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Maize Genetic Diversity and Heterosis Group

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Abstract: The purpose of this article is to collect maize species resources with rich genetic diversity, and divide the resources into heterosis groups to significantly improve breeding efficiency and the advantages of heterosis groups. The genetic diversity of 33 maize inbred lines was studied using SSR markers. A total of 289 allelic variants were detected in the test materials with 72 pairs of primers. 2 to 7 alleles were detected for each pair of primers, with an average of 4.01. The genetic similarity coefficient of the 33 inbred lines varied from 0.5866 to 0.9247, with an average of 0.7056.Although the average genetic similarity coefficient was large, the range of variation was also large, indicating that there were abundant genetic diversity among the tested inbred lines. Sex. The experiment showed that through analysis of genetic distances between the parents of 22 approved maize varieties, it was found that the genetic distance between SSR molecular markers and heterosis showed a certain relationship, and the parents of the extended combination had larger genetic distances, but not the largest genetic distance. High-yielding combinations were assembled among all inbred lines, and the genetic distance of the parents of some combinations of Xiyu 3, Qisan Single Cross, Chuandan 18, Chuandan 13, Chuandan 16 and Yandan 14 was less than the average Genetic distance did not show obvious linear relationship between genetic distance and heterosis. In addition, 119 hybrid combinations were formulated in 24 inbred lines according to the incomplete two-row cross design. Using the UPGMA method, 17 inbred lines were classified into 6 categories based on the special combining ability of yield. The classification results and inbred lines The kinship relationship is basically the same, and the special cooperation force between groups is greater than the special cooperation force within groups.

1. Introduction

The level of maize breeding depends on the degree of innovation of germplasm resources and the depth of understanding and application of germplasm resources [1]. Abundant corn germplasm resources are the basic conditions for generating heterosis, and the narrow genetic basis of

germplasm is a major problem that plagues corn breeding. Therefore, the study of genetic diversity of germplasm can serve to make full use of maize germplasm resources and improve the level and efficiency of maize breeding. Chinese breeders have used traditional methods to systematically study the basis of maize germplasm based on the pedigree source of maize germplasm, the combining ability of major economic traits, and heterosis performance, and divided heterosis groups [2-3]. However, for some materials whose pedigree is not clear, it is difficult to accurately divide the heterosis group according to the field phenotypic characteristics. At the same time, using the heterosis performance of the field hybrid to classify the heterosis group is time-consuming, labor-intensive, and inefficient [4 -5].

The use of heterosis is a major breakthrough in agricultural scientific research in the 20th century, and it is an important measure for countries in the world to significantly increase crop yields. Maize is a typical outcross crop, showing extreme inbreeding decline and heterosis. This makes it one of the first crops to significantly increase yield using heterosis [6]. At present, breeders in various countries are studying heterosis groups adapted to local ecology and production conditions, and establishing heterosis models with high combining ability [7]. The United States is the earliest and most popular country to take advantage of maize heterosis, and it is also the earliest country to form heterosis groups and study heterosis patterns [8]. To date, the longest-used and most widely used U.S. is still the two heterosis groups and the heterosis model composed of them: Reid Yellow Dent and Lancaster Sure Crp. This is the basic heterosis group and heterosis model in temperate regions recognized by corn breeders, and it is also the first and most typical heterosis model formed in corn breeding research [9]. China's research on heterosis groups and heterosis models started late. In the 1980s, three provinces analyzed the hybrid parents for production and application, and divided China's germplasm into two major groups: domestic inbred lines and foreign inbred lines. And consider domestic and foreign inbred lines to be the main heterosis model, and many excellent hybrids such as "Zhongdan 2", "Yandan 14", and "Danyu 13" have adopted this model [10].

