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草莓分子标记技术发展与应用

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摘　要　栽培草莓(*Fragaria*×*ananassa*)为八倍体多年生草本植物，具有复杂的遗传背景，多数农艺和品质性状均为多基因控制，为草莓的遗传育种工作带来了挑战。DNA 分子标记直观反映基因组的差异和变化，对于遗传高度杂合、生产周期长的多年生果树来说，能够有效提高育种效率。近年来，分子标记技术在果树辅助育种、基因定位、遗传图谱构建、QTL 定位、品种鉴定和基因组研究等方面应用广泛，有力地推动了果树分子育种的发展。本文综述了分子标记技术在草莓生产育种中的研究应用现状，以期为后续的研究提供参考，推动草莓的生产和育种。

关键词　草莓，分子标记，基因定位，遗传图谱，QTL 定位，遗传多样性
中图分类号　S603.6　　**文献标识码**　A

Development and Application of Molecular Marker Technique in Strawberry (*Fragaria*×*ananassa*)

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Abstract　The cultivated strawberry, *Fragaria*×*ananassa* (2n=8x=56), is an octaploid perennial herb plant with complicated genetic background. Most agronomic and quality traits of strawberry are controlled by polygenes, resulting in difficulty in breeding. DNA molecular markers showed the genome variances and mutants directly, so it had been widely used in marker associated breeding, gene mapping, genetic map construction, QTL mapping, cultivar identification and genome research since 1990s. And it greatly improved the efficiency of breeding, especially for the perennial fruit trees which were highly heterozygous and had long growth period. In this paper, we reviewed the current status of the applications of molecular marker techniques in strawberry breeding and production, aiming to provide foundations for further studies and push forward strawberry breeding and the whole industry.

Keywords　*Fragaria*×*ananassa*, Molecular marker, Gene mapping, Genetic map, QTL mapping, Genetic diversity

栽培草莓(*Fragaria*×*ananassa*, 2n=8x=56)为八倍体多年生草本植物，是重要的经济作物，在世界范围内广泛栽培。联合国粮农组织(Food and Agriculture Organization, FAO)统计显示，2016 年世界

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草莓总产量约为912万吨。我国草莓总产量约为380万吨，占世界草莓总产量的41.7%，为草莓生产第一大国(FAOSTAT 2016. www.faostat3.fao.org)。栽培草莓起源于智利草莓(*F. chiloensis*)和弗州草莓(*F. virginiana*)的偶然杂交(Staudt, 1962)，为异源八倍体，遗传背景复杂，相较于二倍体作物其遗传育种研究具有一定难度。目前，普遍认为栽培草莓的基因组组成为AAA'A'BBB'B'(Bringhurst, 1990)，起源于多个野生二倍体草莓种，包括森林草莓(*F. vesca*)，饭沼草莓(*F. iinumae*)，绿色草莓(*F. viridis*)，布哈拉草莓(*F. bucharica*)和五叶草莓(*F. pentaphylla*)等(Senanayake, Bringhurst, 1967; Davis, Yu, 1997; Davis et al., 2006)。同时研究发现，栽培草莓中许多分子标记呈现二倍化分离(Kunihisa et al., 2005; Rousseau-Gueutin et al., 2008; Sargent et al., 2009)，简化了栽培草莓的基因组研究。因此，从二倍体草莓入手，利用分子标记等技术，可能是解析栽培草莓复杂基因组、遗传规律、性状调控的有效途径。

分子标记技术，以DNA一级序列的多态性为基础，遗传稳定，不受外界环境影响，位点丰富，多态性高(Agarwa et al., 2008)，尤其在遗传背景复杂，高度杂合的果树研究中具有显著优势。在果树的品种鉴定(Larsen et al., 2017)、遗传多样性分析(Cipriani et al., 2006)、基因定位(Sun et al., 2012)、遗传图谱构建(Sun et al., 2015)、辅助育种(Chagné et al., 2016)等方面得到了广泛的应用，有力地推动了果树分子育种与生物技术研究。草莓相比于苹果(*Malus×domestica*)、桃(*Prunus persica*)等蔷薇科其他果树，具有多倍化特点，国内研究起步较晚，图谱构建、QTL定位等研究鲜有报道。本文综述了分子标记技术在草莓基因定位、遗传图谱构建、农艺品质QTL定位以及品种鉴定、基因组结构、遗传进化等方面的研究现状，以期为我国的草莓遗传育种工作提供参考，推动我国草莓产业和科研的发展。

