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杨梅分子生物学研究进展

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摘 要:杨梅(*Morella rubra* Sieb. et Zucc.)是我国南方重要的特色水果,其果实色泽鲜艳,营养价值高,深受国内外消费者的喜爱。本文综述了杨梅分子生物学的研究进展,主要包括三个方面:杨梅遗传多样性、杨梅基因组学和杨梅雌、雄性别控制遗传模式,展望了杨梅分子生物学今后的研究方向,旨在为杨梅育种及开发利用提供更多的科学参考。

关键词:杨梅;分子生物学;遗传多样性;基因组学;分子标记

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A review for molecular biology of *Morella rubra*

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Abstract: Red bayberry (*Morella rubra*), is an economically important, subtropical evergreen fruit tree in southern China. The fruit has bright color and high nutritional value which make it popular with the domestic and foreign consumers. The fruit is not only eaten fresh, dried and canned, but is also widely used for making wine and juice. With the development of red bayberry market, more and more problems have emerged. Firstly, the breeding cycle of red bayberry is long, and the breeding method mainly depends on the natural variation and bud mutation. Secondly, red bayberry is not strongly resistant and is easily affected by environmental factors. For example, too much rain in early spring will affect pollen dispersal and reduce the fruit-setting rate. In addition, red bayberry are not covered by the exocarp, and the edible part is soft and easy to be damaged by machinery. Storage and transportation of the fruit after harvest are also unfavorable factors in the production and industrial development of red bayberry. The above problems will seriously inhibit the future development of the red bayberry industry. These problems are closely related to the genetic characteristics of red bayberry. Therefore, it is significant that explore the inherent determinants of the genetic characteristics of red bayberry. The old process of red bayberry breeding was slow and the cycle was long. The genomes are fundamental to molecular biology, genetics and breeding research on Morella species. The completion of red bayberry genome sequencing marks the shift from phenotypic studies to genotype studies, from single-gene studies to genome-wide association analysis. This paper reviews the following three aspects in red bayberry molecular biology: 1. The genetic diversity research progress of red bayberry. DNA molecular marker is a comparatively ideal genetic markers developed after the shape marker, cellular marker and biochemical marker. A

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large number of previous studies about DNA molecular marker in bayberry have provided reliable auxiliary materials for the genetic breeding and molecular research of red bayberry and laid a foundation for the follow-up research. 2. The genome research progress of red bayberry. The publication of the whole genome sequencing results of red bayberry will help us to understand the structure and function of red bayberry, which is a great guiding significance to explore the origin and evolution of red bayberry, clone the important functional genes, and accelerate the process of molecular breeding. At the same time, the genome sequence of red bayberry can be used as a reference for other Myricaceae plants' genome sequencing (The Myricaceae is a small, sub-cosmopolitan family of about 50 species) to accelerate the process of genetic breeding of other plants. 3. The sex differentiation mechanism of red bayberry. In order to explain the phenomenon of male flowers in the female trees of red bayberry and find the genes that determine the sexual differentiation of bayberry, Jia combined with the whole genome sequencing information of red bayberry, sequenced the two mixing pools of 100 female red bayberry and 100 male red bayberry, and resequenced the three female and three male red bayberry respectively, and found that: there is a Female specific region (FSR) with a length of about 59 kb on chromosome (No.8) of red bayberry. It is deduced that the sex determination mechanism of red bayberry is Z/W type. FSR segment contained seven predicted genes, four gene (*MrASP2*, *MrCPS2*, *MrSAUR2* and *MrFT2*) in FSR segment were associated with sexual determination. The researchers believe that the expression level of FSR region gene determines the final direction of sex differentiation of red bayberry. When the gene expression level of FSR region is induced by environmental factors, male flowers of female red bayberry will appear. This study reveals the genetic basis of sex determination of red bayberry and is helpful to the further development of genetic breeding of red bayberry. Finally, we prospected the future development direction of molecular biology research. Future red bayberry molecular biology research mainly revolves around the following aspects: firstly, making full use of the rich information resources of genome-wide of red bayberry, developing and applying new experimental methods, analyzing all the gene function of red bayberry genome at the genome or system level comprehensively, studying the gene expression and its regulation mode to reveal the growth, environmental response interaction molecular network, metabolism and other molecular mechanisms of red bayberry. The gene expression and its regulation pattern were studied to reveal the molecular mechanism of red bayberry growth, development, the environmental response, interaction molecular network and metabolism and so on. Secondly, in the actual production, the red bayberry resistance is poor, so producing with excellent properties such as cold resistance, drought resistance to meet the needs of production is urgent. Therefore, it will also be one of the research directions in the related research fields to study the physiological mechanism of red bayberry under stress, explore anti-stress genes, and obtain transgenic red bayberry with promotion prospects. This could provide new materials for the cultivation, processing and development of medical care products of red bayberry.This study was aimed at providing more scientific references for the breeding and exploitation of red bayberry.