Corn is an important food, feed and industrial raw material in China, and it plays a vital role in the national food security strategy and socio-economic development. "In the world of grain security, seed casting foundation", germplasm resources are the material basis of corn breeding. The excavation, discovery and utilization of excellent corn germplasm resources are the key to the success of corn breeding [11]. Since the concept of "hybridity" was put forward and applied to the practice of corn breeding, with the promotion of corn hybrids, the lack of corn germplasm resources and the narrow genetic basis have become the consensus of the corn breeding community. According to the analysis of relevant experts, by 2019, China's corn demand will exceed 150 billion kilograms. According to the current domestic corn production and marketing situation, in the next 5 to 10 years, China's corn will be in short supply, with a gap of more than 60 million tons; at the same time, cultivated land will continue to decrease, the population will continue to increase, and people's living standards will increase. The bigger the rice will be [12]. However, there are a large number of favorable genes in heterosis clusters, and large heterosis can often be obtained when inbred lines between groups are crossed. Therefore, the division of heterosis groups is of great significance for inbred line improvement and hybrid selection and cross breeding.

In this experiment, the genetic diversity of the American germplasm, local germplasm, and innovative germplasm suitable for the environment was analyzed, and the heterosis relationship was analyzed to divide the dominant groups. The genetic relationship provided theoretical basis for the improvement of maize inbred line breeding methods and the selection of strong dominant combinations. At the same time, the relationship between the genetic distance of SSR molecular markers and heterosis was further explored, and accumulated experience for molecular marker-assisted breeding in maize was provided, which provided a reference basis for Lichuan to broaden the corresponding germplasm base and enrich germplasm resources. The experimental

analysis and verification of the combining ability provide a solid theoretical basis for breeding practice, and provide a rich germplasm basis for corn breeding and production in the Southwest and China.

2. Proposed Method

2.1. Heterosis Groups and Heterosis Models

Heterotic group refers to a breeding base group with a wide genetic basis, rich genetic variation, more favorable genes, higher general combining ability (GCA), and excellent breeding properties. It is a living gene bank formed by repeated selection and germplasm infiltration under the action of natural selection and artificial selection. From these, excellent inbred lines with high combining ability can be continuously separated and selected. Heterotie pattern refers to a combination pattern in which two different heterosis groups have a higher genetic interaction effect and a higher special combining ability (SCA). The odds of strong dominant hybrids among the excellent inbred lines selected from the two dominant groups are also correspondingly high. The research on heterosis groups and heterosis models is a strategic basic construction work in corn breeding.

(1) Common methods for dividing heterosis groups

Pedigree analysis method The United States divided the germplasm of maize belt into two heteroid groups of Reid and Lancaster based on long-term breeding experience and pedigree sources. Based on this, the first heterosis model was constructed. The new white-bred lines selected in the future are classified into the Reid group or non-Reid group according to the genealogical source, thereby improving the efficiency and purpose of hybrid breeding and germplasm improvement.

Quantitative genetics methods can be used to classify heterosis groups based on the heterosis performance and degree of mutation of the offspring of germplasm crosses. The performance of hybrids can be decomposed into the general mating ability and special combining ability of the parents: the special mating force between the parental inbred lines has certain rules to follow, which can be used as the basis for group classification. The special combining ability of inbred lines can be analyzed by the two-row hybridization method for group classification, and it is very effective. Its reliability depends on the number of white lines tested and the extensive genetic basis. However, for the analysis of canine inbred lines, the huge workload makes it lack of feasibility in practical operation.

The molecular marker method performs principal component analysis (PCoA) or cluster analysis (CA) on the genetic distance determined by molecular markers. The tested germplasm can be divided into different groups. The principal component analysis is suitable for the division of population inbred lines. Cluster analysis can reliably reveal the close relationship between white crosses. Due to the complementarity of the two cyans, simultaneous use of two types of analysis can extract the maximum amount of information from the molecular marker data.

