

Application of SSR Molecular Markers in Breeding and Crop Genetics

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Abstract: SSR is a widely used molecular marker based on PCR technology. Its main advantages are high polymorphism, abundant content, and codominance. In this paper, the analysis steps of SSR molecular markers are studied, and the use of SSR molecular markers in crop genetics and breeding is analyzed.

Genetic markers refer to genes used in genetic analysis, that is, marker genes. It can track chromosomes and certain genes and segments to pass on any genetic characteristics in the family. Currently, widely used genetic markers include biomarkers, morphological markers, and cell and molecular markers. The first three types of genetic markers all express genes indirectly, and they have fewer marker sites and poor polymorphisms. They are affected by seasons and the environment, so they develop slowly. Analytical markers are ideal genetic markers, and SSR (Simple Sequence Repeat Length Polymorphism) is widely used [1]. Therefore, this article analyzes the use of SSR molecular markers in crop genetics and breeding.

1 Principles of SSR Molecular Marker Technology

SSR is also called microsatellite DNA, simple sequence length polymorphism and short tandem repeat. It refers to a DNA sequence that uses 2-5 nucleotides as a unit for multiple tandem repeats. Nucleotides serve as tandem repeat units. Similar microsatellite DNA can be distributed in different locations in the genome, and the length is less than 100bp. Because there are different numbers of repetitions, each site is polymorphic. Each SSR is a conservative single copy sequence at both ends. This sequence is used to implement complementary oligonucleotide primers and PCR amplification of SSR. Because SSR polymorphisms are mostly caused by differences in the number of simple sequence repetitions, generally: Codominant [2]. SSR amplification products are detected using high concentration agarose gel or polyacrylamide gel electrophoresis. Compared with other molecular markers, the main advantages of SSR markers are: one is that they are abundant and cover the entire genome, revealing high polymorphisms; the other is that the co-dominant inheritance is not easy to be eliminated by natural selection and artificial selection. Mendel's genetic law; Third, it has multi-allele characteristics and provides a relatively high amount of information; Fourth, by designing primers to sequence the site design, it is convenient for different laboratories to exchange and cooperate to develop primers; fifth, it is convenient for PCR technology. For analysis, the requirements for DNA quality are relatively low, and the amount is relatively small. It is not necessary to use isotopes, and even partially degraded samples can be analyzed [3].

2 SSR Molecular Marker Analysis Steps

2.1 Getting the Primers

Find the five SSR flanking sequences or primers you need from the public DNA sequence database, DDBJ, or published articles. SSR locus sequences are similar and conserved among genera and species within the family. Studies by ZHAO et al. Reported that rice SSR primers can achieve maize amplification. Primer cross-species and kinship are closely related. Whether a primer can be used across species depends on specific experiments and analysis [4].

If this method fails to screen the required primers, a genomic library must be created. Realize

genomic DNA digestion and create small fragment gene libraries. Positive clones were screened using specific SSR sequence probes to determine the DNA sequence. Because the SSR sequences on both sides of the same species are highly conserved, if the appropriate SSR site sequence is obtained, the design of highly transferable SSR primers can be achieved with flanking sequences, thereby realizing PCR amplification of genomic DNA and obtaining specific sites. SSR DNA products are polymorphic monitored by electrophoresis.

2.2 PCR Amplification

Once primers are in place, SSR regions can be amplified using standard PCR procedures. For different primer pairs, optimize professional reaction conditions. Generally, reliable and clear bands are obtained by changing the buffer Mg^{2+} concentration, annealing temperature, etc. [5].

2.3 Electrophoresis

PCR product separation and electrophoresis includes polyacrylamide denaturing sequence gel electrophoresis, agarose electrophoresis, polyacrylamide gel electrophoresis, etc., because the amplified fragments are relatively short and the differences between genes are small, high-resolution polyacrylamide Gel electrophoresis.

2.4 Data Processing

Co-dominant inheritance of SSR loci will produce one or two main bands. Generally, the paternal value is 1, the heterozygous value is 2, and the maternal value is 3. Various material band patterns were recorded to make them into 0,1 data, and genetic analysis was performed using computer programs [6].

3. SSR Molecular Marker Use in Crop Genetics and Breeding

In the process of human genetics and breeding, the superiority of molecular marker technology has received much attention, and related research has become more and more mature.

