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# Application of Molecular Marker Technology in the Study of Forest Tree Species

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**Abstract** Due to its unique advantages, molecular marker technology is widely applied in the research of forest tree species. This paper reviewed the application of molecular marker technology in tree species resource diversity, germplasm identification, genetic map construction, gene mapping and marker-assisted selection (MAS) breeding. In addition, it elaborated the great significance of molecular marker technology to promote the sustainable development of forestry production in China.

**Key words** Molecular marker, Species resource identification, Randomly amplified polymorphic DNA (RAPD), Forest tree breeding

## 1 Introduction

With the rapid development of life science and technology, various biotechnologies have been widely applied in the field of tree species research. In recent years, molecular marker technology has developed rapidly and has become a powerful tool for forest tree species research. It has brought great changes to traditional genetic improvement and greatly improved people's understanding of forest tree species at the molecular level. As a new type of genetic marker, molecular marker is directly expressed in the form of DNA. It is an ideal form of genetic marker developed after palynological markers, morphological markers, biochemical markers and cytological markers. It has the characteristics of not being easily affected by external environmental factors, large amount of information, accurate and rapid detection, simple operation, and has nothing to do with gene expression. Therefore, the molecular marker technology has been widely applied in the study of diversity of germplasm resources of forest tree species, germplasm identification, construction of genetic maps, gene mapping and marker-assisted selection (MAS) breeding.

## 2 Diversity and genetic relationship analysis of forest tree germplasm resources

In essence, the diversity of forest tree germplasm resources is the variation of biological genetic material. Exploring genetic diversity is the basis for studying plant evolution and kinship. A large number of varieties of forest trees has appeared in the continuous evolution stage, which has greatly enriched the genetic diversity of forest tree species and provided an important foundation for the cultivation of a large number of new tree species. In recent years, researchers have introduced molecular marker technology into forest tree species classification and genetic diversity research, and have made remarkable achievements.

When analyzing the plants of *Millettia dielsiana* in different geographical conditions, Li Li *et al.* [1] used molecular marker technology to study the genetic diversity of wild colony of *M. dielsiana* plant originating from different provinces and cities in South China and found that there was a high genetic differentiation among the populations of *M. dielsiana*. Using the molecular marker technology can correctly evaluate the genetic diversity of forest trees, bring predictive help to the prediction of genetic variation, parent selection and heterosis value in the future, and then achieve the goal of promoting breeding[2-3]. The similarity at the DNA level can be used as direct evidence of kinship. On the basis of establishing the DNA fingerprints of varieties, cluster analysis can be carried out to discuss the classification and kinship of varieties. Using fluorescent AFLP technology to study 20 new pear hybrid varieties and their parents, Wang Fei *et al.* [4] constructed the fingerprints of 43 varieties, analyzed the genetic relationship and genetic relationship between the new varieties and their parents at the molecular level, and classified 20 new varieties at the molecular level, most of which were consistent with the traditional classification of morphological characteristics. Using randomly amplified polymorphic DNA (RAPD), Teng *et al.* [5-6] analyzed the genetic relationship between Xinjiang pear (*Pyrus sinkiangensis*) varieties and 92 main pear varieties in East Asia. All varieties were clustered into 5 groups, among which Chinese white pears and sand pears were grouped into one group, and main Japanese pears were clustered into one group. The results also showed that the genetic relationship between Western pear varieties and Asian pear varieties was relatively distant. Using RAPD molecular marker technology, Shen Yuying *et al.* [7] analyzed the genetic relationship of 6 sand pears and 36 Japanese pears from Fujian and Zhejiang, which were divided into 4 sand pear groups and 5 Japanese pear groups, of which 4 Japanese pear groups contained varieties or types from Kochi Prefecture in Japan. Due to sampling bias, Chinese pears and Japanese pears were not mixed into a large group in

