生 物 工 程 学 报
Chinese Journal of Biotechnology
http://journals.im.ac.cn/cjbcn
DOI: 10.13345/j.cjb.200702

Aug. 25, 2021, 37(8): 2703-2718

·综 述·

植物外源基因组成分鉴定方法的研究进展

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谢中艺, 党江波, 温国, 等. 植物外源基因组成分鉴定方法的研究进展. 生物工程学报, 2021, 37(8): 2703-2718.
Xie ZY, Dang JB, Wen G, et al. Advances in identification methods of alien genomic components in plants. Chin J Biotech, 2021, 37(8): 2703-2718.

摘 要: 具外源基因组成分 (外源染色体/染色体片段/基因) 植株是目前进行基因组学研究以及作物改良的重要材料。迄今为止，已建立了基于性状观测、染色体分析、特异蛋白、DNA 序列 4 种鉴定外源基因组成分的策略。其中，基于 DNA 序列的分子标记技术是当前鉴定外源基因组成分的主要手段，文中归纳了用于小麦、甘蓝等重要作物外源基因组成分的分子标记，且对简单重复序列 (Simple sequence repeat，SSR)、插入缺失 (Insertion-deletion，InDel)、单核苷酸多态性 (Single nucleotide polymorphism，SNP) 等 9 种标记进行了系统的比较。相比单一的鉴定方法，组合法更全面精准，文中对各组合法的应用情况进行统计和分析，提供了小麦族、芸薹族等作物的最佳鉴定组合。新一代分子标记 InDel、SNP 易实现高通量检测，对于外源渗入基因的精细定位展现了一定的优越性。此外，可以考虑一些新鉴定方法的加入，如微阵列比较基因组杂交 (Microarray-based comparative genomic hybridization，array-CGH)、抑制差减杂交 (Suppression subtractive hybridization，SSH)。

关键词: 性状观测，染色体分析，特异蛋白，分子标记，组合法，微阵列比较基因组杂交

Received: November 2, 2020; **Accepted:** March 12, 2021
Supported by: National Key Research and Development Program of China (No. 2019YFD1000905), Fundamental Research Funds for the Central Universities, China (No. XDJK2019AA001), Chongqing Science and Technology Commission, China (Nos. cstc2018jscx-mszdX0054, cstc2019jscx-gksbX0133), Innovation Research Group Funds for Chongqing Universities, China (No. CXQT19005), Chongqing Science and Technology Project of China National Tobacco Corporation (No. NY20170401070001).
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国家重点研发计划 (No. 2019YFD1000905)，中央高校基本科研业务费专项资金 (No. XDJK2019AA001)，重庆市科技局项目(Nos. cstc2018jscx-mszdX0054，cstc2019jscx-gksbX0133)，重庆市高校创新研究群体项目 (No. CXQT19005)，中国烟草总公司重庆市公司科技项目(No. NY20170401070001)资助。
网络出版时间：2021-04-12 网络出版地址：https://kns.cnki.net/kcms/detail/11.1998.Q.20210412.0955.007.html

Advances in identification methods of alien genomic components in plants

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Abstract: Plants with alien genomic components (alien chromosomes / chromosomal fragments / genes) are important materials for genomic research and crop improvement. To date, four strategies based on trait observation, chromosome analysis, specific proteins, and DNA sequences have been developed for the identification of alien genomic components. Among them, DNA sequence-based molecular markers are mainly used to identify alien genomic components. This review summarized several molecular markers for identification of alien genomic components in wheat, cabbage and other important crops. We also compared the characteristics of nine common molecular markers, such as simple sequence repeat (SSR), insertion-deletion (InDel) and single nucleotide polymorphism (SNP). In general, the accuracy of using a combination of different identification methods is higher than using a single identification method. We analyzed the application of different combination of identification methods, and provided the best combination for wheat, brassica and other crops. High-throughput detection can be easily achieved by using the new generation molecular markers such as InDel and SNP, which can be used to determine the precise localization of alien introgression genes. To increase the identification efficiency, other new identification methods, such as microarray comparative genomic hybridization (array-CGH) and suppression subtractive hybridization (SSH), may also be included.

