

Genetic Diversity Research of Yam Germplasm

Resources

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Abstract: *Yam (Dioscorea spp.), a versatile economic crop used both as food and medicine, is widely distributed globally, particularly in tropical and subtropical regions. Its abundant germplasm resources provide a crucial foundation for genetic diversity research. This paper reviews the latest advances in the genetic diversity study of yam germplasm resources, focusing on research methods and outcomes based on morphological traits, cytological markers, and molecular markers. By analyzing the application and effectiveness of these research methods, this paper summarizes key findings in yam genetic diversity research and proposes future research directions, aiming to provide theoretical support and technical guidance for yam genetic improvement and germplasm resource conservation.*

Keywords: *Yam germplasm resources, genetic diversity, morphological markers, cytological markers, molecular markers, genetic breeding*

Introduction

Yam (*Dioscorea* spp.) belongs to the genus *Dioscorea* in the *Dioscoreaceae* family. It has been cultivated in China for over 2,500 years, with various regions engaging in cultivation and exchange of varieties^[1]. There are numerous cultivated and wild varieties, making germplasm resources very rich. However, due to the lack of a germplasm resource classification system, many yam varieties are named based on their shape or origin. The diversity of yam germplasm resources provides a rich gene pool for genetic breeding. Yet, modern agricultural structural adjustments and environmental changes pose risks of losing local yam varieties. Additionally, long-term asexual reproduction leads to variety mixing, quality degradation, severe pest issues, continuous cropping obstacles, and unclear efficacy components, severely restricting the breeding of new yam varieties and the high-quality development of the yam industry. Studying yam's genetic diversity not only helps reveal its genetic structure and evolutionary patterns but also provides scientific evidence for germplasm resource conservation and improvement.

1 Yam Genetic Diversity Research Based on Morphological Characteristics

1.1 Classification and Identification of Yam Morphological Traits

Yam's morphological traits are fundamental to the classification and identification of its germplasm resources. These traits include aspects of the rhizome, vine, leaves, and bulbils, showing significant differences among varieties. Specific classification and identification methods are as follows:

1.1.1 Rhizome Morphology

The rhizome is yam's primary economic organ, with characteristics including shape (e.g., stick-like, cylindrical, spindle-shaped), color (e.g., white, yellow, purple), skin smoothness, and parameters like length, diameter, weight, number of fibrous roots, and head features. These traits are crucial for yam variety identification.

1.1.2 Vine Characteristics

Yam vines exhibit diverse morphology, including vine color (green, purple), direction of coiling, presence of ridges, and cross-sectional shape. These characteristics affect yam's growth habits and can be used for variety distinction.

1.1.3 Leaf Morphology

Leaves are essential photosynthetic organs in yam, with traits including shape (e.g., heart-shaped, spear-shaped, trident-shaped), color, lobing, vein distribution, petiole length, and leaf size. Different yam varieties show marked diversity in leaf morphology.

1.1.4 Bulbils

Traits of yam bulbils, such as presence, shape, size, and weight, can also be used for variety identification.

1.2 Collection and Analysis Methods of Yam Morphological Data

To systematically study yam's genetic diversity, scientific collection and analysis of morphological data are essential. Specific methods include:

1.2.1 Sample Selection and Collection

Select representative yam varieties and types from different geographic regions and ecological environments to ensure sample diversity and representativeness. Measure and record various parts of the plant (e.g., leaves, vines, bulbils, rhizomes) during the growth and maturation periods. These samples should cover the main morphological traits of yam to comprehensively reflect its genetic diversity.

1.2.2 Data Measurement and Recording

Use standardized measurement tools and methods for precise measurement of yam morphological traits. All measurement data should be accurately recorded in specialized data collection sheets to ensure data accuracy and reproducibility. Qualitative traits should follow the maximum similarity principle, with results recorded and assigned values. Additionally, record environmental conditions of the sampled sites, such as soil type, light, and moisture, for correlation analysis between environmental factors and morphological traits.

