

Research Article

# Application of SSR Molecular Marker Technology in Rice Variety Identification

Shasha Zhang\*

Panjin Center for Inspection and Testing, Panjin, China

## Abstract

This study mainly describes the principles and advantages of SSR molecular marker technology, focusing on the analysis and discussion of the application of SSR molecular marker technology in rice variety identification, as well as the technical points of using SSR molecular marker technology to identify rice varieties, including the study of rapid DNA extraction methods, optimization of PCR amplification procedures and reaction systems, improvement of PCR amplification product detection methods, primer screening and construction of DNA fingerprint maps.

## Keywords

Rice, Paddy, Variety Identification, SSR Molecular Marker

## 1. Introduction

Paddy is one of the major crops in the world and the most important crop in China. The population of China rely on rice as their staple food accounts for more than 50% of the total population. Paddy ranks first in terms of planting area and total output among China's grain crops. [1]. As the proportion of rice planting area in China increases year by year, the quality inspection and supervision of rice seeds are particularly important. In the early days, due to the lack of a complete, mature and effective identification method for rice resources, confusion between the same species and different names and the same name and different species of rice often occurred in China, which greatly hindered the full utilization of rice resources. Rice seed variety identification is an important part of seed market supervision departments and variety approval and access. Variety identification has important significance and application value in the circulation process of seed production, processing, storage

and trade. Especially in the circulation process of rice seeds, in order to save costs and shorten time, the importance of rice variety identification is often ignored, resulting in the occurrence of rice seed adulteration incidents, causing certain losses to rice production enterprises.

At present, the main methods for rice variety identification include morphological identification, biochemical identification, and DNA molecular biology identification. Morphological identification generally uses the most intuitive method, observing the shape of the grain to distinguish between indica paddy and japonica paddy, but this is not accurate [2]. For a long time, farmers have been observing the appearance of seeds, mainly relying on the phenotype of paddy to identify and select varieties. This requires a high level of experience from agricultural workers, is easily affected by subjective factors and interference from environmental factors, and requires multiple technical

\*Corresponding author: 250456075@qq.com (Shasha Zhang)



measurements, which is not only time-consuming but also laborious. Therefore, it is necessary to seek more accurate variety identification methods on this basis.

DNA molecular marker technology has been widely used in research such as germplasm identification, kinship identification, and the construction of molecular fingerprints [3]. DNA molecular biological identification is not restricted by the environment and seasons and is not affected by human factors. It also makes up for and overcomes the shortcomings of morphological identification and biochemical identification. The DNA molecular biological identification methods that are widely used in rice variety identification mainly include RAPD, RFLP, AFLP, SSR, and SNP. Since the emergence and development of SSR molecular markers, it has played an important role in promoting the identification of rice varieties. Compared with ordinary marker methods, SSR markers are not affected by the surrounding environmental conditions and related factors. They have the advantages of stable amplification conditions, wide distribution in the genome, good polymorphism, and simple operation, and can effectively distinguish different varieties. At present, SSR has become one of the most common identification technologies in the field of germplasm resource identification [4]. This study will also analyze and discuss the basic principles of SSR markers, the application of SSR molecular markers in paddy variety identification, and the technical points of using SSR molecular markers to identify rice varieties.

## 2. Principles and Advantages of SSR Markers

Crops have specific genome compositions, but because each SSR sequence has certain differences, the loci of the gene also have polymorphism. The key link of SSR markers is to fully understand and analyze the nucleotides on both sides of the SSR locus and to form specific protection for its basic structural sequence. SSR molecular markers mainly include four steps: DNA extraction, PCR amplification, gel electrophoresis, and imaging observation. The detection procedure is relatively simple. A sample can be completed from sampling, detection to reading results within a few hours and is not affected by the external environment and season. The sequences at both ends of SSR marker are mostly relatively conservative single-copy sequences [5]. In this process, it is also possible to search for SSR sequences in the existing DNA database, and then further design and amplify the existing SSR sequences based on the search results using a pair of specific primers. After the SSR sequence is designed and amplified, it is analyzed according to the corresponding results after a series of staining and electrophoresis treatments.

DNA molecular markers are genetic markers based on the polymorphism of biological macromolecules, which can

directly reflect the differences between genomic DNA. In eukaryotic organisms, there are a large number of non-coding DNA sequences in the genome, and their variation is not or is less restricted by natural selection. Compared with other identification methods, SSR molecular markers have the following advantages: 1) They can be detected in all developmental stages and tissues of organisms, are not restricted by seasons and environmental conditions, and do not have problems such as whether they are expressed or not [6]; 2) DNA samples can be stored for a long time at low temperatures, which is convenient for sample re-examination, which is very beneficial for arbitration or traceability identification [7]. Therefore, SSR molecular marker technology is widely used in the identification of crop varieties.