3.1 To Create A Molecular Marker Genetic Map

Genetic map refers to using a certain marker site as the basis, looking for polymorphic markers at a certain distance in the SSR fragment, and using the linkage analysis of the markers to fully display the relative relationship of the analyzed markers. The created genetic map can provide a theoretical basis for the research of plant germplasm resource bank, molecular cloning and breeding, and molecular marker technology is also used in the creation of common crop genetic maps.

3.2 Gene Mapping

Gene mapping is the main use of genetic maps in plant genetics and breeding, mainly including gene mapping of quantitative and qualitative traits. SSR molecular markers are used to separate population group analysis and near-isogenic line analysis to use a wide range of methods for qualitative gene mapping. Resistance genes such as tomato antiviral gene Tm-2a and rice semi-dwarf gene s-dg are passed Isogenic lines locate genes. Genes identified by the method of segregated population grouping include wheat powdery mildew resistance genes, rice blast resistance genes, etc. [7].

3.3 Selecting Molecular Marker Assist

The selection of molecular marker assist in crop breeding is to determine the existence of the target gene by analyzing molecular markers closely linked to the target gene. If the target gene is closely linked to a molecular marker, the molecular marker genotype is used to obtain the target genotype. The main advantage of molecular marker assisting compared with traditional breeding methods is that they will not be disturbed by external conditions and the breeding cycle is short, so they can do more with less. SSR markers have a significant role in the use of crop heterosis,

backcross breeding, etc., especially in crop resistance breeding. For example, the SSR marker RM262, which is closely linked to the rice blast disease resistance gene *Pe-d (t)*, was used to achieve MAS selection in the F₂ population of rice and susceptible varieties Jiangnan Xiangnuo and 8987 with this disease resistance gene. The results showed that the accuracy rate of selecting resistant plants with pure and heterozygous bands for this marker was more than 98%. In addition, SSR marker-assisted wheat-barley yellow dwarf virus marker-assisted selection and SSR-resistant wheat aphid-assisted breeding of wheat were studied [8].

3.4 Identification of Variety Purity

The reaction of genome-specific DNA fragments between biological individuals and populations through electrophoretic bands is the main way of DAN molecular marker technology to identify variety purity. DNA molecular markers can be used to identify the purity of varieties, which can avoid the traditional field morphological identification method using the individual plant traits in the sample variety, remove the individual plants with different performances, and then estimate the uncertainty of the overall purity data, and avoid environmental impact on plants. For example, after SSR molecular marker detection was performed using DAN extracted from different parts of whole seeds, seed embryos and seedlings, it was indicated that primary leaves and bud tips were the most suitable parts for identifying the purity of corn varieties using SSR molecular marker technology. In addition, SSR molecular marker technology is a good way to identify the purity of hybrid rice [9].

3.5 Molecular Marker Assisted Breeding

The combination of molecular marker-assisted selection and modern biology, traditional breeding and other technologies can quickly and accurately analyze the genetic composition of individuals based on molecular level, directly select genotypes, and promote the breeding process based on original breeding technologies. The advantages of molecular marker-assisted breeding selection are: one is to solve the linkage of bad traits and introduce distantly good genes; the other is to aggregate multiple favorable genes to improve breeding efficiency; the third is to distinguish between heterozygous and homozygous. Contributory markers are identified by genotype of the plant in isolated generations. Fourth, environmental changes and gene expression do not affect target trait selection during plant development. For example, in the production of straw mushrooms, a conventional refrigerated temperature of 4 ° C will cause the mycelium to dissolve and liquefy and soften the fruiting bodies. The production environment of straw mushroom is high temperature and high humidity, which improves the artificial selection of new strains of straw mushroom with low temperature and high yield. Using mating gene molecular markers, it is possible to quickly create molecular markers for straw mushrooms, and to assist in the hybrid breeding technology system to cultivate new strains of excellent straw mushrooms at low temperature and high yield [10].

Conclusion

The development of modern molecular biology and genetics has promoted the development of plant genetics and breeding. The molecular marker technology combined with the rich experience of breeders can facilitate the breed selection. However, in the current actual work, the operation of molecular labeling technology is more complicated and the cost is higher, which limits its use. Therefore, how to simplify the operating procedures and reduce the cost is a new topic for the application research of molecular marker technology. Through long-term analysis, in the process of continuous development of science, technology and economy, molecular marker technology is the main auxiliary breeding method in plant.

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