Key words: *Morella rubra*; Molecular biology; Genetic diversity; Genome; Molecular markers

杨梅（*Morella rubra*/*Myrica rubra* Sieb. et Zucc.）是亚热带常绿果树，属于杨梅科（Myricaceae）杨梅属（*Myrica*）小乔木或灌木。英文名字为 Chinese bayberry、Waxberry 或者 redbayberry 等。其性喜温较耐寒，主要生长在我国长江以南和西南地区。一般在年平均温度 15~20 ℃，年降水量 1 000 mm 以上的湿润地区，杨梅树丰产，经济寿命可达 100 a（年）[1]。杨梅作为南方重要的特色果树，在经济、生态、医疗等领域都有非常重要的应用。在经济方面，杨梅树易于栽培，生产成本低，被人们称为“绿

色银行”“摇钱树”[2]。杨梅果实为果中珍品，风味独特，色泽诱人，可鲜食，也可制作果汁、蜜饯、果酱和果酒等[3]，深受消费者的青睐。杨梅产业的发展，吸引国内外商客纷至沓来，带动了种植区经济水平的提高，据统计浙江省仙居县2018年杨梅产量9万t，产值高达6.67亿元[4]；2019年浙江省杨梅产值更是超过50亿元[5]。在生态方面，杨梅树耐旱、耐瘠，省工省肥，非常适合在山地进行退耕还林改造，是保持生态环境的理想树种[6]。据统计，截至2017年，重庆市杨梅种植面积达3 720 hm^2，种植区主要分布在万州、渝北、涪陵和南川等区县，占重庆市杨梅种植总面积的90%以上[7]，为当地带来经济效益的同时，也发挥着非常重要的生态效益。在医药方面，杨梅果实富含维生素[8]、膳食纤维[9]等营养物质以及人体所必需的8种氨基酸和17种矿物元素，堪称“百果之王”[10]。近年来，杨梅中的酚类化合物[11]因具有抗氧化[12]、抗糖基化[13]、改善高血糖[14-15]等功能而备受关注，其酚类化合物的主要来源包括花青素、酚酸、黄酮醇[16]。随着杨梅生产与市场的发展，越来越多的问题显现出来：首先杨梅的育种周期较长，育种方式主要是依赖于植株自身的实生变异和芽变；其次是杨梅果实无外果皮包被，其可食用部分柔软，易受机械损伤，采后果实品质劣变迅速，极不耐贮运[17]。这些问题都与杨梅的遗传特性密切相关，因此，探讨杨梅遗传特性的内在决定因素，对发展杨梅产业具有重要的理论指导意义。分子生物学技术的不断完善与发展，为杨梅的育种改良提供了新的思路和有利工具。目前，对杨梅的全基因组测序已经完成，这对认识杨梅的遗传机制从而进行深度开发利用具有重要意义。笔者基于目前取得的研究成果，总结了杨梅分子生物学最新的研究进展，并探讨了未来杨梅分子生物学的发展方向和研究重点。