Keywords: trait observation, chromosome analysis, specific proteins, molecular marker, combination method, array-CGH

具外源基因组成分的材料是目前研究基因组学和作物改良有力的工具，针对外源基因组成分的鉴定方法不断地革新，但就其本质来说可分为基于性状观测、染色体观测 (核型/带型分析、原位杂交)、特异蛋白、DNA 序列这 4 大类鉴定策略。传统的鉴定方法存在很多缺点：性状标记不准确、易受环境影响；核型分析分辨率低且易受人为主观判断的影响，难以实现对异源基因的鉴定；原位杂交操作难度大，费时费力；生化标记适用范围窄。分子标记可实现异源基因的精细定位，是目前鉴定异源基因的主要手段，但传统的分子标记如限制性片段长度多态性(Restrictive fragment length polymorphisms，RFLP)、扩增片段长度多态性 (Amplified fragment length polymorphism，AFLP)、随机扩增多态性 DNA (Random amplified polymorphic DNA，RAPD) 等存在操作烦琐、重复性差、密度低等缺点。高通量测序技术的发展促进了对于 SNP、InDel 等高密度分子标记的快速获取，使得渐渗系异源基因的高通量鉴定与定位成为可能。单个核苷酸变异的检测难以实现对更大的结构基因组变异的研究，array-CGH 的发展加快了研究者对于渐渗基因广度和深度的探索[1]，是研究近等基因系间基因组结构变异与异源基因渗入边界的首选方法。此外，用于富集差异 cDNA 的鉴定方法抑制差减杂交法[2]，在外源 DNA 片段的筛选上展现了一定的可能性。相比单一的鉴定方法，组合法具有更高的准确性且可获得更多异源基因相关信息，但具体的参考组合方案还未明确。文中对植物外源基因组成分的鉴定方法进行详细的综述与统计，归纳了常用于鉴定作物外源基因的分子标记；针对各方法的优缺点进行了系统的比较，并提出了明确的鉴定方法组合建议以及推荐了更精准的鉴定技术，为小麦、甘蓝等重要作物高效鉴定外源基因

组成分提供参考信息。

1 外源基因组成分的鉴定方法

目前，人们可通过远缘杂交、回交等方式创制具外源基因组成分的材料[3]，如异源附加系、异源渐渗系。对其外源基因组成分的鉴定方法众多，但始终围绕性状观测、染色体分析、特异蛋白、DNA序列展开，鉴定方式由表及里、由浅至深 (图 1)。

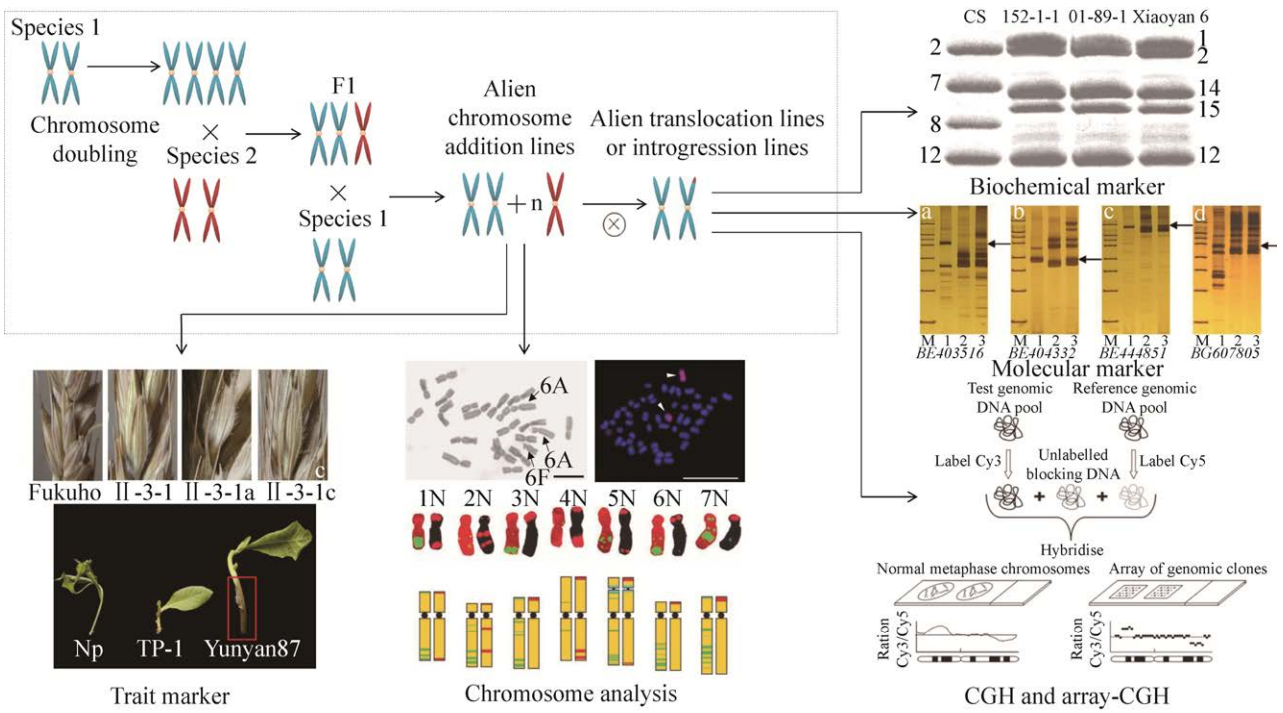

图 1 外源基因组成分的鉴定方法示意图[4-11]