(2) Technical route to divide heterosis groups

Based on the commonly used method of r heterosis grouping, AMBlONET China Laboratories has developed a technical route based on molecular markers. Double-column hybridization and NC-1I technology are used to molecularly mark and double-rank 15 inbred lines. Based on the practical experience of breeders and the latent analysis of typical inbred lines, the cross analysis was used to select 15 inbred lines in this order for preliminary research of heterosis groups. The selection of these materials widely represents the current status of the five germplasms in China. The pedigree is clear. It is very suitable for the study of heterosis groups and heterosis models. The tested Baiwen department was clearly divided into five groups (Sipingtou, Lufu Red Bone, Lancaster, BSSS, and PA), and the results were basically consistent with the genealogical analysis.

At the same time, the two-column hybridization analysis also obtained the same results as the molecular marker analysis and divided it into five corresponding fecal groups, thereby verifying the reliability of the molecular marker technology. The above research has initially determined the basic framework of maize germplasm in China, and selected SSR markers as the most suitable molecular marker system for the division of heterosis groups.

2.2. Genetic Differences in DNA Molecular Markers and Heterosis

(1) Limitations of conventional methods for dividing heterosis groups

The pedigree analysis method is based on the analysis of the germplasm basis, divides the heterosis group according to breeding practice experience and blood relationship, and then builds the heterosis model. However, because many inbred lines are mixed in China and there is no reliable pedigree record, this method is basically not applicable to the classification of corn germplasm in China. With the development of quantitative genetics, the heterosis groups can be divided according to the heterosis performance and the degree of mutation of the germplasm crosses. The performance of hybrids can be decomposed into the general combining ability and special combining ability of the parents; the special combining ability between the parental inbred lines basically reflects the genetic differences between the hybrid parents, which can be used as the basis for group classification. Most heterosis models are now based on special combining ability analysis. However, the determination of the special combining ability of the hybrid combination is heavy due to the field experiment workload and easy to be affected by the environment, which results in a low repetition rate, which is the defect of this method.

Isoenzymes are different types of enzyme molecules that catalyze the same reaction but have different structure and physicochemical properties. They are the products of gene expression. The variation of isoenzymes between parents reflects the differences between genotypes. Therefore, isozyme technology can also be used for the research of genetic diversity analysis and heterosis division. However, there are few isozyme detected sites and low polymorphisms, and there are only 10 to 20 polymorphisms of isozyme sites in plants.Therefore, for inbred lines with closer kinship, isozyme analysis The differences between inbred lines cannot be revealed, and it is difficult to divide heterosis groups to establish corresponding heterosis patterns.

(2) Several commonly used DNA molecular markers

Since the emergence of restriction fragment length polymorphism (RFLP) in 1980, molecular markers have developed greatly [13]. There are many types of molecular markers that have been applied, but they can be roughly divided into two categories: Core molecular markers, such as the analysis method of RFLP and various repeat sequence clones as probes; the second is molecular markers based on PCR technology, which is divided into single primer PCR markers, such as RAPD, SPARS, and DAF, and double primers. PCR markers, such as AFLP, SSR, STS, ISSR, etc. Among them, restrictive fragment length polymorphism (RFLP), random amplified polymorphism DNA (RAPD), increased fragment length polymorphism (AFLP), single sequence repeat (SSR), and ISSR markers are commonly used molecular markers .

(3) DNA molecular marker methods and heterosis grouping

At present, RFLP, RAPD, SSR and AFLP molecular marker technologies have been widely used in the study of maize heterosis groups and heterosis models. The analysis results of the distribution level are basically consistent with the genealogy. This method can not only divide many inbred lines of unknown origin to corresponding heterosis groups based on genetic distance, but also accurately separate many groups with unclear boundaries. Using 46 RFLP probe enzyme combinations, 148 maize inbred lines commonly used in Shaanxi production were successfully divided into two major groups, LSC and BSSS, and 11 subgroups. A total of 116 RFLP probe

enzyme combinations were used to divide 116 common inbred lines in Europe and North America into two types: horse-tooth type and hard c-type, with 12 subgroups. In our country, 145 RFLP probe enzyme combinations were used to divide 41 inbred lines widely used in the southern maize region of China and 4 Shaanxi inbred lines representing the major corn heterosis groups in the United States into 6 groups. Numerous studies have shown that RFLP markers can be successfully used to divide heterotic groups in maize germplasm, and the results are basically consistent with the genealogical source.

