

# Analysis of Waxy Maize Germplasm Resources In Southwest China Based On SNP Markers

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## Research Article

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# Abstract

Waxy maize (*Zea mays* L. var. *ceratina*) is an important economic crop in China, most of which are distributed in Southwestern China. In this study, 30 main waxy maize inbred lines in Southwest China were used as materials, and genetic analysis of the tested waxy corn materials was carried out using high-quality single nucleotide polymorphism (SNP) marker technology. A total of 15111 SNPs were obtained from 30 test materials, and the genetic similarity coefficient varied from 0.4568 to 0.9974. The results of population genetic structure and principal component analysis showed that the tested waxy maize materials and the commonly used common maize classification representative inbred lines (B73, ZI330, Mo17, Huangzao 4, etc.) could not be effectively aggregated. The 30 waxy corn materials can be divided into five groups separately. The application of the genetic relationship between materials identified by SNP analysis will enable breeders to select different parents to develop high-yield varieties with improved quality traits.

## Introduction

Waxy corn (*Zea mays* L. var. *ceratina*) is a special type of corn formed by a recessive genetic mutation in common corn[1]. Southwest China is the origin center of waxy corn[2]. The starch in waxy corn endosperm is mainly amylopectin (small molecular weight, high edible digestibility), which is a special corn with high nutritional value and economic value[3]. Single nucleotide polymorphism (SNP) genotyping has the advantages of wide distribution, dimorphism, high-throughput, strong genetic stability and easy automated analysis[4], it has been applied to analyze the genetic diversity of crop germplasm resources[5], and research germplasm kinship[6], and provide a new idea for selecting new crop varieties and using the genetic diversity of germplasm[7].

This study used SNP markers to analyze the genotypes of the main waxy maize resources in Southwestern China, and analyze the population genetic structure and genetic diversity, in order to understand the blood relationship, population genetic structure and evolutionary groups of waxy maize materials. It aims to provide a theoretical reference for using the genetic diversity of waxy maize germplasm resources, the breeding of new varieties and the creation of new materials.

## 1 Materials And Methods

### 1.1 Plant materials

The test materials were 30 waxy corn inbred lines, which were planted in the experimental field of the Dianjiang Scientific Research Base of Chongqing Academy of Agricultural Sciences. Three rows were planted for each material, 20 plants per row, two plants were planted, and self-pollination was strictly bagged. The test materials were numbered from C1 to C30, and the strain name, pedigree source and grain character were shown in Table 1.

### 1.2 SNP genotyping

The young leaves of the test material were taken from the jointing stage of the plant, and 10 plants from each strain were taken. After mixing, the DNA of the test material was extracted by the CTAB method[8], and the quality of the extracted DNA samples was checked by 1% agarose gel electrophoresis. The sample concentration was measured by DNA concentration analyzer, and the sample DNA concentration was diluted to the standard concentration according to the measurement results. The above-mentioned genetic material was scanned for the whole genome using the Maize SNP 56K Chip (developed by Affymetrix) on the Axiom® Maize56K SNP Array platform (developed by Zhongyujin Mark (Beijing) Biotechnology Co., Ltd.) to obtain the original markers. The redundant data of SNPs with minor allele frequency (MAF) <5% and deletion rate>20% were filtered out[9, 10], and finally 15,111 high-quality SNP loci were obtained for subsequent analysis.

### 1. 3 Statistical Analysis

The genetic similarity coefficient was calculated by NTSYS-pc 2.11[11]. Population genetic structure refers to a non-random distribution of genetic variation in a species or population[12]. Based on the selected SNP markers, Structure 2.2 was used to analyze the population genetic Structure of the tested materials[13]. The genetic distance among strains was calculated by Nei's algorithm[14]. Based on Nei's genetic distance, NTSYS-pc 2.11 software was used for principal component analysis(PCA), and two-dimensional space and three-dimensional space cluster graph were drawn. The NJ cluster diagram is constructed according to the neighbor joining method for cluster analysis[15].