1 分子标记类型与开发

基于分子杂交、PCR以及基因组测序等技术，已经建立了多种分子标记技术体系(表1)。在草莓研究中，以随机扩增多态性DNA(random amplified polymorphic DNA, RAPD)，扩增片段长度多态性(amplified fragment length polymorphism, AFLP)，序列特征扩增区域(sequence characterized amplified region, SCAR)，简单重复序列(simple sequence repeats, SSR)和单核苷酸多态性(single nucleotide

表1 已建立的分子标记技术

Table 1 Established molecular marker techniques

缩写 Abbreviation	全称 Full name	参考文献 Citation
RFLP	限制性片段长度多态性 Restriction fragment length polymorphism	Botstein et al., 1980
SSCP	单链构象多态性 Single strand conformation polymorphism	Orita et al., 1989
RAPD	随机扩增多态性DNA Random amplified polymorphic DNA	Williams et al., 1990
AP-PCR	随机引物PCR Arbitrarily primed-PCR	Welsh, McClelland, 1990
SCAR	序列特征扩增区域 Sequence characterized amplified region	Williams et al., 1991
SSR	简单序列重复 Simple sequence repeats	Hearne et al., 1992
CAPS	酶切扩增多态性序列 Cleaved amplified polymorphic sequence	Lyamichev et al., 1993
STS	序列标签位点 Sequence tagged sites	Fukuoka et al., 1994
ISSR	简单重复序列间隔 Inter-simple sequence repeats	Zietkiewicz et al., 1994
AFLP	扩增片段长度多态性 Amplified fragment length polymorphism	Vos et al., 1995
RBIP	基于反转录转座子的扩增多态性 Retrotransposon-based amplified polymorphism	Flavell et al., 1998
IRAP	反转录转座子间扩增多态性 Inter-retrotransposon amplified polymorphism	Kalendar et al., 1999
SNP	单核苷酸多态性 Single nucleotide polymorphism	Sachidanandam et al., 2001
SRAP	相关序列扩增多态性 Sequence-related amplified polymorphism	Li, Quiros, 2001
GSM	基因特异标记 Gene-specific markers	Sargent et al., 2007
DArT	多样性阵列技术标记 Diversity Array Technology (DArT) markers	Sánchez-Sevilla et al., 2015

polymorphism, SNP)五种分子标记技术应用最多。近十年来，基于稳定检测、操作简便和高通量等方面的要求，分子标记研究则主要采用SSR和SNP标记。SSR标记在基因组中数量丰富、重复性好且为共显性遗传，在育种应用中有很大的优势，可用于群体的连锁分析、图谱构建、图位克隆等。SNP标记蕴藏的遗传信息更为广泛，可通过质谱、高分辨率溶解曲线(high-resolution melting, HRM)、测序等手段检测(Mahoney et al., 2016; Lee, Lee, 2017; Vining et al., 2017)，具有快速、高通量的特点，适应于大群体、多标记的育种研究。

对于RAPD、AFLP等依赖随机引物和限制性内切酶的分子标记，开发时参照已经发表的方法，选用适宜的随机引物和内切酶组合即可。在草莓品种‘Ever Berry’和‘Toyonoka’中，利用175对随机引物开发RAPD标记，其中71对引物获得的89条具有重复性和多态性的扩增条带可用于后续的群体连锁关系研究(Sugimoto et al., 2005)。参考Vos等(1995)的方法，通过*Eco*RⅠ和*Mse*Ⅰ酶切，产生了69对AFLP引物，在草莓杂交组合‘Tribute’×‘Honeoye’的127株F_1实生苗中进行扩增，获得了1 065条多态性片段，可作为标记进行遗传图谱构建(Weebadde et al., 2008)。由于特异性和转用性方面的局限，在不同草莓群体和品种中均开发了RAPD和AFLP标记，有些还通过测序转化为序列特异的SCAR标记(Haymes et al., 1997; Haymes et al., 2000; Lerceteau-Köhler et al., 2003; Lerceteau-Köhler et al., 2005)。