## 3. Application of SSR Molecular Marker Technology in Rice Variety Identification

SSR molecular marker is a type of marker with genetic characteristics based on paddy molecular polymorphism. They can directly reflect and analyze the differences in DNA within the molecule. Compared with ordinary genetic marker methods, SSR molecular markers are not restricted by the paddy development stage and development environment, but also the high polymorphism level directly enhances the effect of the marker, which is conducive to the identification and analysis of excellent rice varieties. Therefore, SSR markers have extremely high application value in rice variety identification.

Since 2006, the identification of related rice varieties and the establishment of SSR marker fingerprint databases have been launched gradually, and very important research results have been achieved. Xiao Xiaoyu et al. [8] used 208 pairs of SSR primers to amplify and screen the main hybrid rice parents in Sichuan Province of China, and found that 123 pairs of primers were polymorphic. From them, 18 pairs of primers with high polymorphism were selected and a SSR fingerprint database of 26 main hybrid rice parent materials in Sichuan Province was established. This is the first time that a systematic work on the construction of a DNA marker database for major rice varieties has been reported across the world. Cheng Benyi et al. [9] conducted DNA fingerprint detection on the main rice varieties in Zhejiang Province of China and established a DNA fingerprint database of 253 rice varieties. In 2009, Chen Yinghua et al. [10] selected 10 pairs of core primers from 500 pairs of SSR primers and used them to construct DNA fingerprints of 79 varieties in regional trials in Northeast China; In 2010, Ma Hongbo et al. [11] used 24 hybrid rice varieties and 18 hybrid rice parents in Fujian Province of China as materials to establish a DNA fingerprint of 504 SSR markers, providing a basis for the purity and authenticity identification of rice varieties. In 2011, Xiao Zifa et al. [12] used 48 pairs of SSR

primers to complete the construction of DNA fingerprints of 77 hybrid rice varieties approved by Hunan Province of China, and successfully established a variety fingerprint database, which can effectively identify hybrid rice varieties. In 2013, Ren Hui [13] conducted SSR molecular marker identification on 8 hybrid rice varieties, optimized the DNA extraction method and PCR amplification reaction system, and compared the SSR molecular marker identification results with the field identification results in 5 different provinces of China. Statistical analysis found that SSR molecular marker identification results were easier to distinguish heterozygous seeds, while field identification results were difficult to distinguish similar heterozygous plants. In 2014, the Ministry of Agriculture of China promulgated a new agricultural industry standard NY/T1433-2014 “SSR Marker Method for Rice Variety Identification Technical Regulations”. In 2016, Zeng Xiaoshan [14] et al. used 48 pairs of primers to perform DNA fingerprint analysis on 27 parents. The genomic DNA of the 27 rice parent materials was PCR amplified in turn. After development and photography, a set of SSR primer maps were obtained. Based on the constructed fingerprint map, the authenticity of a certain variety can be identified. In 2022, Fan Jiameng et al. [15] used Fengliangyou 3305 and Rundao 118 as experimental materials and screened out SSR molecular markers suitable for purity identification of these two varieties. Wang Yankun et al. [16] used 40 pairs of SSR primers to study genetic diversity, constructed 12 fingerprint maps, and calculated their genetic distances and generated cluster maps, providing better genetic information for breeding at the molecular level. In summary, the detection technology for identifying rice varieties through SSR molecular markers has matured. However, with the emergence of new varieties every year, the specificity of SSR primers may be lost, and SSR molecular marker identification system needs to be continuously optimized and updated. Therefore, SSR molecular marker identification methods can identify rice varieties more quickly and accurately.

## 4. Key Technical Points for Rice Variety Identification Using SSR Molecular Markers

### 4.1. Research on Rapid DNA Extraction Methods

DNA extraction and purification are important and basic steps in rice variety identification. Rice DNA can be extracted from rice seeds or rice leaves. Extracting DNA from rice seeds is not limited by time and space. However, since rice seeds contain a large amount of polysaccharides, proteins and other substances, the required extracting solution is relatively complex and the steps are relatively more complicated [17]. The SDS method and CTAB method currently used to extract DNA from rice leaves have high quality and purity, which accord with the requirements of

PCR detection. However, most of them require the use of liquid nitrogen to freeze the plant tissue and grind it, which is a complicated operation [18]. Even if a relatively simple kit is used, the process of grinding the plant tissue with liquid nitrogen cannot be avoided. In addition, the number of DNA test samples required for variety identification is large, and the traditional DNA extraction method has complicated operation steps and is not suitable for the needs of variety identification. Our laboratory has developed a rapid, efficient and high-quality DNA extraction method. Instead of extracting DNA from leaves, rice seeds are directly ground, sieved and crushed into rice flour. The CTAB method is used to extract genomic DNA from rice. The extraction efficiency is high and can fully satisfy PCR amplification.

