Application of DNA molecular markers in citrus research

Zhu Weiqing[1, a, *], Li Minjian[1, b],

[1]Huazhong Agricultural University, No.1 Shizishan Street, Hongshan District, Wuhan City, Hubei Province, 430070, China
a.zhuweiqing_zwq_221@outlook.com, b.lmj_liminjian_mj@163.com
***Corresponding Author**

Abstract: Citrus is a globally important economic fruit tree, and DNA molecular marker technology has become a key tool for molecular research in citrus. This article reviews the mainstream types of DNA molecular markers—including RFLP, RAPD, AFLP, SSR, ISSR, and SRAP—and their respective technical characteristics; it also summarizes domestic and international research examples and advances in the application of these technologies to the analysis of citrus genetic diversity, variety identification, the elucidation of germplasm kinship, and the localization of genes associated with stress and disease resistance. Current research indicates that each type of molecular marker has its own advantages and limitations, and no single marker can meet all research needs. With the development of high-throughput sequencing and third-generation sequencing technologies, the development of novel molecular markers, the construction of germplasm molecular databases, and marker-assisted breeding will become key research directions in the future, thereby providing a theoretical reference for the conservation, genetic breeding, and utilization of citrus germplasm resources.

Keywords: DNA molecular markers, genetic diversity, variety identification, kinship, gene mapping

1. Introduction

Citrus fruits are an important economic crop worldwide and occupy a highly significant position in international fruit trade; they are now widely cultivated in tropical and subtropical regions. In recent years, as molecular biology research has continued to deepen and practical applications have rapidly developed, the application of DNA molecular marker technology in citrus research has become increasingly widespread, primarily focusing on genetic diversity analysis, variety identification, kinship analysis, and gene mapping. Based on the current state of research both domestically and internationally, this paper provides a systematic review and analysis of recent advances in the application of DNA molecular marker technology in citrus research, with the aim of providing a reference for citrus research and its development and utilization.

2. Overview of DNA molecular marker technology

DNA molecular marker technology, developed in the 1960s, is a type of genetic marker based on the polymorphism of biomacromolecules, especially genetic materials (nucleic acids). It is free from the influences of growth stages, organ and tissue differences and environmental constraints, and features abundant quantity, wide distribution, high polymorphism, good stability, rapid detection and simple operation. For these merits, it is regarded as an ideal genetic marker technology and has been rapidly popularized and applied in recent years [1].

DNA molecular markers can be classified according to the adopted molecular biological techniques into those centered on Southern hybridization and those centered on PCR technology. The former is mainly represented by Restriction Fragment Length Polymorphism (RFLP) and DNA fingerprinting techniques; the latter mainly includes Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Inter-simple Sequence Repeat (ISSR), Simple Sequence Repeats (SSR), etc [2]. At present, the molecular markers widely used in citrus research mainly include RFLP, RAPD, AFLP, ISSR,

https://doi.org/10.62852/csa/2026/308
Copyright (c) 2026 Cambridge Science Advance

SRAP (Sequence Related Amplified Polymorphism), SCAR (Sequence Characterized Amplified Regions) and SSR.

3. Application of DNA molecular marker technology in citrus research

3.1. Analysis of genetic diversity

Genetic diversity is a major indicator reflecting the environmental adaptability of a species, and its analysis can provide guidance for parent selection and genetic breeding [3]. In recent years, DNA molecular marker technology has been widely applied to analyze the genetic diversity of citrus. Li Min et al. [4] adopted AFLP technology to study the genetic diversity of citrus cultivars used for dried tangerine peel and analyzed the genetic background of 46 citrus samples collected from five provinces. A total of 548 DNA bands were amplified, among which 472 were polymorphic bands, with the percentage of polymorphic loci reaching 86.13%, indicating abundant genetic diversity among citrus cultivars for dried tangerine peel. Liu Tong et al. [5] used SSR and SRAP molecular markers to analyze the genetic diversity of 34 wild citrus germplasms and local cultivars from Guangxi. The results showed that SSR and SRAP markers yielded 221 and 215 polymorphic bands respectively, with an average of 10.1 and 19.5 polymorphic bands per primer pair. Clustering based on SSR and SRAP revealed that the genetic similarity coefficients between every pair of the 34 citrus germplasms ranged from 0.78 to 0.96 and 0.60 to 0.92, respectively. Gao Hengjin et al. [6] employed SSR, InDel (insertion-deletion) and lncRNA (long non-coding RNA)-derived molecular markers to assess the genetic diversity of 45 wild, semi-wild and local loose-skin citrus resources from different regions. Thirty-one primer pairs generated 81 marker loci and 280 polymorphic bands, with an average of 9 amplified bands detected per primer pair. The average Shannon's information index and average expected heterozygosity of polymorphic marker loci were 0.651 and 0.385, respectively, demonstrating that some bred cultivars and hybrid citrus resources of loose-skin citrus possess relatively rich genetic diversity. This study verified that wild loose-skin citrus originating from the Nanling Mountains share relatively similar genetic backgrounds and exhibit lower diversity than modern cultivated loose-skin citrus due to the absence of artificial domestication and exogenous gene introgression, which provides scientific evidence for the hypothesis that the Nanling Mountains serve as the origin center of loose-skin citrus in China.