SSR标记开发需要已知的DNA序列，因此最初的SSR标记都来自基因组文库构建。利用森林草莓‘Ruegen’的基因组文库和森林草莓的AC富集基因组文库，分别获得了21和45个SSR标记(Hadonou et al., 2004; Cipriani et al., 2006)。利用野生八倍体弗州草莓的基因组文库，获得了8个SSR标记，通过群体验证发现这些标记呈高度的二倍化分离，在弗州草莓、智利草莓和栽培草莓3个八倍体种间转用效率高(Ashley et al., 2003)。通过草莓品种‘Strawberry Festival’的cDNA文库构建，获得了208个SSR标记，可在蔷薇科基因组数据库(Genome Database for Rosaceae, GDR)(https://www.rosaceae.org/)中下载引用(Folta et al., 2005)。为了使SSR标记的应用更具目的性，Bombarely等(2010)利用草莓品种‘Carisma’、‘Chandler’以及‘Elsanta’等不同发育时期的果实不同部位构建cDNA文库，结合RNA测序，挖掘383个SSR位点，应用于遗传及分子生物学研究。随着EST等公共数据库的发展，SSR标记开发的成本显著降低，且更加简单易行，标记数量大幅提升(Chen et al., 2006)。Zorrilla-Fontanesi等(2011b)利用ESTs数据库，开发SSR标记122个。2013年，从ESTs数据库中获得草莓SSR标记4 349个(Isobe et al., 2013)。随着全基因组测序的发展，标记覆盖范围更加广泛，利用二倍体森林草莓‘Hawaii 4’的基因组序列，获得了10 071个SSR标记(Sargent et al., 2011)。此外，由于SSR标记具有通用性，不同物种间同源保守序列区域的SSR标记可以相互转用，这也是草莓SSR标记的一个来源(Gasic et al., 2009)。

SNP标记的开发则依赖于高通量测序和大数据库。森林草莓‘Hawaii 4’全基因组序列的公布(Shulaev et al., 2011)、GDR等大型基因组数据库的不断丰富以及草莓转录组测序、基因组重测序的进行，是草莓大量SNP标记开发的基础。利用草莓果实不同发育时期的RNA测序序列，获得372个SNP位点(Bombarely et al., 2010)。Bassil等(2015)对二倍体饭沼草莓和19个八倍体品系进行测序，与‘Hawaii 4’参考基因组比对，过滤后集成了一张包含95 063个SNP标记的芯片(90 K Affymetrix® Axiom® array)，可以广泛应用于草莓遗传图谱构建、品种鉴定和种群分析等领域，显著提升了检测通量和效率。兴起的子代测序(next-generation sequencing, NGS)也是SNP开发的策略之一，无需基因组信息，可以同时实现标记开发和子代基因分型，适用于大群体的图谱构建和性状关联分析(Davey et al., 2011)。酶切测序(restriction site-associated DNA sequencing, RADseq)作为NGS策略的一种(Miller et al., 2007)，已经应用于果树(Sun et al., 2015)，草莓中采用该技术获得了1 098个SNP标记，并将907个定位在了遗传图谱上(Davik et al., 2015)。

2 分子标记与基因定位

分子标记与生物学形状的连锁分析，是分子标记应用的方向之一。通过杂交群体的标记分型以及表型数据的调查，计算遗传交换率，确定标记与性状的连锁关系。紧密连锁标记可用于分子标记

辅助育种，进行目标性状的早期筛选；也可通过标记锚定基因组位置，进行相关基因的挖掘和图位克隆。果树中，基因定位的主要方法有集群分离分析法(bulked segregent analysis, BSA)，又称近等基因池法(Nandi et al., 1997)和主基因连锁分析法(Sun et al., 2012)。目前，研究获得了许多与草莓抗性和开花特性等性状连锁的分子标记(表2)。

在草莓抗病性基因定位方面，主要病害均获得了与抗性基因连锁的分子标记。利用‘Md683’×‘Senga Sengana’杂交群体，获得了4个与红中柱根腐病抗性基因(Phytophthora fragariae resistance gene, *Rpf1*)连锁的RAPD标记(表2)，并将其中的1个转化为SCAR，遗传距离为3.0 cM(Haymes et al., 1997; Haymes et al., 2000)。与炭疽病抗性基因(Anthracnose resistance gene, *Rca2*)连锁的分子标记为STS-Rca2_417和STS-Rca2_240，在43个抗感品种中的验证结果显示，验证率分别为81.4%和62.8% (Lerceteau-Köhler et al., 2005)。利用‘达赛莱克特’×‘甜查理’群体，获得了与灰霉病抗性和白粉病抗性相连锁的SSR标记(曹娴等, 2011; 刘建成等, 2012)。