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the cluster analysis. However, the similarity coefficient between Chinese sand pear and some Japanese pears, especially Japanese pears from Kochi Prefecture, was higher than that with Chinese pears. Lin *et al.* [8] used the amplified fragment length polymorphism (AFLP) technology to analyze the genetic relationship of 10 species of *Pyrus* in China. The clustering results showed that when the genetic distance was 0.66 – 0.69, they were divided into two categories, one was 8 species native to China, among which the genetic distance of white pear and sand pear was the closest, and they clustered together first and Western pears and apricot pears are another category. Molecular markers are effective tools for detecting genetic diversity and genetic relationship analysis of forest tree germplasm resources. With the development of molecular biology techniques, the application of molecular markers in genetic diversity and genetic relationship analysis of forest tree germplasm resources will be more extensive.

## 3 Resource identification of forest tree species varieties and strains

The identification of forest tree species is generally divided into identification among species, identification of different species within a population, and identification of different geographical groups. Due to the large genome differences between species, it is easy to identify and distinguish them by morphology and various markers. The identification and distinction of different genera, different geographical groups or hybrid strains within a species is more complicated. Traditional methods cannot distinguish, and there are a large number of polymorphic markers among varieties of the same species. A variety has unique marks that distinguish it from other varieties, that is, the combination of some specific DNA segments is called the "fingerprint" of the variety[9]. It was found that the good asexual genetic polymorphism and genetic relationship of vegetation varieties were analyzed by molecular marker technology, and the standardized DNA and electrophoretic patterns of such high-quality forest species were established through the determined primers with high resolution and best repeatability, which made the identification of forest species more accurate and rapid[10]. Therefore, molecular markers are an efficient identification method. Japanese scholar Yamamoto *et al.* [11] used apple SSR molecular markers to identify 36 pear varieties including 19 Japanese pears, 7 Chinese pears, 5 European pears, 3 wild varieties and 2 hybrid varieties. The results showed that all varieties except mutants could be distinguished from each other at the detected 79 allelic loci. Based on the amplification of 12 pairs of SSR primers, Mo Wenjuan *et al.* [12] screened 3 pairs of primers with clear amplification patterns and amplified a total of 22 bands from 9 materials, all of which had polymorphisms and the average polymorphism ratio was 100%. The similarity coefficients of the nine Xingaoli varieties were 0.47 – 0.90 by cluster analysis, and the nine Xingaoli varieties could be distinguished. The results showed that the molecular markers were used for the identification of *Pyrus* varieties with high polymorphism, and could distinguish all the tested materials, and had a high degree of discrimination ability. Kimura *et al.* [13] used 9 SSRs to identify 60 Asian pears of 6 species, of which 7 SSRs could distinguish 58 varieties; in 2003, 20 SSRs were used to analyze the offspring of 8 crosses within or between species.