Fig. 1 Schematic diagram of identification methods of alien genomic components[4-11].

1.1 基于性状观测

性状标记包括能够明确显示遗传多样性的形态学标记或借助简单测试即可识别的性状差异，如株高、叶型、花色、生理特性、病虫害抗性等。随着人们对杂交育种的深入研究，性状标记被作为鉴定植物是否渗入外源基因的第一步。研究发现冰草颖片脊上的刚毛可作为其 1P 染色体的重要形态标记[4]。Dang 等[5]通过野草烟草抗黑胫病这一性状在大量回交后代中初步筛选出具有蓝茉莉叶烟草 *Nicotiana plumbaginifolia* Viv 染色体的异源附加系。近年来，“基因组显性”这一概念的提出为性状标记的鉴定提供了理论支持[12]。随着性状标记的发展，小麦[13]、玉米[14]、草莓[15]等作物中的一系列形态学标记被应用于杂交种或抗病材料筛选，为后续外源染色体的鉴定提供了有利的工具。

1.2 基于染色体观测、分析

1.2.1 染色体核型分析

1921 年由 Belling 发明的乙酸洋红染色体压片法问世，为染色体形态、结构和行为变异的观察提供了有力的技术支撑。染色体的核型分析是指通过对染色体形态、相对长度以及臂比等参数来定义和鉴别染色体，如对于大白菜背景下引入的甘蓝 C 组染色体的鉴定[16]。此外，花粉母细胞减数分裂时期的染色体配对分析(单价体、二价体、多价体的有无及出现的频率)可进一步探究外源染色体的同源性。迄今，核型分析已被应用于枇杷[17]、甘蓝[18]、甘蔗[19]等作物染色体的鉴定。但对于某些染色体差异小的物种的鉴定，往往要借助于染色体显带技术。

1.2.2 染色体带型分析

随着高分辨率电子显微镜的出现以及显微操作技术的进步，染色体显带技术 (C-带、N-带、G-带、R-带、Q-带) 问世，基于不同个体间的染色体带型的差异揭示了染色体的内部结构分化，得以实现初步鉴定外源染色体片段。这对于染色体形态无较大差异但带纹明显的近缘物种染色体的区分十分有利。伴随着小麦标准带型的建立，C-显带技术成为初步鉴定小麦外源染色体的首选方法，至今已鉴定了小麦背景下的黑麦[9]、中间偃麦草[20]、山羊草[21]等染色体。染色体核型、带型、配对情况的综合分析可实现小片段染色体的定位。Cai 等[22]最早用双端二体测交分析和染色体分带技术相结合，准确地鉴定出异源易位染色体 1R/1B。染色体分带技术具有带型稳定、结果可靠等优点，但其鉴定本质立足于染色体的化学成分，难以实现对不显带或带型无明显差异染色体的鉴定，这极大地限制了显带技术的应用范围。

1.2.3 基因组原位杂交

基因组原位杂交技术 (Genome *in situ* hybridization，GISH) 突破了染色体核型/带型分析分辨率低这一限制，该技术以染色体供体种基因组 DNA 为探针，受体种基因组 DNA 为封阻，以杂交信号来精准定位外源染色体。该技术无需特异性探针即可直观地通过杂交信号判断外源染色体的具体条数，因此被广泛地应用于各物种染色体的计数，如山羊草[23]、玉米[24]、甘蓝[25]。但 GISH 难以鉴别具体引入的是供体种的哪一条染色体，因此通常与 C 显带技术或分子标记技术结合应用。Chen 等[7]通过 GISH 技术和 SSR 分子标记相结合鉴定了陆地棉背景下澳洲棉的每一条 G 染色体。