四季结果性状或者日中性性状因其能够实现草莓的周年生产，在草莓研究中备受关注。因此，开展了许多与开花性状相关的基因定位研究。在森林草莓中，获得了3个与单季开花基因连锁的SCAR标记，其中SCAR2与单季开花基因间的遗传距离为0.0 cM，定位在连锁群相同位置(Albani et al., 2004)。栽培草莓中，Sugimoto等(2005)首先筛选出了2个与四季结果性状连锁的RAPD标记，但遗传距离较远。Castro等(2015)获得了3个与日中

表2　草莓性状连锁标记

Table 2　Markers linked to specific trait in strawberry

性状 Traits	群体 Population	标记类型 Marker type	连锁标记 Linked markers	遗传距离/cM Genetic distance	参考文献 Citations
红中柱根腐病抗性基因*Rpf1* Phytophthora fragariae resistance gene *Rpf1*	Md683×Senga Sengana	RAPD	OPO-08A	1.7	Haymes et al., 1997
			OPO-16A	3.0	
			OPO-16B	3.0	
			OPO-16C	3.0	
		SCAR	SCAR-R1A	3.0	Haymes et al., 2000
炭疽病抗性基因*Rca2* Anthracnose resistance gene *Rca2*	Capitola×Pajaro	SCAR	STS-Rca2_417	0.6	Lerceteau-Köhler et al., 2005
			STS-Rca2_240	2.8	
灰霉病抗性 Gray mold resistance	达赛莱克特×甜查理 Darselect×Sweet Charlie	SSR	UFFxa01H05	15.9	曹娴等, 2011
白粉病抗性 Powdery mildew resistance	达赛莱克特×甜查理 Darselect×Sweet Charlie	SSR	FSS50	1.3	刘建成等, 2012
			FSS121	3.3	
单季开花 Seasonal flowering	*F. vesca* ssp. *vesca*× *F. vesca* ssp. *semperflorens*	SCAR	SCAR1	3.0	Albani et al., 2004
			SCAR2	0.0	
			SCAR3	1.7	
四季结果 Everbearing	Ever Berry×Toyonoka	RAPD	OPE07-1	11.8	Sugimoto et al., 2005
			OPB05-1	15.8	
	Sagahonoka×Sunmmer berry	SSR	FxaACA02I08C	1.1	Honjo et al., 2016
	Sagahonoka×Ever berry		FxaACA02I09C	1.5	
	Hecker×Sagahonoka		FxaACA02I10C	1.1	
日中性 Day-neutrality	Tribute×Honeoye	SSR	ChFaM011-163T	6.8	Castro et al., 2015
			ChFaM148-184T	5.5	
			CX661225-335TH	8.4	
果实黄色 Yellow fruit colour	DNIC×Yellow Wonder	GSM	F3H	0.0	Deng, Davis., 2001
	Yellow Wonder×FRA520	RAPD	B191A	0.0	

性性状连锁的SSR标记，遗传距离显著缩小。2016年，利用3个杂交群体，将标记与四季结果性状的遗传距离缩短至1.5 cM以内，3个SSR标记在群体中的验证率高达98%(Honjo et al., 2016)，可用于辅助育种。

草莓品质性状的基因定位研究较少，果实黄色性状的定位结果显示，在二倍体草莓群体‘DNIC’×‘Yellow Wonder’和‘Yellow Wonder’×‘FRA520’中，基因标记F3H和RAPD标记B191A与果实黄色性状紧密连锁，遗传距离为0.0 cM，定位在连锁群同一位置(Deng, Davis, 2001)。

3 遗传图谱构建

遗传连锁图谱基于分子标记间的连锁关系，呈现标记在染色体上的线性关系，是数量性状QTL定位的基础，也是辅助基因组拼接，研究染色体结构变异的工具，在果树分子育种研究中起重要作用。

由于栽培草莓高度杂合，遗传背景复杂，草莓的遗传图谱构建工作率先在二倍体野生草莓中开展，已发表了具有不同二倍体草莓种系谱的多张遗传图谱(表3)。第一张二倍体草莓遗传图谱发表于1997年，利用森林草莓‘Baron Solemacher’×‘WC6’的F_2群体，共有80个RAPD标记定位在7个连锁群(linkage group, LG)上，图谱总长445 cM(Davis, Yu, 1997)。二倍体草莓的参考图谱，利用‘*F. vesca* 815’×‘*F. nubicola* 601’(后被更正为*F. bucharica* 601)的F_2群体，历经4次修正完善，最终包含411个标记位点，7个LGs，全长442.8 cM(Sargent et al., 2004; Sargent et al., 2006; Sargent et al., 2008; Sargent et al., 2011)。具有绿色草莓系谱的‘815’×‘903BC’群体的遗传图谱发表于2006年，仅含有33个标记位点(Nier et al., 2006)。随着技术发展，利用SSR荧光检测技术，NGS和SNP芯片分型技术，构建了饭沼草莓和森林草莓的遗传图谱，在标记数目、图谱密度以及构图效率等方面均都有了很大的突破(Mahoney et al., 2016; Samad et al., 2017)。