1 杨梅分子标记

遗传多样性（Genetic diversity）是指物种携带的遗传信息总和，对物种的遗传、进化和变异具有决定性作用。一个物种遗传多样性越高，则其对不良环境的适应力越强[18]。对杨梅遗传多样性的相关研究，有助于发掘出更多优异的种质资源，更有效地指导杨梅生产和育种工作。分子标记作为遗传多样性研究的辅助材料，现已广泛应用于分子生物学各领域中，因其直接以DNA的形式表现，具有不受环境条件和发育阶段影响、标记数目多、多态性高等优点，是一种较为理想的遗传标记。目前在杨梅中应用的分子标记有：扩增片段长度多态性(Amplified fragment length polymorhism，AFLP)、相关序列扩增多态性（Sequence-related amplified polymorphism，SRAP）、简单重复序列(Simple sequence repeats，SSR)、单核苷酸多态性(Single nucleotide polymorphism，SNP)等（表1）。杨梅分子标记研究集中于特异性引物开发、遗传多样性检测和系统进化等方面。林旗华等[19]利用SRAP标记技术对18份杨梅种质资源进行遗传多样性分析发现：部分来源相同的杨梅种质资源遗传关系较近，但杨梅资源的亲缘关系与地理来源相关性不甚明显。Zhang等[20]使用AFLP标记对100份杨梅进行遗传多样性分析，得出杨梅品种遗传聚类与地理因素关系密切的结论。陈慧[21]等利用SRAP标记技术对66份杨梅种质进行遗传多样性分析，再次验证了杨梅品种间亲缘关系与地理分布及来源密切相关。以上实验分析均从不同角度证明了杨梅品种的亲缘关系与地理分布关系密切，为进一步对杨梅群体遗传结构和系统进化的研究提供了依据。

表1 杨梅分子标记研究

Table 1 Research on molecular markers in Morella rubra

标记类型 Type of marker	引物组 Number of primer set	样本数 Number of sample	参考文献 Reference
SRAP	8	18	[19]
AFLP	6	100	[20]
SRAP	57	66	[21]
SSR	11	100	[24]
SSR	11	100	[25]
SSR	13	32	[26]
SSR	14	122	[27]
SSR	158	32	[28]
SSR	107	45	[29]
SSR	89	83	[30]
SNP, SSR	1 132, 38	103	[31]
SSR	109	10	[32]
SSR	59	14	[33]

SSR标记是目前杨梅遗传多样性研究中的主流分子标记，具有信息量大、重复性好、多态性高和易检测等优点[18]。朱长青[22]构建了杨梅果实4个成熟度的cDNA文库，并分别从4个cDNA文库中随机挑取500个克隆进行测序，获得了2 000个EST