The above shows that DNA molecular markers are effective in dividing maize heterosis groups, and the results are basically consistent with pedigree sources, and germplasm with unclear pedigree sources can be divided into corresponding heterosis groups, and even new hybrids can be systematically identified. Dominant group. Using molecular markers can quickly and economically divide a large number of test materials into corresponding heterosis groups, which is unmatched by traditional pedigree analysis and quantitative genetics. However, the division of population heterosis groups faces complex yield traits, and it is obviously inappropriate to completely rely on molecular marker technology to abandon field evaluation of breeding materials. For now, molecular markers are used to accurately divide corn populations. Heterosis groups and the construction of heterosis models also need to be combined with quantitative heredity and the practical experience of breeders.

2.3. China's Maize Germplasm Foundation

The ancestors of maize are still unknown, and it is widely believed that maize originated from Xiaoying. No matter at the phenotypic level or the molecular level, maize has abundant genetic variation. In the long-term evolution process, due to the artificial selection of target traits and the "bottleneck effect" at the level of the Keegan group, genetic drift has resulted in the loss of many genetic diversity in corn. Modern corn includes only 88% of the genetic diversity and 76% of the alleles of ruminant grass.

China is not the center of maize diversity, and the genetic basis of maize germplasm is relatively weak. At present, China's maize breeding system relies on five backbone germplasms. In addition to the genetic basis of the red bone of the traveler, the other four germplasms are basically from a few hybrids. The genetic basis of local germplasm in China is also weak. China's corn backbone inbred lines are mainly derived from two domestic heterosis groups (Sipingtou and Traveler Red Bone) and two American heterosis groups (Reid and Lancaster). After ten years of application, these backbone inbred lines Gradually degraded, M017 grain rot has a tendency to aggravate, and the incidence of yellow spot disease and small spot disease is also getting worse. At the same time, with the widespread use of hybrids, China's local varieties have gradually withdrawn from the field of production. Although the yield and lodging resistance of local varieties are not as good as hybrids, they have strong adaptability to the local environment, rich genetic basis, and contain many traits that can be benefited from Lizhou (such as disease resistance, drought tolerance, strong adaptability and better quality). Etc.), is an indispensable germplasm resource. Ignoring the research and utilization of local breeds will, to a certain extent, lose many opportunities to select excellent inbred lines and reduce the efficiency of breeding research. Participated in some places | Again: More than 75% of the tested varieties contained PB germplasm, and the germplasm used in our country basically came from two similar hybrids of American Pioneer. Lan germplasm basically comes from the improved line of M017, Sipingtou and PA germplasm are from two hybrids of unknown white lineage, respectively. This situation restricts the innovative ability of corn breeding. The above facts show that the problem of the narrow foundation of corn germplasm in China is still serious, and there are hidden dangers of genetic fragility and sudden destructive diseases, which

should cause our vigilance.

3. Experiments

3.1. Experimental Data Set

Four to five seeds of 39 inbred lines were cultured under a constant temperature box, and samples were taken when two or three leaves grew, and DNA was extracted by CTAB method. The DNA concentration of the sample was diluted to 60ng / μL and stored in a 20 °C refrigerator for later use. Using the DL2000 fragment as DNAMaker, 90 pairs of SSR primers covering the entire maize genome were selected. According to the SSR primer series information provided by the Maize Genetics and Genome Database (http // www.maizegdb.org), 32 pairs of band-stable, Polymorphic primers.

3.2. Experimental Environment

CERVUS2.0 software was used to calculate gene frequency and polymorphic information content (PIC). The statistical common software SPSS 21.0 was used as the software for statistical development.