八倍体栽培草莓的遗传图谱构建与二倍体野生草莓稍有不同，主要利用F_1杂交群体，通常会分别构建双亲及整合图谱。同时，连锁群数目变化较大，只有极少图谱能够获得与单倍型相一致的28个连锁群(表4)，可能与部分亚基因组研究不足，标记的丰富度缺乏有关。栽培草莓的首张遗传图谱发表于2003年，主要采用AFLP，STS和SCAR标记，分别构建了双亲图谱(Lerceteau-Köhler et al., 2003)。此后，栽培草莓遗传图谱构建发展迅速，发表了多个群体的遗传图谱，且标记类型逐渐转变为以SSR和SNP为主(Isobe et al., 2013; Davik et al., 2015; Castro, Lewers, 2016; Lee, Lee, 2017)。有5个群体的遗传图谱都开展了延续性研究，对标记类型、标记数目进行了更新和补充，提升了应用效率和价值(Lerceteau-Köhler et al., 2003; Rousseau-Gueutin et al., 2008; Sargent et al., 2009; Sargent et al., 2012; Weebadde et al., 2008; Castro et al., 2015; Zorrilla-Fontanesi et al., 2011a; Sánchez-Sevilla et al., 2015; van Dijk et al., 2014; Bassil et al., 2015)。测序技术的发展以及大数据时代的到来，结合标记分型检测技术的不断进步，草莓高密度遗传图谱也陆续发表(Isobe et al., 2013; Nagano et al., 2017)，这些图谱将在QTL定位，性状候选基因挖掘等研究中，发挥更大的作用。

表3 二倍体草莓遗传图谱

Table 3 Genetic linkage maps of diploid strawberry species

作图群体 Mapping population	标记类型 Marker type	位点数目 No. of loci	连锁群数 No. of linkage groups	图谱总长/cM Map length	参考文献 Citation
Baron Solemacher×WC6, F_2	RAPD	80	7	445	Davis, Yu., 1997
F. vesca 815×*F. nubicola* 601, F_2	SSR+Gene+SCAR	76	7	448	Sargent et al., 2004
F. vesca 815×*F. nubicola* 601, F_2	SSR+Gene	182	7	424	Sargent et al., 2006
F. vesca 815×*F. bucharica* 601, F_2	SSR+Gene	296	7	578	Sargent et al., 2008
F. vesca 815×*F. bucharica* 601, F_2	SSR+Gene	411	7	442.8	Sargent et al., 2011
815×903BC, BC1	SSR+Gene	33	7	241.6	Nier et al., 2006
F. iinumae J17×*F. iinumae* J4, F_2	SNP	496	7	451.7	Mahoney et al., 2016
Hawaii 4×FV, F_2	SSR+SNP	2395	7	558	Samad et al., 2017