## 2 Results And Analysis

### 2.1 Genetic Similarity

The results of genetic similarity coefficient analysis of the tested materials were listed in Table 2, which varied from 0.4568 to 0.9974. The materials with the lowest similarity were C30 and C19, and the materials with the highest similarity were C16 and C18. The combinations with genetic similarity coefficients between 0.45 and 0.6 accounted for 68.81%, and the combinations between 0.60 and 0.75 accounted for 23.65%. The genetic similarity between varieties with the same parental origin was higher, and genetic similarity analysis provided a direct basis for material classification[16].

### 2.2 Population genetic structure

Population genetic structure refers to a non-random distribution of genetic variation in a species or population[17]. The analysis of population structure helps to understand the evolution process, and the subgroup to which an individual belongs can be determined by the correlation study of genotype and phenotype[18]. The results of genetic structure analysis of the tested material population are shown in Fig. 1. The test material C10 belongs to the REID group represented by pink; The ZI330 (ZI330 and Luda Honggu blood relationship) group represented by green had no test materials; Material C17 belongs to LAN(Lancaster) group represented by light blue; Since there is no reference inbred line in the blue classification, it is classified as an unknown group and there are 25 test materials.

## 2.3 Principal component analysis

In order to reflect the genetic relationship between different populations, PCA analysis was performed on the groups of tested materials based on SNP markers, and the material clustering was displayed in the form of graphs. The linear distance between materials in PCA was proportional to the genetic distance. Fig. 2 is a plan view of the PCA analysis of 30 test materials, and Fig. 3 is a three-dimensional view of the PCA analysis. The genetic materials of different subgroups in the PCA analysis are marked with different colors. According to the concentration degree of each material, samples C09 and C10 were divided into LAN population and REID population respectively, and samples C17, C26 and C30 were divided into SPT (Tang-Si-Ping-Tou) population. The other materials could not gather together effectively with the existing common corn group materials, so waxy corn materials should be classified separately.

## 2.4 Cluster analysis

Based on the results of genetic similarity test and the test data of representative materials for common maize group classification, 30 samples were mapped with specific representative materials from PB, SPT, REID, ZI330, 335FM(335 male parent blood relationship) and LAN populations, and the results were shown in Fig. 4. Among the 30 materials, 8 materials could be effectively integrated into the classification of common maize group, and 22 materials were clustered together independently, which was consistent with the results of principal component analysis.

In order to clarify the genetic relationship between waxy corn inbred lines and improve their utilization efficiency, a separate cluster analysis was performed on the tested waxy corn materials (Fig. 5). The 30 waxy corn materials can be roughly divided into five major groups, namely, Hengbai, YA, YB, YW and tropical material groups. C11, C15, C21, C23 and C25 belong to Hengbai group; C01, C02, C04, C08, C12, C20, C22 and C24 belong to YW group; C03, C17, C26 and C30 belong to YA group; C05, C09 and C10 belong to YB group; C06, C07, C13, C14, C16, C18, C27, C28 and C29 belong to tropical material group, and C19 could not cluster in these five groups effectively.

# 3 Discussion

Southwest China is the origin center of waxy maize germplasm resources, which is rich in waxy maize germplasm resources. In this study, 30 waxy maize inbred lines commonly used in Southwest China were selected from Chongqing, Sichuan, Yunnan, Guizhou and other places, and the grain color was white, purple, black and other types, which were representative. SNP as the third-generation molecular marker has been applied in the fingerprint detection of common corn[19], but there are still few studies on waxy corn germplasm. Therefore, it is of great significance to use SNP molecular markers to analyze the genetic structure and diversity of waxy maize germplasm in Southwest China.

The results of this study showed that the genetic similarity coefficients between selected waxy corn germplasm resources ranged from 0.456 to 0.997, with an average value of 0.605. The materials with the lowest similarity were C30 and C19, and the materials with the highest similarity were C16 and C18. The

genetic similarity coefficient between 4.7% of the tested materials was between 0.65 and 0.89, and the genetic similarity coefficient > 0.89 accounted for 6.4% of the total. In conclusion, the genetic similarity among waxy maize materials in Southwest China is low, but the genetic relationship between waxy maize materials is different, and the genetic basis is relatively rich, which is generally consistent with the previous results of group classification by SSR markers[20].