序列，首次构建了杨梅果实 EST 文库。对 EST 文库进行分析，可以鉴定和分析基因功能和表达模式。由于 SSR 标记在 EST 中出现频率远远高于全基因组 DNA，因此利用 EST 数据开发 SSR 标记，是进行构建 EST 文库的另一个重要目标[23]。张水明[24]、Zhang 等[25]通过筛选杨梅果实 EST 库开发了 11 对 SSR 标记，为杨梅遗传多样性的研究提供了基础材料。Terakawa 等[26]从基因组文库中开发出 13 对杨梅 SSR 标记，该研究对分析杨梅的物种起源具有较高的价值。Xie 等[27]利用 14 对 SSR 标记对 122 个杨梅品种进行遗传多样性分析，进一步明确了杨梅品种间的亲缘关系。Jiao 等[28]在对杨梅全基因组进行测序工作中开发了 158 对 SSR 标记，可用于构建杨梅连锁图谱和遗传多样性分析。Jia 等[29]基于杨梅全基因组鸟枪法测序数据，开发了 107 个 SSR 标记，并应用于品种间遗传多样性分析及品系鉴定。沈禹彤[30]利用 89 个 SSR 标记构建了杨梅‘荸荠’×‘东魁’的遗传框架图谱，图谱总长 415.6 cM，标记间平均距离 5.1 cM 。贾慧敏[31]利用转录组测序（RAD-seq）技术对杨梅‘荸荠’×‘东魁’亲本及 101 个 F1 代个体进行高通量测序，对前期获得‘荸荠’×‘东魁’SSR 遗传框架图进行加密。亲本及 F1 代测序深度分别为 14X、22 X 和 8X。构建的‘荸荠’×‘东魁’高密度遗传连锁图谱包含了 1 132 个 SNP 和 38 个 SSR 标记，图谱总长 563.01 cM，标记间平均图距为 0.48 cM。该研究为杨梅基因组学研究、基因图位克隆、QTL 定位和分子标记辅助选择奠定了基础。在杨梅的研究中，SSR 标记不足以满足分子育种研究的发展需要，Zhang 等[32]从杨梅转录组序列中开发了 109 对 EST-SSR 标记（eSSR）；张淑文[33]等开发出 59 对基于基因组序列开发的基因组 SSR 标记（gSSR）。以上研究为杨梅遗传育种和分子研究提供了可靠的辅助材料并为后续研究工作奠定了基础。

2　杨梅基因组学

基因组（Genome）指生物整套染色体所含有的全部 DNA 序列，即一个物种所有遗传信息的总和。为了解生物的结构、组成、功能及其进化规律，进行了大量模式植物的基因组测序工作。随着测序技术的发展和测序成本的降低，非模式植物基因组学的研究成果层出不穷，很多果树基因组陆续被测出，如葡萄（*Vitis vinifera* L.）[34]、番木瓜（*Carica papaya* L.）[35]、苹果（*Malus domestica* Borkh.）[36]、草莓（*Fragaria ananassa* Duch.）[37]、海枣（*Phoenix dactylifera* L.）[38]、可可（*Theobroma cacao* L.）[39]、香蕉（*Musa* spp.）[40]、梨（*Pyrus bretschneideri* Rehd.）[41]、甜橙（*Citrus sinensis* (L.) Osbeck）[42]等，这些果树全基因组测序研究为其他果树作物全基因组测序研究提供了大量的参考信息。

杨梅为二倍体（2*n*=16）雌雄异株植物，因此杨梅的雌、雄种质及不同品种在分子标记上存在重要差异，这为杨梅的分子生物学研究提供了重要信息[43]。杨梅基因组测序工作始于 2012 年，Jiao 等[28]通过对杨梅雄株材料‘C2010-55’进行鸟枪法测序，共获得 9.01 Gb 原始数据，覆盖深度为 26 X，通过 K-mer 分析得出杨梅基因组大小为 323 Mb，约为拟南芥基因组（125 Mb）大小的 2.6 倍，其基因杂合度较高，重复序列含量较低，组装获得 255.7 Mb 序列。但此次测序的主要目的是开发 SSR 标记，测序深度及拼接结果（技术限制）均不理想。与杨梅基因组测序同时开展的还有杨梅转录组学和蛋白组学的研究，Feng 等[44]通过对杨梅品种‘荸荠’的茎、叶、花和果的不同组织混合样以及果实 3 个发育时期进行 RNA-seq 测序，共获得 1.92 Gb 原始数据，得到 41 239 个 UniGene，其中 32 805 个可以比对到蛋白库，在果实成熟过程中有 826 个 UniGene 上调伴随着 1 407 个 UniGene 的下调，实验还分析了相关基因在花青苷代谢途径和蔗糖、柠檬酸合成途径中的表达模式，揭示了杨梅果实成熟的分子机制，为进一步探索杨梅果实发育过程提供了理论依据。戚行江等[45]对杨梅基因组进行了重新测序，共得到 13.70 Gb 原始数据，覆盖深度为 45.01 X，数据评估分析得出杨梅基因组大小为 304.38 Mb，GC 含量 37.99%，组装后获得 289.92 Mb 序列。基因重复序列占基因组 45.82%，说明杨梅的基因组属于中等复杂程度。贾慧敏[31]利用二代和三代基因组测序技术对杨梅雌株材料‘Y2012-145’进行测序，共获得 9.6 Gb 原始数据，测序覆盖深度 307 X，通过 K-mer 分析得出杨梅基因组大小为 322.7 Mb，和雄株基因组（323 Mb）大小相似，GC 含量 36.9%，组装获得 255.7 Mb 序列。在杨梅基因组中共检测出 29 414 条基因，其中可注释到的基因占 89.47%。重复序列占基因组 35.38%，其中转座因子占重复序列的