1.2.3 Data Sorting and Statistical Analysis

Input the collected morphological data into electronic spreadsheet software for sorting and preliminary statistical analysis. Common statistical methods include descriptive statistics, variance

analysis, and correlation analysis. Descriptive statistics reveal basic distribution patterns of yam morphological traits, such as mean values and standard deviations; variance analysis detects significant differences in morphological traits among varieties; and correlation analysis explores relationships between different morphological traits. These analyses provide an initial understanding of the distribution and variation of yam morphological traits.

1.2.4 Diversity Index Calculation

To quantify the diversity of yam germplasm resources, calculate diversity indices such as the Shannon-Wiener Index and Simpson Index. These indices reflect the richness and evenness of different morphological traits in the sample population. Additionally, calculate variety richness and evenness indices to further refine diversity evaluation. The calculation and comparison of these indices provide scientific evidence for the conservation and utilization of yam germplasm resources.

1.2.5 Multivariate Statistical Analysis

To deeply understand the complex relationships in yam morphological data, use multivariate statistical analysis methods such as Principal Component Analysis (PCA) and Cluster Analysis [2]. PCA reduces multidimensional data to a few principal components, revealing major sources of variation; cluster analysis groups varieties with similar morphological traits, revealing relationships and classification structures [3]. Combined with multivariate regression analysis of environmental factors, explore interactions between morphological traits and environmental conditions, supporting research on yam germplasm resource environmental adaptability. [4]

1.3 Evaluation of Yam Germplasm Resource Diversity Based on Morphological Traits

Evaluating yam germplasm resource diversity based on morphological traits is a key method to understand genetic diversity. Evaluation methods include:

1.3.1 Cluster Analysis

Use clustering methods (e.g., hierarchical clustering, K-means clustering) to group yam morphological data, classifying varieties and types with similar traits. Cluster analysis reveals the classification structure and relationships among yam germplasm resources.

1.3.2 Principal Component Analysis (PCA)

Use PCA to reduce multidimensional morphological data to a few principal components for visualization and interpretation. PCA identifies traits contributing most to morphological variation, aiding in understanding major sources of variation in yam germplasm resources.

1.3.3 Discriminant Analysis

Use discriminant analysis to establish discriminant functions between morphological traits and variety classification for classifying and identifying unknown varieties. Discriminant analysis verifies the accuracy and reliability of classification standards.

1.3.4 Diversity Evaluation Report

Based on the analysis results of morphological data, compile a diversity evaluation report of yam germplasm resources, detailing morphological traits, variation, and classification structures of different varieties and types. The report should include comprehensive charts and statistical results, providing scientific evidence for the conservation and utilization of yam germplasm resources.^[5]

2 Study on Genetic Diversity of Yam Based on Cytological Markers

2.1 Types and Applications of Yam Cytological Markers

Cytological markers are crucial tools for studying plant genetic diversity. By analyzing characteristics at the cellular level, they can reveal genetic structures and evolutionary relationships of plants. The main types and applications of yam cytological markers include:

2.1.1 Chromosome Number and Morphology Analysis

Chromosomes are carriers of genetic information. By analyzing the number, size, shape, and structure of yam chromosomes, we can understand its genomic composition and evolutionary history. Variations in chromosome number (such as polyploidy) and structural differences (like chromosome breakage and rearrangement) are significant for classifying and identifying yam germplasm resources.

2.1.2 Karyotype Analysis

Karyotype analysis involves detailed description and comparison of chromosomal characteristics during metaphase, including chromosome length, arm ratio, and centromere position. By drawing karyotype maps, different yam varieties can be classified, revealing their genetic relationships and diversity.

2.1.3 Cell Cycle and Mitotic Index Determination

The length of the cell cycle and the mitotic index reflect the activity of cell proliferation. By measuring the cell cycle and mitotic index of yam, we can assess the growth vigor and adaptability of different yam varieties. This method is widely used in yam tissue culture, mutagenesis breeding, and genetic improvement.^[6]

2.2 Acquisition and Processing of Yam Cytological Marker Data

The acquisition and processing of cytological marker data are key steps in the study of yam genetic diversity, mainly including the following aspects:

2.2.1 Sample Preparation and Chromosome Observation

Select appropriate yam tissues (such as root tips and young leaves) for cell fixation, staining, and slide preparation. Common stains include acetic orcein and Giemsa stain. Observing chromosomes under a microscope, we record the number and morphological characteristics of chromosomes.