This study combined principal component analysis and population genetic structure analysis, and found that most of the tested materials and common maize genetic materials could not be effectively clustered together. There is a clear distinction between waxy maize genetic resources in Southwest China and representative inbred lines of commonly used maize in China. Waxy maize materials in Southwest China should be classified separately[5, 21]. Furthermore, 30 waxy corn materials were further divided into five major groups, and the results of group classification can effectively guide the utilization of waxy corn materials and the breeding of new varieties, and improve the breeding efficiency. Chongqing Zhongyi Seed Co., Ltd. used C12 (YW material 715-10) as the female parent and C14 (tropical material 8300-22) as the male parent to bred a new waxy corn variety 'Q-nuo No.5'[22]; Using C10(YB material 46A) as the female parent and C04 (YW material w218) as the male parent, a new large ear waxy corn variety 'Jinnuo 2' was selected and bred[23].

## 4 Conclusion

The genetic basis of waxy corn germplasm resources in southwestern China is rich, and it is clearly distinguished from the commonly used ordinary corn inbred lines. The genetic relationship is relatively far, and a new waxy corn hybrid dominant group classification system can be created. In this study, 30 waxy maize inbred lines from Southwest China were divided into five groups, which provided a theoretical reference for waxy maize material utilization and new variety breeding. It not only effectively improved the breeding efficiency, but also provided help for the utilization and innovation of germplasm resources.

## Declarations

The authors declare that they have no conflict of interest.

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**Author contributions** C.Z. and S.W. design experiments and provide resources for all experiments. C.Z. and S.Z. coordinate experiments, data analysis and write manuscripts. W.Z., L.C. and T.Y. plant and collect materials. C.Z. Extract DNA.

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## Tables

Tables 1-2 are available in the Supplemental Files section.

## Figures

### Figure 1

The results of genetic structure analysis of the tested material population

Pink represents improved REID blood relationship; Green was ZI 330 and Luda Honggu blood relationship; Light green was the Lancaster(LAN) group; Light blue represents the blood relationship of Tang-Si-Ping-Tou(SPT)

### Figure 2

Two dimensional PCA analysis of Waxy Maize Inbred Lines

Yellow indicates the REID population and Improved REID blood related materials in the germplasm resource bank; Pink is ZI330 and Luda Honggu blood relationship group; Brown is the Tang-Si-Ping-Tou(SPT) group; Green is the Lancaster(LAN) group; Orange is PB group; Red is the backbone parent 335 male parent group; Blue is the 335 female parent group; Purple is the test sample in this project.

### Figure 3

Three dimensional PCA analysis of Waxy Maize Inbred Lines

Yellow indicates the REID population and Improved REID blood related materials in the germplasm resource bank; Pink is Zi330 and Luda Honggu blood relationship group; Brown is the Tang-Si-Ping-Tou(SPT) group; Green is the Lancaster(LAN) group; Orange is PB group; Red is the backbone parent 335 male parent group; Blue is the 335 female parent group; Purple is the test sample in this project.

### Figure 4

Phylogenetic tree of Waxy Maize Inbred Lines

The waxy maize inbred lines in this study were unmarked; Gray represents 335 male parent blood relationship and 335 female parent blood relationship; Blue represents Lancaster blood; Pink represents PB blood relationship; Red represents improved REID blood relationship; Green represents the blood relationship of Tang-Si-Ping-Tou(SPT); Yellow was ZI 330 and Luda Honggu blood relationship

### Figure 5

Cluster analysis of Waxy Maize Inbred Lines

Group I is named YB, Group II is named YW, Group III is named YA, Group IV is named tropical material, and Group V is named Hengbai.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.xlsx](#)
- [Table2.xlsx](#)