## 4 Construction of genetic map

Genetic map, also called linkage map, is a map with polymorphic genetic markers as guideposts and the exchange rate of two loci. It can provide a detailed description of the genome structure of forest trees, and provide a large number of genetic markers for population genetic research or monitoring of tree breeding activities. Besides, it can identify DNA transduction insertion sites. Combined with other techniques, it can isolate single genes and chromosome sheets encoding quantitative trait loci. Therefore, the genetic map is not only a powerful tool for forest tree breeding and gene transduction, but also makes it possible to select trees early, improve selection efficiency and shorten the breeding cycle. To construct a genetic map, there should be suitable segregating populations and genetic markers that can reveal parental polymorphisms. The pear Bartlett genetic map constructed by Yamamoto *et al.* [14] in 2007 had a total length of 1 000 cm, 17 linkage groups, and 447 loci. The average distance between 58 pear SSR markers, 60 apple SSR markers and 322 AFLP markers was 2.3 cm. Using 87 $F_2$ individual plants of *Populus deltoides* Marshall × *Populus cathayana* Rehd as a mapping population, Huang Qinjun *et al.* [15] obtained a molecular linkage genetic map with 810 markers (784 AFLP markers and 26 SSR markers), and the total distance of the frame map was up to 3 382.4 cm. Dang Zhiguo *et al.* [16] obtained $F_1$ hybrids by crossing between mango varieties "Jinhuang" and "Guifei". In the hybrid population, they selected 98 $F_1$ plants as the mapping population. Through the linkage analysis of ISSR, SRAP and AFLP markers, they constructed the molecular genetic map of mango, which provided an important basis for further research on the important agronomic traits of mango. Tanaka Chunichi[17] used 46 individuals of the $F_1$ group of the tea tree variety "Soubei" and its bred product line "Jingyi Yinza 131" to be labeled by RAPD, and 105 markers were detected using 166 primers. Among them, 82 markers were observed to segregate in the $F_1$ generation. Among the 82 markers, 32 were from "Soubei", 43 were from "Jingyi Yinza 131", 7 were common markers from both parents. Six linkage groups were detected from "Soubei" and "Jingyi Yinza 131", and there were many independent markers, part of the genetic map of "Soubei" and "Jingyi Yinza131" was plotted. Huang Fuping[18] carried out research on the construction of tea tree molecular genetic map, and first established the tea tree AFLP technology system in China. He used ISSR markers to evaluate the genetic diversity of Chinese cultivated tea tree germplasm resources, explored the segregation mode of ISSR and RAPD markers in the first generation of backcross of tea plants, and preliminarily constructed a partial linkage genetic map of Fuding Dabai tea. From the perspective of the forest tree genetic map constructed so far, the density of the map is still low. It is necessary to locate some insect resistance genes, drought resistance genes and other genes, and to construct high-density genetic maps.

## 5 Location of tree species trait resources

The traits of forest tree species are divided into qualitative

traits and quantitative traits. There are two main methods for mapping quality trait genes: one is to locate based on the existing linkage map; the other is to use near-isogenic Line (NIL) or bulked segregant analysis (BSA). Many plants, especially perennial fruit trees, do not have near-isogenic lines, thus most of them use the BSA method for gene mapping. At present, some markers linked to the main quality trait genes have been found in fruit trees such as apple, peach, citrus, almond and sweet cherry, such as the discovery of the *Venturia nashicola* resistant *Vf* gene[19]. Zhang Sipu *et al.*[20] used the SRAP primer combination Me30 (TGAGTCCAAACCGGACG)/Em7 (GACTGCGTACGAATTCGA) to amplify a stable differential band with a length of 78 bp between the mother plant of pomegranate safflower and the mutant paper strips of white flowers, indicating that the generation of white-flowered variant branches may be related to the deletion of DNA fragments in the mother plant.

## 6 Molecular marker assisted selection breeding

It is the direction that breeding researchers have been working hard to fully explore and utilize the natural excellent gene pool of forest germplasm resources. Using molecular markers to locate the target gene in a specific DNA region, and then obtain the target gene for inter-species or intra-species transfer, is also an important means for molecular markers to be used in breeding and to create new materials. At present, the most valuable practical application of molecular marker technology is molecular marker assisted selection (MAS). It advantages are mainly manifested as follows: (i) reducing linkage drag by realizing directional transfer of favorable genes through backcrossing; (ii) accumulating target genes through gene introgression; (iii) shortening the breeding cycle of perennial plants by early selection using molecular markers. Compared with morphological markers, molecular markers have incomparable advantages, and their application in breeding is increasing day by day.