2.2.2 Image Analysis and Data Recording

Using a microscopic image acquisition system to capture chromosome images, we use image analysis software (such as ImageJ) to measure parameters like chromosome length, arm ratio, and centromere position. Measurement results are recorded in electronic spreadsheets for preliminary statistical analysis.

2.2.3 Cell Cycle and Mitotic Index Measurement

Using flow cytometry or microscopic counting methods, we determine the DNA content and mitotic index of cells. Flow cytometry can quickly and accurately analyze the DNA content distribution of a large number of cells, while microscopic counting involves calculating the number of cells at different mitotic stages to determine the mitotic index.

2.2.4 Data Organization and Statistical Analysis

All cytological marker data are organized and summarized, then entered into statistical analysis software (such as SPSS and R) for analysis. Common statistical methods include descriptive statistics, variance analysis, and cluster analysis.

2.3 Genetic Diversity Assessment of Yam Germplasm Resources Based on Cytological Markers

Based on cytological marker data, the genetic diversity of yam germplasm resources can be assessed, revealing their genetic structure, variation, and evolutionary relationships. Specific methods include:

2.3.1 Chromosomal Variation Analysis

Chromosomal variation is a significant source of genetic diversity. By analyzing the number and morphology of chromosomes, we can assess the chromosomal variations in yam germplasm resources. Steps include: analyzing chromosome numbers to determine the count for different yam varieties, recording variations such as aneuploidy and polyploidy, and analyzing chromosomal structure to reveal types and frequencies of variations. Finally, we draw chromosomal maps to visually display characteristics for comparison and analysis.^[7]

2.3.2 Karyotype Diversity Assessment

Karyotype analysis, by recording chromosomal features like length, arm ratio, and centromere position, calculates karyotype diversity indices such as TF value and A1 value. These indices quantify karyotype diversity, reflecting genomic structural differences among yam germplasm resources. Drawing karyotype maps reveals genetic structure differences and evolutionary relationships.

2.3.3 Cell Cycle and Growth Vigor Analysis

The cell cycle and growth vigor are important indicators of yam's genetic diversity and adaptability. Measuring the cell cycle using flow cytometry or microscopic methods records the proportion of cells at various mitotic stages (G1, S, G2, M phases). Mitotic index calculation involves counting cells in the mitotic phase per unit time, while cell viability staining (using Trypan Blue or MTT assay) assesses cell growth status and health.

3 Study on Genetic Diversity of Yam Based on Molecular Markers

3.1 Application of Molecular Marker Technology in Yam Genetic Diversity Research

Molecular marker technology provides efficient and precise tools for yam genetic diversity research, widely applied in genomic characterization, kinship identification, and genetic diversity assessment. The main molecular marker techniques include:

3.1.1 Random Amplified Polymorphic DNA (RAPD)

RAPD technology uses random primers to amplify polymorphic fragments in the genome, quickly revealing genetic differences among yam varieties. It is cost-effective and species-independent, but has poor repeatability.

3.1.2 Simple Sequence Repeats (SSR)

SSR markers, also known as microsatellite markers, consist of short tandem repeat sequences in the genome. They are highly polymorphic, co-dominant, and highly reliable, widely used in yam genetic diversity research, genetic linkage map construction, and germplasm identification. [8]

3.1.3 Amplified Fragment Length Polymorphism (AFLP)

AFLP technology combines RAPD and restriction fragment length polymorphism (RFLP) advantages, selectively amplifying specific genome fragments to reveal higher polymorphisms. AFLP markers are extensively used in yam genomic analysis and kinship studies.

3.1.4 Single Nucleotide Polymorphisms (SNP)

SNPs are the most common form of genetic variation in the genome, detected and analyzed using high-throughput sequencing technologies. SNP markers, characterized by high density and throughput, are suitable for constructing genetic linkage maps, analyzing population genetic structures, and molecular breeding in yam.