95.6%，转座子通过 DNA-DNA 方式或以 RNA 为介导实现转座，转座因子所占比例较大，这说明转座子转座是导致杨梅基因组加倍的主要原因。

Jia 等[46]采用二代 Illumina 双末端测序结合三代 PacBio 测序进行杨梅雌株‘Y2012-145’、雄株‘H2011-12’两个品系的全基因组测序，结果显示（表 2）：杨梅基因组大小为 320 Mb，组装基因组大小 313 Mb，占预测基因组大小的 97%，预测出蛋白编码基因 32 493 个。雌性植株‘Y2012-145’，Illumina 测序深度为 278 X，PacBio 测序深度为 15 X；雄性植株‘H2011-12’，Illumina 测序深度为 48 X，PacBio 测序深度为 61 X；最后用 HABOT 软件组装，雌雄两个杨梅树的基因组都达到了 313 Mb，覆盖了杨梅基因组（323 Mb）的 96.9%。结合遗传图谱分析，锚定到染色体水平得到最终的雌雄个体基因组大小分别是 280 Mb（90%）和 264 Mb（84%），说明实验组装效果很好。杨梅利用传统育种手段进程缓慢，周期较长，杨梅全基因组测序结果的公布将有助于了解杨梅的基因组结构和功能，对探索杨梅的起源与进化、开展重要功能基因的定位和克隆、加速分子育种进程等都具有重要的指导意义。同时，杨梅的基因组序列可作为其他杨梅科植物基因组测序的参考，加快推动其他植物的遗传育种进程。

表 2 杨梅基因组测序的组装结果

Table 2 The assembly results in *Morella rubra*

类别 Categories	雌性 Female	雄性 Male
基因组大小（K-mer分析）Estimated genome size/Mb	322.70	319.20
N50（Scaffold）/ Mb	1.60	2.00
组装基因组大小 Total assembled size/Mb	312.60	313.50
杂合度 Heterozygosity/%	0.56	0.72

3 杨梅雌、雄性别控制遗传模式

杨梅为雌雄异株，偶有雌雄同株，有的杨梅品种存在雌株雄性化的现象[47]。雌株杨梅的雌花为总状花序，在结果枝上每节叶腋处着生一个花穗；雄株杨梅的雄花为柔荑花序，着生于叶腋，一个雄花枝上一般有 10~20 个雄花穗[48]（图 1）。在自然界中，雌雄异株植物所占比例较少，但却是植物性别决定机制及遗传、演化模式的重要研究材料。通过分子生物学技术分离性别决定的相关基因是揭示雌雄异株植物性别决定的关键问题之一[49]。

a. 雌花；b. 雄花。

a. Female; b. Male.