Using the leaves of the $F_1$ generation of *Populus deltoides* Marshall × *Populus nigra* as materials, Cervera used AFLP molecular marker technology to screen 11 500 polymorphic fragments with a size of 70 – 800 nucleotides with 144 primers, and obtained 3 AFLP markers closely linked to the poplar rust resistance gene[21]. Song Wei *et al.*[22] used the $F_1$ generation segregation materials of "*Pyrus communis* L" × ("Chi pear" + "Xingao pear") to screen and compare 281 pairs of SSR primers from apple and pear genomes with BSA, and obtained the SSR markers CH02f16 and CH02b10 related to pear fruit shape, and the coincidence rates of their round and non-round traits were 96.67% and 91.67%, respectively. Villar *et al.*[23] used the BSA method and adopted a 2 × 2 factorial mating design to detect 5 RAPD markers linked to poplar leaf rust resistance in 189 offspring of 4 poplar families, and they obtained a disease resistance gene marker smaller than 1 cm, making it possible to isolate the gene. Using DNA molecular marker technology to assist forest tree breeding has made certain achievements. However, it is still in its infancy and exploratory stage, and it is necessary to use molecular markers to formulate and implement forest tree breeding strategies. To realize the effective combination of molecular markers and traditional forest tree breeding, there are still many theories and methods to be explored and solved.

## 7 Application prospects and outlook

Modern molecular marker technology is developing very rapidly, and its application fields are becoming wider and wider. At present, the commonly used molecular markers mainly include RFLP, RAPD, AFLP, and SSR, among which RAPD is the most widely used molecular marker technology due to its low cost and other advantages. Besides, DNA chip technology and Messenger RNA differential display (mRNA DD) provide new tools for forest tree species research. The research of application of molecular markers in forest trees will play an immeasurable role. Molecular marker technology is a new technology with potential and application prospects. With the rapid development of molecular biology, the application of various molecular marker systems in forest trees will become more and more extensive and play an enormous role. The successful application of molecular markers will provide more valuable information for tree species selection and breeding, greatly improve the purpose and accuracy of breeding, shorten the breeding cycle, and improve breeding efficiency. In addition, molecular markers can accurately and quickly provide information on the genetic structure of forest tree populations, and provide theoretical guidance for the preservation of forest natural resources, especially the preservation of rare and endangered tree species, which is of great significance for promoting the sustainable development of forestry production in China.

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**4.4.3** Supporting and operating a number of dairy cooperatives. It is necessary to strengthen dairy farmers' understanding of the economic functions of cooperatives, build a bridge of communication with the government through cooperatives, and truly guide and support the operation of a number of demonstration cooperatives, to enhance the voice in negotiations with relevant enterprises and reduce the costs of forage, technical services and veterinary ser-vices, which is conducive to building a negotiated pricing mechanism for raw milk, improving the benefit distribution mechanism of the industrial chain, and promoting the dairy industry to become an industry in stable development.

**4.5 Strengthening policy guarantee to promote the high-quality development of dairy industry** In the new development stage, the realization of high-quality development of dairy industry needs more precise and stable policies and measures, and scientifically determining the key areas supported by policies is the key.

**4.5.1** Increasing policy support aimed at key areas that restrict high-quality development. (i) To support the development of high-quality forage industry. It is necessary to speed up the construction of modern forage industry system, adjust measures to local conditions, and optimize forage planting structure and dairy cow diet structure. (ii) To support the green transformation of dairy development mode in Henan Province. It is necessary to gradually increase policy support for standardized breeding, waste resource utilization and harmless treatment of sick and dead livestock. (iii) To support the construction of quality monitoring system for raw milk and dairy products. It is necessary to establish a quality traceability system covering feed, production, processing, transportation, storage and sales, fully assume quality and safety responsibilities, and ensure the whole process quality of products.

**4.5.2** Paying special attention to the policy effect evaluation of the important links to ensure high-quality development. (i) Pre-evaluation. As to whether the projects supported by policies are suitable for the region and whether there are environmental risks, risk assessment and policy implementation effect pre-judgement should be carried out. (ii) Process evaluation. It is necessary to track whether there are policy implementation deviations in the implementation of the project, so as to provide a basis for timely correction. (iii) Post-evaluation. The effect should be evaluated in accordance with the implementation of policy objectives to ensure the effectiveness of policies to promote high-quality development.

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