	0.179	0.197	0.011
48	Cang344	0.537	0.005	0.009	0.416	0.026	0.007
49	Jinfen3A	0.006	0.351	0.034	0.007	0.004	0.597
50	Jin15A	0.005	0.64	0.342	0.004	0.004	0.004
51	Datong28	0.007	0.105	0.876	0.004	0.003	0.005
52	Zheng9188	0.754	0.004	0.122	0.004	0.09	0.025
53	Datong14	0.338	0.009	0.634	0.005	0.006	0.008
54	Zhengkang2	0.024	0.07	0.885	0.005	0.007	0.009
55	Dungu1	0.068	0.003	0.004	0.007	0.899	0.019
56	Yugu No.6	0.166	0.014	0.328	0.293	0.147	0.052
57	Datong30	0.047	0.011	0.905	0.006	0.006	0.026
58	Chenggu12	0.082	0.01	0.11	0.003	0.613	0.181
59	Chigu No.10	0.013	0.066	0.907	0.003	0.004	0.007
60	Datong27	0.007	0.01	0.972	0.004	0.004	0.004
61	Gu3A	0.168	0.05	0.015	0.633	0.005	0.129
62	An4852	0.26	0.005	0.023	0.016	0.017	0.679
63	Shi207286	0.009	0.016	0.011	0.071	0.015	0.879
64	Shi02530	0.094	0.007	0.525	0.079	0.237	0.059
65	Shi207393	0.02	0.033	0.02	0.106	0.243	0.579
66	Shi207382	0.165	0.027	0.033	0.023	0.164	0.587
67	Chigu8	0.006	0.034	0.925	0.007	0.016	0.012

Continued

68	Shi98700	0.135	0.018	0.368	0.035	0.012	0.432
69	Shi02521	0.106	0.186	0.119	0.021	0.011	0.556
70	Gu6A	0.574	0.036	0.008	0.155	0.171	0.057
71	Shi97672	0.028	0.015	0.26	0.005	0.441	0.251
72	Shi98622	0.018	0.006	0.141	0.032	0.342	0.462
73	Jingu No.16	0.007	0.924	0.016	0.009	0.039	0.006
74	Chaolv-1	0.007	0.026	0.95	0.006	0.005	0.006
75	Changgu No.1	0.115	0.665	0.082	0.006	0.005	0.126
76	K546	0.961	0.005	0.014	0.005	0.007	0.009
77	Gu10A	0.481	0.006	0.006	0.007	0.05	0.451
78	Richaogu	0.017	0.015	0.407	0.004	0.017	0.54
79	Meiguodatou	0.004	0.007	0.795	0.004	0.167	0.023
80	K660	0.506	0.006	0.005	0.337	0.134	0.012
81	K1011	0.004	0.005	0.244	0.248	0.494	0.004
82	Gu11A	0.005	0.711	0.034	0.011	0.23	0.008
83	Bagu214-2	0.006	0.009	0.972	0.006	0.004	0.003
84	K1130	0.893	0.01	0.015	0.039	0.032	0.011
85	Jigu19	0.014	0.006	0.021	0.005	0.021	0.933
86	Xiaoxiangmi	0.63	0.007	0.015	0.33	0.011	0.007
87	Gu38A	0.038	0.024	0.055	0.683	0.08	0.121
88	Shi06-439	0.203	0.01	0.27	0.495	0.01	0.011
89	Gufeng2	0.009	0.005	0.024	0.697	0.124	0.14
90	Gu57A	0.048	0.011	0.434	0.353	0.143	0.01
91	Shi181-5	0.562	0.003	0.009	0.401	0.006	0.019
92	Jigu21	0.051	0.004	0.004	0.011	0.92	0.01
93	Gu65A	0.06	0.081	0.795	0.02	0.013	0.031
94	Jigu22	0.036	0.035	0.02	0.186	0.025	0.698
95	Jigu20	0.005	0.005	0.111	0.039	0.241	0.598
96	Gu66A	0.022	0.014	0.02	0.925	0.005	0.015
97	Yangu No.13	0.004	0.334	0.632	0.01	0.016	0.005
98	Shi06-766	0.329	0.038	0.091	0.065	0.378	0.099
99	Jigu No.12	0.01	0.01	0.232	0.011	0.008	0.73
100	Bao182	0.582	0.04	0.181	0.127	0.062	0.007
101	Jinangu11	0.18	0.012	0.041	0.063	0.693	0.011
102	Jinangu12	0.374	0.006	0.015	0.492	0.081	0.033
103	Chaogu15	0.005	0.011	0.133	0.005	0.832	0.014
104	Lugu No.10	0.005	0.004	0.099	0.729	0.01	0.153

Continued

105	Ji9409	0.006	0.006	0.293	0.062	0.039	0.594
106	Jigu27	0.003	0.006	0.98	0.005	0.003	0.003
107	Ji9403	0.14	0.012	0.161	0.125	0.01	0.552
108	Heng968	0.006	0.009	0.726	0.01	0.006	0.243
109	Gu83A	0.21	0.025	0.094	0.636	0.03	0.006
110	Taixuan4	0.012	0.578	0.22	0.127	0.017	0.046
111	Chaogu14	0.04	0.009	0.302	0.334	0.171	0.144
112	Jingu No.29	0.004	0.285	0.527	0.164	0.011	0.009
113	Gu95A	0.156	0.007	0.104	0.703	0.014	0.017
114	Jingu No.21	0.012	0.853	0.109	0.004	0.008	0.014
115	Taixuan2	0.498	0.071	0.322	0.008	0.007	0.093
116	Taixuan5	0.081	0.512	0.345	0.012	0.039	0.01
117	Jingu No.35	0.078	0.755	0.149	0.004	0.008	0.005
118	Changnong35	0.006	0.89	0.089	0.005	0.006	0.005
119	Jigu28	0.031	0.048	0.707	0.023	0.142	0.049
120	Changnong36	0.01	0.336	0.428	0.168	0.018	0.041
121	Jigu24	0.023	0.005	0.005	0.021	0.057	0.888
122	ZA1	0.31	0.034	0.028	0.615	0.005	0.008
123	C178	0.249	0.005	0.214	0.036	0.012	0.484
124	Shi202242	0.113	0.018	0.032	0.042	0.011	0.784
125	An2367	0.536	0.064	0.028	0.027	0.324	0.02
126	203184zao	0.178	0.005	0.032	0.004	0.519	0.262
127	C164	0.728	0.011	0.025	0.178	0.045	0.013
128	Gonggu65	0.006	0.006	0.875	0.011	0.008	0.095

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