1.2.4 以特异 DNA 序列作为探针

相比于基因组 DNA，以特异序列作为探针则更具精度上的优势。特异性 DNA 探针主要来自重复序列、单拷贝序列、寡核苷酸等，染色体微切割、微分离和微克隆技术是获取特异探针的主要方式之一。Deng 等[26]利用显微切割技术将小麦-中间偃麦草二体附加系中疑似中间偃麦草的 7Ai-1 端体切割扩增作为探针，与中间偃麦草基因组杂交后确定了该染色体的来源。特异 DNA 探针在不同个体染色体上建立荧光原位杂交核型排列图，以不同强度或不同位置的荧光信号作为每条染色体的标记，从而实现专一地鉴定某一外源染色体或染色体片段[27]。rDNA 是目前最常用的重复序列探针，其检出率高、重复性好。5S rDNA、25S rDNA 常作为探针鉴定芸薹属植物外源染色体，如芜菁背景下甘蓝 5 号、8 号、9 号染色体[28]。基于重复序列设计的寡核苷酸探针，可进一步反映串联重复序列在染色体不同部位的分布和结构特征。针对小麦染色体所设计的 Oligo-Ku、Oligo-3B117.1 等一系列的寡核苷酸探针，通过非变性荧光原位杂交 (Non-denatured fluorescence *in situ* hybridization，ND-FISH)[29]的方式被应用于小麦背景下簇毛麦、百萨偃麦草等近缘物种染色体的识别[30-31]。此外，利用多个探针进行多色荧光原位杂交 (Multicolor fluorescence *in situ* hybridization，mFISH) 可实现多种外源基因的高效识别[32]。

1.3 基于特异蛋白的鉴定方法

在电泳技术的支持下，以蛋白质为遗传标记的鉴定方法被应用于小麦等物种上，包括种子贮藏蛋白和同工酶两类，该类蛋白也被称为生化标记。基因的差异所引起的蛋白质分子结构的差异，使其电泳后产生的多态性指纹图谱可作为遗传标记。目前该方法的应用主要集中于小麦族作物外源染色体的鉴定，这与小麦异源多倍体性质有关。小麦中大量异源同工酶结构基因如 *Aadh1F*、*β-amy-R1*、*Ph-2* 被定位于小麦各近缘物种的特异染色体上[33-34]，加速了人们对于异源附加系的选育。由于同工酶基因在同源群染色体上具共线性特点，故可作为外源染色体的部分同源群归属的重要依据。SKDH、GPI、MDH 等同工酶标记被证明可用于检测小麦背景下山羊草 $5S^1$、黑麦 1R

染色体[35-36]。此外，小麦族物种中高分子量谷蛋白亚基 (High molecular weight glutenin subunit，HMW-GS)、醇溶蛋白同样被应用于相关异源易位系或渐渗系的鉴定，如用于鉴定中间堰麦草 1St#2 染色体上的特异 HMW-GS 基因 *glu–1st#2x*[37]。

1.4 基于 DNA 序列的鉴定方法

1.4.1 特异 PCR 扩增

对于小片段外源基因的鉴定，在已知外源基因序列的情况下对其设计引物进行扩增无疑是最便捷的鉴定方式。反向 PCR 技术(Inverse PCR, IPCR)[38]通过对目标序列的侧翼进行扩增以实现对外源基因整合位点的分析。利用实时荧光定量 PCR 建立外源基因拷贝数检测体系[39]可实现拷贝数的检测，该方法相比于其他定量技术如 Southern 印迹杂交，更省时省力和节约成本。任爽等[40]利用 Taqman 定量 PCR 技术建立了基因编辑番茄植株中外源基因拷贝数的检测体系。特异 PCR 扩增法是目前转基因植物目的基因检测的主要手段，它能实现已知序列的外源基因的鉴定，但难以满足对于染色体片段的鉴定。

1.4.2 分子标记

自 Mcgrath 等[41]最早以 RFLP 技术鉴定了甘蓝型油菜外源染色体的替换与重组以来，分子标记逐渐成为鉴定外源基因组成分的主要手段。到如今发展了基于随机引物扩增分子标记技术 RAPD，基于限制性片段与 PCR 扩增的分子标记技术 AFLP，基于序列差异的分子标记 SSR、InDel、相关序列扩增多态性 (Sequence-related amplified polymorphism，SRAP)、序列特异性扩增 (Sequence-characterized amplified region，SCAR)，基于单核苷酸变异的分子标记 SNP。以上各标记在操作难度、精确度以及成本等方面各有不同，现将目前常用分子标记的特点整理至表 1。