### 4.2. Optimization of PCR Amplification Procedure and Reaction System

The optimization of PCR amplification program is mainly to shorten the reaction time and improve work efficiency while ensuring good amplification effect. When Zhan Qingcai [19] used SSR markers to identify the purity of hybrid paddy seeds, he changed PCR amplification program, changed the denaturation and annealing time to 15 s, the extension time to 30 s, and amplified 35 cycles to shorten PCR reaction time. After repeated experimental optimization, our laboratory found Panjin rice SSR marker PCR reaction system for amplifying 11 varieties. The specific amplification program is 10  $\mu$ mol primers, 1.0  $\mu$ L each, 50 ng/ $\mu$ L DNA template, 1.0  $\mu$ L, 1 U/ $\mu$ L 2\*San Taq PCR 10.0  $\mu$ L, dd H<sub>2</sub>O 7.0  $\mu$ L, and a total reaction volume of 20.0  $\mu$ L. PCR reaction program was as follows: pre-denaturation at 94 °C for 5 min; each cycle consisted of pre-denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min, and extension at 72 °C for 1 min, 35 cycles; and finally extension at 72 °C for 8 min, which resulted in the ideal amplification result.

### 4.3. Improvement of PCR Amplification Product Detection Method

The main methods for detecting PCR amplification products include agarose gel electrophoresis, denaturing polyacrylamide gel electrophoresis, non-denaturing polyacrylamide gel electrophoresis, and DNA sequencers [20]. At present, most laboratories still use agarose gel electrophoresis or polyacrylamide gel electrophoresis to detect PCR amplification products. Agarose gel electrophoresis is easy to operate, but the result is low. Another disadvantage is that it requires contact with the strong carcinogen EB. Although polyacrylamide gel electrophoresis and non-denaturing polyacrylamide gel electrophoresis have improved resolution and avoided the risk of contact with the carcinogen EB, they are cumbersome to operate and are still difficult to apply in general laboratories. Our laboratory used a microchip electrophoresis

instrument developed by Shimadzu Corporation of Japan to detect PCR amplification products. It has high sensitivity and the fragment size is intuitive and accurate. The microchip electrophoresis instrument uses its own software to analyze experimental data accurately and reliably, greatly improving work efficiency. However, the instrument is relatively expensive and the required reagents cost is slightly high. Currently, any molecular laboratory with certain conditions can own it.

#### 4.4. Primer Screening and DNA Fingerprint Construction

Primers used for authenticity identification of rice varieties are required to distinguish the identified varieties from combinations or parents of other rice varieties that may be mixed in them. Therefore, primer screening is the core of SSR molecular marker method for variety identification. Since the polymorphism of different SSR markers is different, how to screen out primers that are easy to identify and have good stability as core primers can greatly reduce the workload of screening primers and greatly improve the efficiency of primer identification. There are many rice varieties in China. By collecting a large number of conventional varieties that are widely used in production, the identification markers of each variety are screened out, and the SSR fingerprint database of each region is established. In addition to establishing a DNA fingerprint database of existing varieties, the fingerprint database should be regularly updated and newly approved varieties should be added on the original basis. Only in this way, SSR molecular marker technology can be effectively applied to rice variety identification.

Our laboratory designed and constructed 48 pairs of primer sequences for identifying 11 major rice varieties in Panjin, Liaoning Province, with reference to Chinese National Standard, “NYT1433-2014 Technical Regulations for Identification of Rice Varieties SSR Marker Method” and other literature. These 48 pairs of primers detected a total of 438 alleles in 11 varieties of Panjin rice, with an average of 9 alleles detected per pair of primers. Four pairs of core primers RM551, RM1195, RM1, and RM7102 were screened and obtained, which can completely distinguish 11 varieties of Panjin rice. At the same time, according to the results of rice primer amplification and microchip electrophoresis in Panjin area, the electrophoresis pattern of specific amplification of 48 pairs primers was used to record the characteristics of polymorphic fragments. Referring to the requirements of Chinese National Standards “GB/T18347-2001128 Barcode” and “GB/T 18284-2000 Quick Response Matrix Code”, combined with the numerical values assigned to different varieties of Panjin rice, DNA fingerprint of Panjin rice varieties was encoded using online barcode and QR code generation software to construct a unique ID for Panjin rice varieties, generating unique

identity information for Panjin rice.

## 5. Conclusion

Chinese paddy breeding work is in a leading position in the world. Although China has achieved certain results in rice variety identification, there are still some problems. For example, SSR molecular marker technology started late in China and developed slowly. In order to ensure the continuous promotion and planting of new rice varieties in China, it is more important to use SSR molecular marker variety identification methods to study the authenticity detection of rice variety quality, and the traceability evaluation and protection of rice seed quality. Therefore, it is necessary to continuously explore the accuracy and effect of SSR molecular marker technology, and at the same time further strengthen technical exchanges with experts in rice breeding, so as to achieve Chinese agricultural science and technology progress through the joint efforts of all parties.

## Abbreviations

RAPD	Random Amplified Polymorphic
RFLP	Restriction Fragment Length Polymorphism
AFLP	Amplified Fragment Length Polymorphism
SSR	SSR Molecular Markers
SNP	Single Nucleotide Polymorphism

## Author Contributions

Shasha Zhang is the sole author. The author read and approved the final manuscript.

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## Conflicts of Interest

The author declare that they have no conflicts of interest.

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## Biography

**Shasha Zhang**, female, senior engineer, PhD, engaged in the research of food microbiological inspection technology.