表 4　八倍体栽培草莓遗传图谱

Table 4　Genetic linkage maps of octaploid strawberry cultivars and selections

作图群体 Mapping population	标记类型 Marker type	图谱类型 Map type	位点数目 No. of loci	连锁群数 No. of linkage groups	图谱总长/cM Map length	参考文献 Citation
Capitola×CF1116, F_1	AFLP+STS +SCAR	母本 Female	235	43	1604	Lerceteau-Köhler et al., 2003
		父本 Male	280	43	1469	
Capitola×CF1116, F_1	AFLP+STS +SCAR+SSR	母本 Female	344	28	2582	Rousseau-Gueutin et al., 2008
		父本 Male	379	26	2165	
Redgauntlet×Hapil, F_1	SSR+Gene+AFLP +RAPD	母本 Female	170	32	1675.3	Sargent et al., 2009
		父本 Male	182	37	1440.7	
		整合 Consensus	315	69	3116	
Redgauntlet×Hapil, F_1	SSR+GSM+AFLP +RAPD	整合 Consensus	549	28	2140.3	Sargent et al., 2012
Tribute×Honeoye, F_1	AFLP	整合 Consensus	429	43	1541	Weebadde et al., 2008
Tribute×Honeoye, F_1	SSR+SCAR	母本 Female	119	34	1390.1	Castro et al., 2015
		父本 Male	179	42	1902.8	
232×1392, F_1	SSR+AFLP+GSM +SSCP	母本 Female	154	26	693.5	Zorrilla-Fontanesi et al., 2011a
		父本 Male	126	25	590.5	
		整合 Consensus	338	37	1259.8	
232×1392, F_1	SNP+SSR+GSM	整合 Consensus	2089	33	2490	Sánchez-Sevilla et al., 2015
0212921, S_1	SSR	整合 Consensus	822	34	1508.3	Isobe et al., 2013
02-19×Sachinoka, F_1	SSR	母本 Female	575	32	1668.9	
		父本 Male	556	34	2166.6	
Kaorino×Akihime, F_1	SSR	母本 Female	294	32	1103.4	
		父本 Male	318	33	951.4	
		集成 Integrated	1856	28	2364	
Holiday×Korona, F_1	SSR	整合 Consensus	508	28	1846	van Dijk et al., 2014
Holiday×Korona, F_1	SNP	整合 Consensus	6594	28	2050	Bassil et al., 2015
Sonata×Babette, F_1	SNP	整合 Consensus	902	31	1581.5	Davik et al., 2015
Delmarvel×Selva, F_1	SSR+SCAR	母本 Female	292	41	1529.3	Castro, Lewers., 2016
		父本 Male	242	36	1317.5	
Reikou, S_1	SNP+SSR	整合 Consensus	11574	31	2816.5	Nagano et al., 2017
Sulhyang×Senga sengana, F_1	SNP	整合 Consensus	208	30	800.8	Lee, Lee., 2017

4　QTL 定位研究

QTL 定位依托于遗传连锁图谱，结合表型性状调查与分子标记基因分型，可将表型性状的遗传控制位点锁定在连锁图谱的某个区段内，更加有利于相关连锁标记和基因的分析挖掘。草莓的 QTL 定位研究起步较晚，近几年才开展了较为广泛的研究，定位的性状主要包括抗性性状、开花行为或四季结果性状以及品质相关性状(表 5)。

抗性性状方面，与炭疽病抗性相关的 QTL 定位在 LG3，LG5 和 LG6 上(李静等, 2012)；与黄萎病抗性相关的 QTL 连续三年定位在 LG1、LG2、LG3、LG6 和 LG7 上，具有年份稳定性(Antanaviciute et al., 2015)。利用‘Strawberry Festival’×‘K12-10’和‘Strawberry Festival’×‘K08-17’群体对角斑病抗性进行 QTL 定位研究，发现角斑病抗性受单显性基因控制，主效 QTL 位点定位在 LG6 上，与标记 HRM6D_33.083 和 HRM6D_33.110 紧密连锁。标记的验证结果显示，可用于抗性单株的初步筛选(Roach et al., 2016)。通

表 5　草莓主要抗性、农艺和品质性状 QTL

Table 5　QTLs for main resistance, agronomic and quality traits in strawberry

分类 Category	定位性状 Specific traits	作图群体 Mapping population	QTL 位置[a] QTL position	参考文献 Citation
抗性 Resistance	炭疽病抗性 Anthracnose resistance	Hokowase×Sweet Charlie	LG3, LG5, LG6	李静等, 2012
	黄萎病抗病性 *Verticillium dahliae* resistance	Redgauntlet×Hapil	LG1, LG2, LG3, LG6, LG7	Antanaviciute et al., 2015
	角斑病抗病性 Angular leaf spot resistance	Strawberry Festival×K12-10 Strawberry Festival×K08-17	LG6	Roach et al., 2016
开花行为 Flowering behavior	开花周数 Weeks of flowering	Tribute×Honeoye Delmarvel×Selva[b]	LG4 LG4	Castro et al., 2015; Castro, Lewers., 2016;
	开花时间 Flowering time	Hawaii4×FV Holiday×Korona	LG4, LG6, LG7 LG4	Samad et al., 2017; Verma et al., 2017
	持续开花 Perpetually flower	Tribute×Honeoye	LG4	
	匍匐茎发生数 No. of Runners	Hawaii4×FV	LG4, LG5	
品质性状 Quality traits	单果重 Fruit weight	232×1392[c] Capitola×CF1116[c] Holiday×Korona	LG1 LG2 LG2	Zorrilla-Fontanesi et al., 2011a; Lerceteau-Köhler et al., 2012; Sánchez-Sevilla et al., 2014;
	糖度相关 Sugar related traits	232×1392 Capitola×CF1116 Delmarvel×Selva Holiday×Korona	LG6 LG6, LG7 LG6 LG6	Castro, Lewers., 2016; Verma et al., 2017
	酸度相关 Acidity related traits	232×1392 Capitola×CF1116 Holiday×Korona	LG5 LG5 LG2, LG4, LG5	
	总花色苷含量 Total anthocyanin content	232×1392 Capitola×CF1116 Delmarvel×Selva	LG5 LG2, LG6 LG5, LG6	
	总酚含量 Total phenolics	Delmarvel×Selva	LG7	
	γ-癸内酯含量 γ-decalactone content	232×1392	LG3	