3.2. Cultivar identification

As perennial woody plants, citrus has a long cultivation history. Interspecific hybridization and asexual propagation occur relatively easily, making it often difficult to identify cultivars with similar traits using conventional methods. In contrast, cultivar identification via DNA molecular markers boasts high accuracy, high efficiency and abundant information, and has thus been widely adopted in recent years. Zeng Yanru et al. [7] used RAPD molecular markers to identify 16 citrus cultivars produced in Lishui City, Zhejiang Province. The results revealed differences among these citrus cultivars at the DNA level and confirmed that RAPD markers could be used for citrus identification at the DNA level. Ji Qianhua et al. [8] extracted genomic DNA from local citrus cultivars in Zhaoqing using a modified CTAB method, established an optimal reaction system for RAPD-PCR analysis, and screened a set of primers with high polymorphic amplification bands and good stability for the identification of these local citrus cultivars. Lei Tiangang et al. [9] screened 10 pairs of SSR primers featuring abundant polymorphic loci, clear band patterns and good repeatability across major citrus types. Two efficient primer combinations were determined for pomelo and hybrid citrus cultivars, respectively. Using these two primer sets, characteristic DNA fingerprints were successfully constructed for 5 main pomelo cultivars and 7 main hybrid citrus cultivars widely promoted in China. Gel 2.0, an automatic fingerprint recognition software, was applied to identify the fingerprints of each cultivar and build the corresponding fingerprint database, which provides a rapid and accurate method for citrus cultivar identification.

3.3. Analysis of genetic relationships

In the study of citrus genetic relationships, molecular markers including SSR, AFLP, RAPD and SRAP have been widely adopted in recent years. Wang Fusheng and Jiang Dong [10] used cpSSRs and EST-SSRs combined with morphological evidence to clarify the phylogenetic relationships of citrus and its related

https://doi.org/10.62852/csa/2026/308
Copyright (c) 2026 Cambridge Science Advance

genera, and confirmed that Mangshan wild mandarin, Yuanju and Ichang papeda have close genetic relationships. Yang Haijian et al. [11] carried out cluster analysis on 25 citrus materials including 9 Xiangcheng accessions by SRAP and SSR markers as well as morphological differences of leaves and thorns, to explore the genetic variations among Xiangcheng resources in Sichuan-Chongqing area and the genetic relationships between Xiangcheng and various citrus types. The results showed that the citrus types in order of genetic relatedness to Xiangcheng were mandarin, sweet orange, Ichang papeda, calamondin, lemon, pomelo and trifoliate orange. It was inferred that Xiangcheng might be a hybrid of mandarin and Ichang papeda. Moreover, to identify the parents of Zigui ju orange, Li Junfeng et al. [12] analyzed its genetic relationships using AFLP, cpSSR and nSSR markers combined with ploidy detection and morphological markers. AFLP clustering results showed that Zigui ju orange was genetically intermediate between orange and mandarin, and cpSSR and nSSR markers further verified that the female parent of Zigui ju orange was sweet orange and the male parent belonged to mandarin. Shi Weishu et al. [13] performed PCR amplification on genomic DNA of 24 sweet orange germplasms with ISSR primers. The similarity coefficients among these 24 sweet orange germplasms ranged from 0.815 to 0.983, indicating close genetic relatedness, which was basically consistent with the results reported by Fang et al. [14]. Meanwhile, cluster analysis grouped blood oranges and navel oranges into one cluster, revealing their close genetic relationship and supporting the opinion that blood oranges essentially belong to navel oranges.