3.3. Experimental Steps

Using phenol.chloroform (C1) extraction method, the specific operation is as follows: put 2 × CTAB extract in a 65 °C water bath for 5 minutes to preheat; weigh 0.89 young leaves, add liquid nitrogen in a mortar to quickly grind into powder The powder was carefully scraped into a 5ml centrifuge tube, and 2xCTAB 2.0ml preheated at 65 °C was added. Invert and mix well; incubate in a 65 water bath for 40 minutes, carefully shake a few times during it; remove the centrifuge tube and leave it at room temperature for several minutes, add chloroform. 2 ml of isoamyl alcohol, shake upside down for 20 minutes, then centrifuge at 10,000 r / rain for 10 minutes; The supernatant is added to another 5ml sterilized centrifuge tube, and an equal volume of chloroform-isoamyl alcohol is added, shaken upside down for about 5rain, and then centrifuged at 9000r / rain for 10min; take the supernatant to another 5ml sterilized centrifuge tube, Add an equal volume of isopropanol or 2.5 times the volume of absolute ethanol to obtain a flocculent DNA precipitate; use a glass rod to pick out the DNA into a 1.5ml sterilized centrifuge tube, wash it with 70% ethanol three times, and wash it once with absolute ethanol. Air-dry on a clean bench (do not over-dry, otherwise it is extremely difficult to dissolve), add appropriate amount of TE to dissolve; add RNaseA to a final concentration of 10ng / ml or slightly more, 37. C is kept for 1 hour, and .20’C is refrigerated for later use. LambdaDNA was used as a Marker and electrophoresis was performed on a 0.8% agarose gel to check the integrity of the DNA. The concentration of the DNA was measured using a spectrophotometer and diluted to 20nedul for later use.

4. Discussion

4.1. Genetic Polymorphisms of SSR Markers

(1) From the 251 SSR markers in the maize genome, select 88 SSR markers with good polymorphism and good detection effect. They are evenly distributed on 10 chromosomes of corn. As shown in Figure 1, in 10 inbred lines, a total of 466 alleles were detected for these markers, and each marker could detect 2-17, with an average of 5.31. The polymorphic information content (PIC)

of each SSR locus ranges from 0.213 to 0.965, with an average of 0.586. The maximum bnlgl194 locus is 0.965 and the smallest umc127l locus is 0.213.

Table 1. Genetic polymorphisms of SSR marker loci in maize inbred lines

Mark	Chromosome position	Number of alleles	Polymorphism
umc1619	1.02	2	0.332
bnlg1014	1.03	5	0.510
bnlg1084	0.98	8	0.854
bnlg1866	0.89	5	0.673
phi083	0.96	4	0.736
nc030	0.97	6	0.282
phi1014	2.01	12	0.456
umc1185	2.03	4	0.453
umc1736	2.10	6	0.674
umc1696	2.32	8	0.734