a: QTL 位置不区分亚基因组；b: ‘Delmarvel’×‘Selva’群体只统计高于全基因组 LOD 阈值的 QTL 位点；c: ‘232’×‘1392’和‘Capitola’×‘CF1116’群体只统计重复 2 年以上的 QTL 位点

a: The QTL positions didn’t distinguish subgenomes; b: QTLs above the genome wide threshold were showed in ‘Delmarvel’×‘Selva’mapping population; c: QTLs detected more than two years were showed in ‘232’×‘1392’ and ‘Capitola’×‘CF1116’ mapping population

过 3 个抗性性状的 QTL 定位结果比较发现，均在 LG6 上得到了 QTL 位点，说明 LG6 上可能存在抗性基因聚集现象或广谱抗性基因。

草莓的四季结果性状对实现周年生产具有重要意义，草莓中花序与匍匐茎属于同源器官，具有四季性状的草莓品种一般匍匐茎的发生会减少(Dale et al., 2002)，因此草莓开花行为、匍匐茎发生相关性状的 QTL 定位研究，是学者们关注的热点。研究发现，无论是在二倍体草莓‘Hawaii4’×‘FV’群体中，还是在栽培草莓‘Tribute’×‘Honeoye’，‘Holiday’×‘Korona’和‘Delmarvel’×‘Selva’群体中，开花行为相关性状(包括开花周数, 开花时间,

持续开花)以及匍匐茎发生数的QTL均定位在LG4上(Castro et al., 2015; Castro, Lewers, 2016; Samad et al., 2017; Verma et al., 2017),为进一步的深入研究提供了坚实的基础。

草莓品质性状QTL定位是另一个研究热点,主要品质性状包括:单果重、糖度、酸度、总花色苷含量以及总酚含量等。通过多个群体和表型性状的多年持续调查研究,在LG1和LG2上获得了与单果重连锁的QTL位点;与糖度相关的主效QTL位于LG6;与酸度相关的主效QTL位于LG5。同时,在LG7、LG2和LG4上,也获得了与果实糖酸性状相关的QTL位点(Zorrilla-Fontanesi et al., 2011a; Lerceteau-Köhler et al., 2012; Castro, Lewers, 2016; Verma et al., 2017)。利用'Delmarvel'×'Selva'群体,对果实总花色苷含量和总酚含量进行了QTL定位,分别定位在LG5、LG6以及LG7上。其中总花色苷含量的QTL定位结果与在群体'232'×'1392'和'Capitola'×'CF1116'中所得的结果相一致(表5)。γ-癸内酯是决定草莓桃香味的重要挥发性物质,在'232'×'1392'群体中,与γ-癸内酯含量连锁的QTL被定位在LG3上。在此基础上,采用最新的分池RNA测序策略,在QTL区间内进行差异基因筛选,并利用基因表达量进行QTL定位,最终发现脂肪酸去饱和酶(fatty acid desaturases, FAD)基因*FaFAD1*与脂肪酸羟化酶(fatty acid hydroxylase, FAH)基因*FaFAH1*的表达量与γ-癸内酯含量密切相关,并定位在相同位点上,揭示它们参与了草莓果实中γ-癸内酯含量的调控(Sánchez-Sevilla et al., 2014)。此外,还获得了许多品质性状的QTL位点,包括果实颜色、产量、硬度等。草莓果实品质性状的形成是极其复杂的过程,还需要开展更加广泛、深入的研究。

5 遗传多样性研究

5.1 品种鉴定

草莓主要通过匍匐茎进行营养繁殖,长期的变异积累以及频繁引种,容易造成草莓苗木混杂和名称混乱。分子标记技术在植物品种同一性及纯度鉴定、植物遗传多样性分析方面应用广泛(Govan et al., 2008; Brunings et al., 2010),有利于追溯品种亲缘关系,实施品种保护,保障产业发展。目前,在欧美、日本已经将分子标记鉴定结果作为草莓新品种申请、保护的依据。