3.4. Gene mapping

Gene mapping serves as the prerequisite and foundation for marker-assisted selection breeding and gene cloning, occupying a vital position in genetic research. Xiao Jinping et al. [15] adopted cDNA-AFLP technology with 81 primer combinations to investigate the molecular regulatory mechanism of citrus under drought stress. Seventy differentially expressed TDFs (Transcript-derived fragments) were selected for cloning and sequencing. The results showed that 33 of them shared high homology with known genes, whose functions were involved in signal transduction, energy metabolism, protein synthesis and other processes. Guo Peng et al. [16] took citrus as experimental materials under boron toxicity stress. Using cDNA-AFLP technology and optimizing the reaction system, they obtained electrophoretic profiles with abundant polymorphism and achieved prominent separation of differentially expressed genes, which provides an effective method for screening genes of citrus in response to boron toxicity stress.

In the research on citrus canker, Xiang et al. [17] identified molecular marker loci closely associated with citrus canker resistance based on candidate disease-resistance gene sequences of receptor-like kinases. Peng Zhuchun et al. [18] constructed a resistant segregating population and carried out field resistance tests. Combined with SSR molecular marker technology, they screened and verified molecular markers linked to citrus canker resistance and obtained one marker CCR-110 associated with citrus canker resistance. In addition, Deng et al. [19] used RAPD and SCAR molecular markers to map the region containing the locus for citrus tristeza disease resistance and screened bacterial artificial chromosome libraries harboring this region, which laid a solid foundation for disease-resistant breeding of citrus.

4. Discussion

In recent years, with the rapid development of the citrus industry, biotechnology has played a crucial role in the breeding of new citrus varieties and genetic improvement, among which DNA molecular marker technology occupies a very important position. Nevertheless, for DNA molecular marker technology itself, different types of DNA molecular markers possess distinct advantages, and every single DNA molecular marker has certain limitations. At present, no molecular marker can satisfy all criteria for an ideal molecular marker [1]. Therefore, in future citrus research using DNA molecular markers, appropriate markers should be selected according to experimental objectives and available conditions. Meanwhile, marker methods with higher reliability and efficiency should be actively explored to achieve optimal research results.

In terms of future development trends, the application of DNA molecular marker technology in citrus research will become increasingly extensive. Researchers may focus on advancing the development of new practical molecular markers, the establishment of molecular databases for core germplasm and backbone parents, and the construction of molecular marker-assisted selection systems and high-resolution genetic maps. Meanwhile, as DNA sequencing enters the high-throughput era and the emergence of

https://doi.org/10.62852/csa/2026/308
Copyright (c) 2026 Cambridge Science Advance

third-generation DNA sequencing technology based on single-molecule reading, DNA sequencing will develop toward faster speed and lower cost. This makes it possible to obtain genomic information of biological individuals efficiently, accurately and economically [20], and opens broader prospects for the application of molecular markers in citrus research.

5. Conclusion

With advantages such as high polymorphism, stable detection, and freedom from interference by environments and plant developmental stages, DNA molecular marker technology has become a core technical tool for citrus germplasm resource evaluation, cultivar identification, phylogenetic relationship analysis and functional gene mining. Using various markers including RFLP, RAPD, AFLP, SSR and SRAP, existing studies have clarified the genetic diversity characteristics of citrus germplasms, elucidated the phylogenetic origins and hybrid parental relationships of some citrus resources, realized rapid fingerprint identification of morphologically similar citrus cultivars, and screened molecular loci associated with citrus stress and disease resistance traits. These outcomes provide abundant basic data and technical support for citrus germplasm conservation, parent selection and marker-assisted breeding. Nevertheless, each type of molecular marker has its own drawbacks, and a single marker can hardly meet all research requirements. Reasonable combination of marker types is needed in practical research according to experimental objectives and conditions. With the continuous advancement of high-throughput sequencing and third-generation sequencing technologies, new molecular markers are constantly developed. The molecular database of citrus core germplasms, genetic maps and marker-assisted breeding systems will be gradually improved. In the future, relying on genomic information, DNA molecular marker technology will be further deeply integrated into citrus genetic improvement, facilitating the excavation of elite citrus germplasms and directional breeding of new cultivars, and promoting germplasm innovation and sustainable development of the citrus industry.

6. References

[1] Liu Lili, Li Jianhui, Cheng Haixiang. Application of molecular markers in the study of population historical dynamics[J]. Agricultural Science and Technology Communication, 2014,7:207~211.