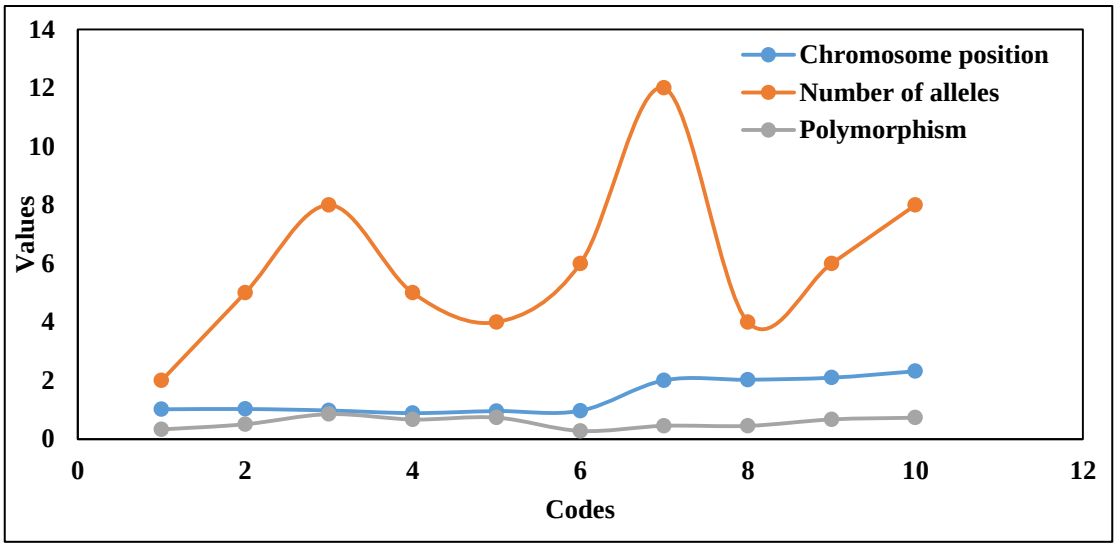

Figure 1. Diversity of marker sites

(2) P/C is an important indicator for measuring the degree of mutation of DNA marker loci. When a DNA marker seat PIC> 0.5, it is a highly polymorphic seat; when 0.25 <PIC <0.5, it is a moderate polymorphic seat; when PIC <0.25, it is a low polymorphic seat. The average PIC value of a DNA marker site can be used to estimate the level of genetic diversity in a population. The

higher the average PIC value of a population, the higher the degree of variation in the population's DNA marker sites, and the richer the population's genetic diversity. The average PIC value of 88 SSR loci of 10 inbred lines in this study as shown in Figure 2 is 0.586, indicating that Guizhou maize inbred lines have rich genetic diversity.

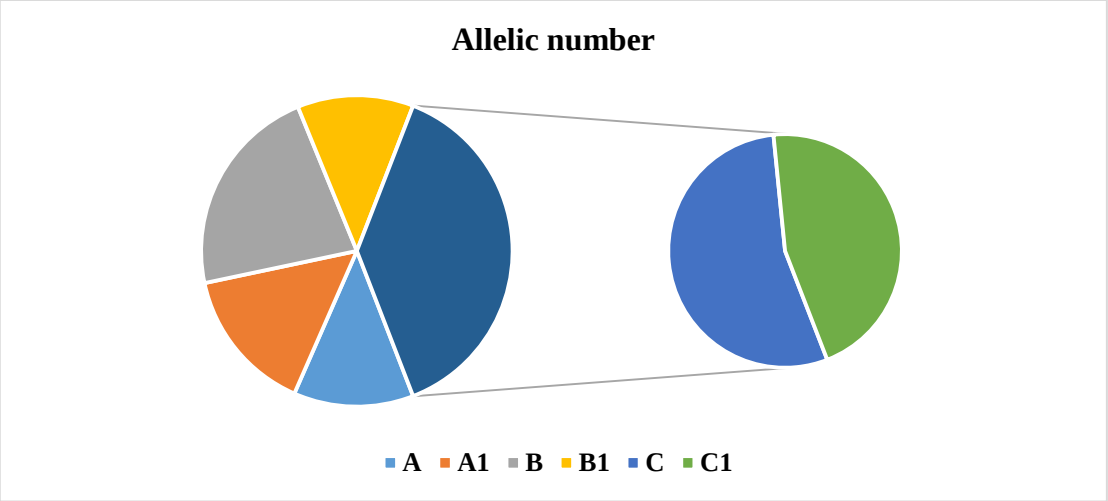

Figure 2. Group polymorphisms as points

4.2. Genetic Variation Analysis and Cluster Analysis

(1) Using 135 InDel markers to calculate the genetic similarity coefficient among 491 maize inbred lines, the range of which is between 0.004 and 0.967, with an average of 0.469. Based on the genetic similarity coefficient matrix of 491 maize white cross materials, the above materials were clustered using the UPGMA method, and the 491 materials were divided into 8 large groups as shown in Figure 3. According to the heterosis groups mainly used in Chinese corn production represented by the standard test species, the clusters classified by cluster analysis include the Reid group (B73), Lancaster group (Mo17), Sipingtou group (Chang 7 ~ 2, Huang Zao four).