3.2 Methods for Collecting and Analyzing Molecular Marker Data

Collecting and analyzing molecular marker data are core steps in yam genetic diversity research, including:

3.2.1 DNA Extraction and Purification

Young leaves from vigorously growing yam plants are used to extract genomic DNA using methods like CTAB, SDS, or commercial DNA extraction kits. The quality and purity of the extracted DNA are checked using agarose gel electrophoresis to ensure accuracy in subsequent experiments.

3.2.2 PCR Amplification and Marker Detection

Specific primers are designed or selected for PCR amplification based on the chosen molecular marker type. Amplified products are separated by agarose or polyacrylamide gel electrophoresis and detected using silver staining, fluorescence staining, or autoradiography. High-throughput sequencing or SNP chips are used for SNP marker detection.

3.2.3 Data Recording and Preliminary Analysis

Molecular marker information is recorded in electronic spreadsheets, including presence/absence of markers, fragment sizes, and genotypes. Preliminary analyses calculate allele frequencies, polymorphism information content (PIC), and marker polymorphisms.

3.2.4 Genetic Diversity Analysis

Specialized software (such as POPGENE, Arlequin, MEGA) is used to analyze genetic diversity from molecular marker data. Common methods include genetic distance calculation, gene diversity index (H), Shannon diversity index (I), and molecular variance analysis (AMOVA).

3.2.5 Cluster and Population Structure Analysis

Cluster analysis (such as UPGMA, NJ tree) and population structure analysis (using STRUCTURE software) reveal genetic structures and kinship among yam germplasm resources, constructing phylogenetic trees and population structure diagrams.

3.3 Genetic Diversity Assessment of Yam Germplasm Resources Based on Molecular Markers

Genetic diversity assessment using molecular markers provides deep insights into the genetic background and variations in yam germplasm resources. Main evaluation methods include:

3.3.1 Allele Frequency and Polymorphism Analysis

By calculating allele frequencies and polymorphism information content (PIC) for each marker site, we assess the genetic diversity levels of yam germplasm resources. High polymorphism markers with evenly distributed allele frequencies indicate rich genetic diversity.^[9]

3.3.2 Gene Diversity Index and Genetic Differentiation Analysis

Calculating gene diversity index (H), Shannon diversity index (I), and genetic differentiation coefficient (Fst) assesses the genetic variation and differentiation among yam populations. High gene diversity and low genetic differentiation coefficients indicate high genetic diversity and gene flow.^[10]

3.3.3 Phylogenetic Tree and Cluster Analysis

Constructing phylogenetic trees (such as UPGMA and NJ trees) and performing cluster analysis based on molecular marker data reveals the genetic relationships and population structures of yam germplasm resources. Clustering results help understand the genetic background and evolutionary relationships of yam germplasm resources.^[11]

3.3.4 Analysis of Molecular Variance (AMOVA)

AMOVA evaluates the distribution of genetic variation among and within different yam populations^[12]. Results reveal the genetic structure and sources of variation in yam germplasm resources, providing scientific basis for germplasm conservation and utilization.

3.3.5 Genetic Linkage Map Construction and QTL Analysis

Using high-density molecular marker data, we construct genetic linkage maps and conduct quantitative trait locus (QTL) analysis to locate genes associated with important agronomic traits. These findings provide significant references for yam genetic improvement and molecular breeding.^[13]

Conclusion

This article reviews the latest advances in the study of genetic diversity in yam germplasm resources, exploring in detail the application and results of various research methods from the perspectives of morphological characteristics, cytological markers, and molecular markers. Firstly, future research should focus on the integration of genomics and epigenetics, aiming for an in-depth analysis of yam's genetic mechanisms using advanced technologies to identify key functional genes. Secondly, there should be a strengthened emphasis on the conservation and utilization of yam germplasm resources to maintain and enrich genetic diversity. Finally, promoting international collaborative research is essential to integrate global yam germplasm resource information and establish a sharing mechanism to collectively address the challenges brought by global economic integration.

Project

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