[2] Agarwal M, Shrivastava N, Padh H. Advances in molecular marker techniques and their applications in plant sciences[J]. Plant Cell Reports,2008,27:617~631.

[3] Li Aili, Ma Zhiying. AFLP molecular markers and crop improvement[J]. Journal of Hebei Agricultural University,2001,24(1):89~94,112.

[4] Li Min, Liu Rong, Yang Li, et al. Study on genetic diversity of medicinal citrus cultivars for dried tangerine peel based on AFLP technology[J]. China Fruits,2015,2:11~14.

[5] Liu Tong, Deng Chongling, Cheng Yufang, et al. Genetic diversity analysis of citrus germplasms in Guangxi using SSR and SRAP markers[J]. Journal of Huazhong Agricultural University, 2016,35(2):23~29.

[6] Gao Hengjin, Chu Chunrong, Wang Xiaoke, et al. Genetic diversity analysis of wild and local loose-skin citrus resources[J]. South China Fruits,2016,45(2):6~14.

[7] Zeng Yanru, Hu Ying, Si Jinping, et al. RAPD analysis of 16 characteristic citrus cultivars in Lishui, Zhejiang[J]. Journal of Zhejiang Forestry College,2005,22(5): 481~485.

[8] Ji Qianhua, Guo Yanjun, Li Zefeng. Preliminary study on molecular methods for identification of local citrus cultivars[J]. South China Fruits,2011,40(3):6~10.

[9] Lei Tiangang, He Yongrui, Wu Xin, et al. Identification of pomelo and hybrid citrus cultivars using SSR markers[J]. Molecular Plant Breeding,2007,5(5):720~724.

[10] Wang Fusheng, Jiang Dong. Genetic background analysis of special citrus germplasm resources using cpSSR and EST-SSR markers[J]. Acta Horticulturae Sinica,2010,37 (3):465~474.

[11] Yang Haijian, Zhou Xinzhi, Gong Yalin, et al. Genetic diversity and phylogenetic relationships of yuzu resources in Sichuan-Chongqing region[J]. Journal of Fruit Science,2014,31(4): 544~550.

[12] Li Junfeng, Xiang Changhai, Deng Xiuxin. Exploration of the origin of Zigui ju orange, a natural citrus hybrid[J]. Journal of Fruit Science,2009,26(4):425~430.

https://doi.org/10.62852/csa/2026/308
Copyright (c) 2026 Cambridge Science Advance

[13] Shi Weishu, Wang Jiangbo, Li Kaituo, et al. ISSR analysis of 24 sweet orange germplasm resources[J]. Chinese Journal of Tropical Crops,2010,31(6):902~907.
[14] Fang D Q, Roose M L, Krueger R R, et al. Fingerprinting trifoliate orange germplasm accessions with isozymes,RFLPs,and inter-simple sequence repeat markers[J]. TAG Theoretical and Applied Genetics,1997,95(1):211~219.
[15] Xiao Jinping, Chen Junwei, Zhang Huiqin, et al. cDNA-AFLP analysis of gene expression profiles in citrus leaves under drought stress[J]. Acta Horticulturae Sinica,2011,38 (3):417~424.
[16] Guo Peng, Yang Lintong, Chen Lisong. Screening of differentially expressed genes in citrus under boron toxicity by cDNA-AFLP[J]. Zhejiang Citrus,2013,30(1): 18~ 20.
[17] Xiang X, Zheng QF, Huang S, et al. Development of RLK-derived molecular markers associated with the resistance to citrus canker [Xanthomonas axonopodis pv.Citri(Xac)] Disease[J]. Molecular Plant Breeding,2005,3(6):825~828.
[18] Peng Zhuchun, Gong Guizhi, Chen Shanchun, et al. Screening of SSR markers associated with citrus canker resistance[J]. Acta Horticulturae Sinica,2010,37(3):383~389.
[19] Deng Z, Huang S, Ling P, et al. Fine genetic mapping and BAC contig development for the citrus tristeza virus resistance gene locus in Poncirus trifoliate (Raf.) [J]. Molecular Genetics and Genomics,2001,265(4):739~747.
[20] Syed F, Grunenwald H, Caruccio N. Next-generation sequencing library preparation:simultaneous fragmentation and tagging using in vitro transposition[J]. Nature Methods,2009,6(11):1~2

https://doi.org/10.62852/csa/2026/308
Copyright (c) 2026 Cambridge Science Advance