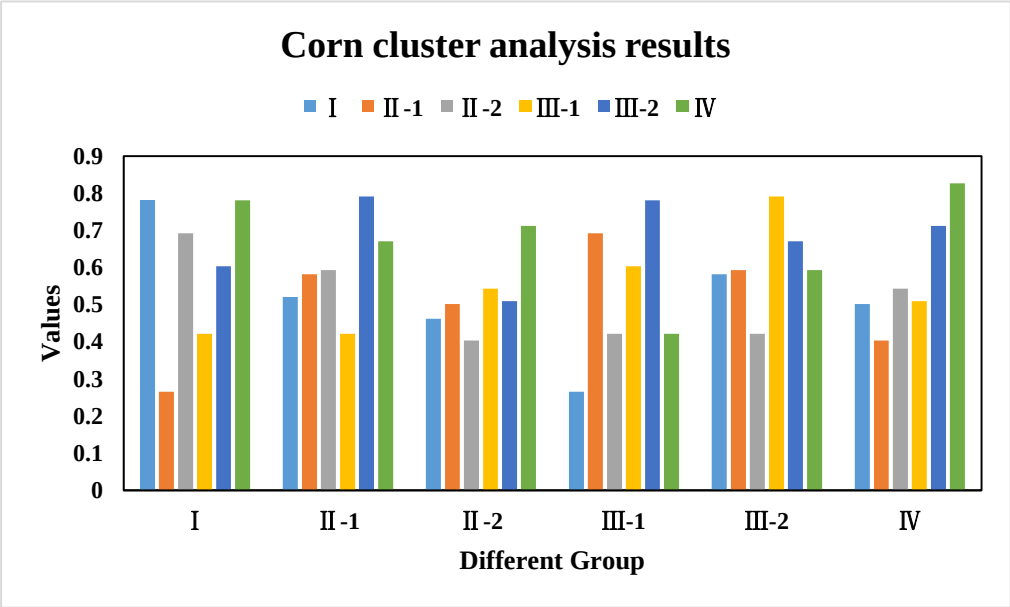

Figure 3. Corn clustering results

(2) According to SRAP marker data, 29 commonly used maize inbred lines were clustered and divided into 7 categories (or subclasses). The average genetic similarity coefficients of SRAP molecular markers of various maize inbred lines after clustering were calculated as shown in Table 2. ; From Figure 4, it can be seen that the average genetic similarity coefficient between classes is smaller than the average genetic similarity coefficient within the class, indicating that classification is conducive to selecting a suitable inbred line to formulate a combination, to avoid crosses between inbred lines within the group, and to reduce the workload of breeding.

Table 2. Mean similarity coefficients of SRAP markers among 7 groups of maize inbred lines

Groups	Ⅰ	Ⅱ-1	Ⅱ-2	Ⅲ-1	Ⅲ-2	Ⅳ
Ⅰ	0.782	0.521	0.462	0.265	0.582	0.502
Ⅱ-1		0.238	0.503	0.692	0.593	0.403
Ⅱ-2			0.632	0.421	0.421	0.543
Ⅲ-1			0.728	0.603	0.791	0.509
Ⅲ-2				0.781	0.671	0.712
Ⅳ					0.593	0.827