图 1 杨梅雌、雄花形态

Fig. 1 Flower morphology of Chinese bayberry

我国早在 1995 年就启动了对杨梅性别决定机制的研究，但由于当时实验设备和测序水平的限制，只对杨梅雌雄株的性别控制遗传模式进行了初探。李国梁等[50]研究发现，杨梅雌雄株的过氧化物酶同工酶在快带区存在差异并表现出规律性；液相色谱图中，杨梅雌雄株不仅表现出共同的物种峰，而且呈现出 3 个性别差异峰；在水溶性酚类总量上，雌株总是大于雄株，该实验揭示出杨梅雌雄株存在差异与性别相关。Jiao 等[28]对杨梅雌株突变雄花序现象进行分析，推断杨梅性别决定机制可能是由微小开关控制的。贾慧敏[31]对 113 份杨梅雌株和 99 份杨梅雄株进行 STRUCTURE 和 Neighbour-joining 聚类分析，结果一致：杨梅材料被划分为两个亚群，分别为雌株（Female dominated）和雄株（Male dominated）。并利用 84 对 SSR 标记分析比较了 212 份杨梅材料在遗传多样性水平上的差异，雌株群体显示出较高的遗传多样性，并发现 2 个 SSR 位点（ZJU062 和 ZJUl30）的 Fst 值为 0.455 和 0.333，这两个位点导致雌雄性别群体的遗传结构分离，因此推断这两个 SSR 位点与杨梅的性别决定机制相关。以上实验，从不同角度出发初探了杨梅雌、雄性别控制遗传模式，为后续实验提供了相关信息。

随着杨梅全基因组测序的完成，开启了进一步探索杨梅雌、雄性别决定机制的篇章。为解释杨梅雌树开雄花的现象并找到决定杨梅性别分化的基因，Jia 等[46]结合杨梅全基因组测序信息，分别对 100 个雌性杨梅和 100 个雄性杨梅的两种混池进行测序，对 3 个雌性杨梅和 3 个雄性杨梅进行重测序分析，发现：在杨梅的 8 号染色体上存在一段长约 59 kb 的雌性杨梅特有片段（Female specific region，FSR），

由此推导出杨梅的性别决定机制为Z/W型。在该决定机制中，雄性（ZZ）为纯合子，雌性（ZW）为杂合子。FSR区段包含7个预测的基因，其中4个基因（*MrASP2*、*MrCPS2*、*MrSAUR2*和*MrFT2*）与雌雄花性别决定相关；同时还在8号染色体上发现这些基因的旁系同源位点，这表明FSR区域可能来源于同源基因的复制和重排。研究者认为FSR区域基因的表达水平决定了杨梅雌雄性别分化的最终方向；当FSR区域基因表达水平受到环境因素的诱导时，就会出现雌性杨梅开雄花的现象。该研究揭示了杨梅性别决定的遗传基础，有助于杨梅遗传育种研究工作的深入开展。

4　结　语

杨梅基因组测序的完成，标志着杨梅研究从依赖表型转向基因型，从单一基因转向全基因组关联分析。但目前尚有很多不足，在科研方面，因杨梅为小宗水果，科研力量的投入较大宗水果还相对薄弱；又因其为核果类果树，该类果树的共性问题是高效稳定的遗传转化体系一直未能建立[51]。在生产方面，杨梅存在坐果率受环境影响较大[52]，育种周期长，供应时间短，果实不耐贮等问题。

未来杨梅的分子生物学研究将主要围绕以下几方面展开：第一是充分利用杨梅全基因组丰富的信息资源，积极发展并应用新的实验手段，在基因组或系统水平上全面分析杨梅基因组中全部基因功能，研究其基因的表达及其调控模式，以揭示杨梅生长发育、环境应答互作分子网络、代谢等分子机制；第二是在实际生产中，培育出抗寒、抗旱、抗虫等优良性状的杨梅品种来满足市场需求，研究杨梅的逆境生理机制并发掘抗逆基因，构建杨梅遗传转化体系，获取具有推广前景的转基因杨梅，为杨梅的种植、加工和医疗保健品开发提供新材料。

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