利用分子标记在草莓品种鉴定方面开展了大量的研究工作,品种鉴定的通量及效率显著提高。董清华等(2011)利用草莓EST数据库开发了60个SSR标记,利用其中的11个可完成20个草莓品种的鉴定。Chambers等(2013)利用森林草莓基因组序列开发SSR标记,并建立了多重PCR检测体系,对219个草莓基因型进行了分析,可区分姊妹系和遗传相近的不同倍性材料。利用高通量SNP芯片,从中筛选出24个SNP标记,可对109个来源于不同国家的草莓品种进行检测鉴定,检测效率得到了提升(Jung et al., 2017)。另一方面,分子标记也应用于草莓属种质资源的鉴定,利用20对SSR引物对草莓属83份资源进行检测,初步分析了种质资源之间的亲缘关系,对草莓的起源及类型有了进一步的认识(韩柏明等, 2012)。

5.2 种群进化与基因组结构分析

分子标记能够直接反应DNA序列上的差异,因此在草莓种群进化、基因组辅助拼接及染色体结构分析等方面也有重要的应用价值。利用RAPD标记对37个八倍体草莓种群中的318个基因型进行研究,可将所有基因型分为三大类,分别为*F. virginiana* ssp. *virginiana* and ssp. *glauca*,*F. virginiana* ssp. *platypetala*和*F. chiloensis*,同时发现*F. virginiana* ssp. *platypetala*与*F. chiloensis*更为接近(Harrison et al., 1997)。同样采用RAPD标记,对意大利埃特纳火山区域的66个草莓品系进行种群分析,可分为三个类群,并推测其起源于共同的祖先'Madame Moutot'(Milella et al., 2006)。

基因组结构分析方面,Sargent等(2011)利用SSR标记构建遗传图谱,完成了93个之前未定位的测序scaffolds的组装定位,全长28.2 Mb,占森林草莓全基因的13%。将'Redgauntlet'×'Hapil'群体遗传图谱与二倍体草莓参考图谱中的标记顺序进行比较,发现LG1与LG6之间存在重复区段,推测草莓种间发生了染色体的复制与易位事件(Sargent et al., 2009)。采用相似的研究思路,在'Holiday'×'Korona'群体的遗传图谱构建中,发现亚基因组之间具有很高的同源性,且在LG2上存在染色体倒位现象。此外,利用蔷薇科保守同源标记(RosCOS markers),能够对蔷薇科主要果树的共线性进行研究,草莓与苹果的分析结果显示,两种果树之间存在21个共线性区段(Bushakra et al., 2012)。

6 展望

分子标记技术作为分子育种的关键技术之一，已经广泛应用于果树育种生产。研究发现，苹果红色果皮性状由单基因控制，通过连锁分析获得了标记ss475879531，可以对目标性状进行筛选，缩短育种年限，提高育种效率(Chagné et al., 2016)。同时，遗传图谱构建、QTL定位结合测序技术也成为解析果树性状遗传规律，挖掘性状调控基因，从而实现定向育种和选择的有效手段。近几年，通过该策略已经获得了苹果果实酸度、果肉质地及果肉红色等性状的相关调控基因(Sun et al., 2015; Longhi et al., 2013; Umemura et al., 2013)。相比之下，栽培草莓的相关研究还具有较大差距，但是通过借鉴其他物种的研究方法，加之新方法、新技术的不断开发，草莓分子标记和遗传育种研究大有可为。

目前，由于栽培草莓基因组的多倍化和复杂性，遗传连锁图谱的连锁群数大多不能与染色体数目相对应，且存在较多缺失和断层，所包含的遗传信息不完善，限制了QTL定位的效果及后续性状调控机制的深入研究。另一方面，研究群体较为集中，遗传变异范围小，获得的标记筛选效率低，不同群体间通用性较差。此外，我国在草莓分子标记基因定位、遗传图谱构建、QTL定位等领域研究缺乏，亟待进一步加强。针对这些问题，在今后的研究中，应发挥我国草莓野生资源丰富的优势，从栽培草莓的二倍体祖先入手，加快栽培草莓全基因组序列的解析。开展高密度遗传图谱构建研究，辅助基因组的组装拼接、染色体结构变异分析和QTL定位。同时，综合应用测序、SNP芯片、全基因组关联分析等手段，立足于大群体，多组合，进行广泛的性状连锁分析和标记、基因挖掘，提高分子育种的精确度和应用价值，推动草莓产业发展和提升。

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