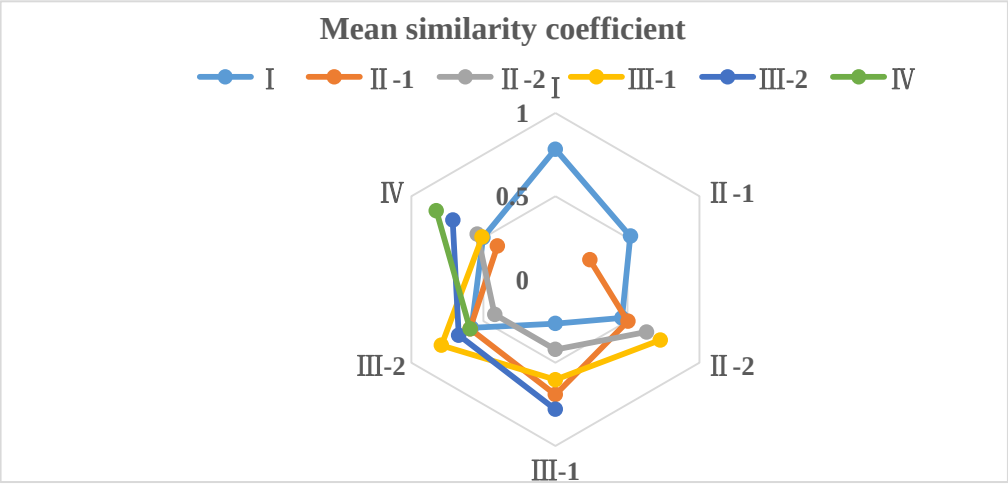

Figure 4. Comparison of average similarity coefficients

5. Conclusion

Dividing excellent maize inbred lines into different heterosis groups, and then establishing a heterosis model for corn, has become an important research direction for corn breeding at home and abroad. Due to repeated crosses and multi-directional breeding between excellent maize inbred lines, the pedigree relationship is not very clear, so it is more difficult to simply use the pedigree relationship to classify heterosis groups. In this experiment, SRAP markers were used to study the genetic diversity of 29 commonly used maize inbred lines. 33 inbred lines were classified into groups, and the classification results were basically consistent with the genealogical relationships. The internal average genetic similarity coefficient indicates that the classification is reasonable; in addition, from the perspective of the current high-yielding hybrid combinations popularized in production, their parents are basically in different taxa and subgroups, and intraclass crosses have almost no excellent hybrid combinations. The practical point of view shows that the SRAP marker technology is feasible in the division of maize heterosis groups. The division of heterosis groups

can provide a theoretical basis for the selection of strong dominant hybrid combinations. Some inbred line division results are inconsistent with pedigree division.The reason may be that the materials used in this study are mostly two-ring lines bred by hybrids, which have more than two parents.The pedigree is not as clear as the one-line line, and because of heterozygous chromosomes With the independent distribution and linkage of genes, it is difficult to determine which side of heredity is preferred. The percentage of positive markers associated with heterosis among the molecular marker sites selected in this study may be low.

Based on the existing research, the selected marker system was used to systematically study the backbone inbred lines and new lines with good application prospects in China's main corn producing areas, and further improve the research of heterosis groups in China. Maize breeding has important guiding significance. At the same time, the narrow foundation of maize germplasm in China has become the primary limiting factor for sustainable development of breeding research. The introduction of improved tropical and subtropical germplasm from CIMMYT is an important approach for germplasm expansion. But germplasm expansion, improvement and innovation must follow the principle of heterosis model. Therefore, while studying the heterosis groups of germplasm within the loquat, we should also start to study the genetic diversity of tropical and subtropical maize inbred lines and populations and the relationship with heterosis groups in China's germplasm; We are going to use the selected standard test species to perform NC-11 analysis on domestic and foreign white lines and populations. We will also use the selected molecular marker system to analyze genetic diversity and divide heterosis groups. For further utilization The domestic and foreign germplasm resources construction technology is almost Taiwan.

The classification results of maize inbred lines are basically the same as those of the predecessors; after classification, the parents of all excellent hybrids prepared by these maize inbred lines are in different major and subclasses. The average genetic similarity coefficients between inbred lines are smaller than the average genetic similarity coefficients within the class; most parents of high-yielding combinations currently produced and promoted have large genetic distances, while inbred lines with smaller genetic distances are not matched A high-yield combination indicates that the classification is effective and has certain guiding significance for corn breeding.The SRAP marker can be used to classify heterosis groups of maize inbred lines.

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Data Availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Conflict of Interest

The author states that this article has no conflict of interest.

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