针对 RFLP、AFLP 和 RAPD 标记操作烦琐、重复性差、DNA 用量大等缺点，常将该类标记扩增的特异条带回收、克隆和测序后转化为 SCAR、序列标签位点 (Sequence tagged site，STS) 等易操作、合成稳定的标记。新一代分子标记 InDel、SNP 在精度与密度上展现了一定的优越性，更适合于高通量的检测分析，可实现对重要性状的渐

表 1 9 种分子标记的比较

Table 1 Comparison of the characteristics of 9 molecular markers

Characteristics	RFLP	RAPD	AFLP	SSR	InDel	STS	SRAP	SCAR	SNP
Operability	Difficult	Simple	Middle	Simple	Simple	Simple	Simple	Simple	Difficult
Codominant marke or not	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Cost	High	Low	High	Middle	Middle	High	Middle	Low	High
Polymorphism	Low	Low	High	High	Middle		Middle		Highest
Repeatability	High	Low	High	High	High	High	High	High	High
Known sequence or not	No	No	No	Yes	Yes	Yes	No	Yes	Yes
Frequency	Random	Random	Random	0.5–5 kb	~3 kb	One time	Random	Random	10–200 bp
High throughput or not	No	Yes	No	Yes	Yes	No	Yes	No	Yes
Species often identified	Wheat, rice	Wheat, radish	Wheat	Wheat, Chinese cabbage, tobacco	Chinese cabbage, cabbage	Wheat	Chinese cabbage, cabbage type rape	Wheat, onion	Wheat

渗基因的鉴定。Ma 等[42]首次基于 SNP 标记技术开发了小麦背景下的冰草 P 基因组特异分子标记，分别鉴定出携带多花多粒性状和高抗白粉病性状的小麦-冰草渐渗系。近年来，各物种 SNP 阵列的开发加速了多个品种差异位点的追踪，但该类标记主要建立在基因组测序基础之上，尚难在未测序植物上应用。InDel 标记相对于 SNP 更为简单经济，且稳定性好、准确性高、扩增产物带型清晰，是未来非常有应用前景的一类标记。朱东旭等[43]利用结球甘蓝 C02 连锁群特异 InDel 标记明确了外源甘蓝染色体片段的物理位置在 18 096 729–18 786 494 bp 之间的 689 764 bp 区域内。

迄今为止，已有小麦、白菜、烟草等物种利用分子标记技术成功地鉴定了外源基因组成分，各标记的应用情况如表 2 所示。其中 SSR、RAPD、STS、RFLP 标记的使用较为普遍，且各物种 RFLP、SSR、InDel 标记遗传图谱的建立加速了外源基因组成分的鉴定与其归属的同源群分析。如小麦族 7 个部分同源群 RFLP 连锁图谱的建立，可用于鉴定小麦背景下的大部分近缘物种染色体及其归属同源群。

表 2　用于鉴定不同作物外源基因组成分的分子标记

Table 2　Molecular markers used to identify alien genomic components of different crops

Receptor species	Alien species	Alien chromosomes	Markers (Numbers)
Triticum aestivum	*Agropyron cristatum*	1P–7P	SSR (2), EST-SSR (2)[44]; EST-SSR (2), SCAR (1)[45]; specific marker (24)[46]; STS (255)[47]; STS (55)[48]; SSR (3), SCAR (3)[49]
	Psathyrostachys huashanica	1Ns–7Ns	EST-STS (8), SCAR (2)[50]; EST-STS (12)[10]; SSR (2)[51]; EST-STS (10)[52]; EST-SSR (2), EST-STS (6)[53]; EST-STS (1), EST-SSR (1)[54]; EST-STS (5)[55]
	Elytrigia intermedium	2Ai–2	RAPD (1)[56]
	Lophopyrum elongatum	St	SNP (93)[57]
	Elymus rectisetus	1–6 homologous group	RFLP (29)[58]
	Thinopyrum bessarabicum	1J–7J	EST-STS (55) [59]; AFLP (267), RAPD (14)[60]
	Leymus mollis	7Ns	EST-STS (2),PLUG (6)[61]
		6Ns	SSR (2), EST-SSR (1), EST-STS (3), PLUG (3)[62]
		Ns genome	SNP (6317)[63]
	Haynaldia villosa	1V–7V	SSR (7)[64]; IT (232)[65]
	Leymus racemosus	9 chromosome	SNP (3656)[66]
	Hordeum disticum	2H	SSR (10)[67], RFLP (1)[68]
	Aegilops uniaristata	1N, 2N, 3N, 4N, 5N, 7N	RAPD (12), SSR (17)[8]
	Aegilops comosa	1M	PLUG (3)[69]
	Leymus multicaulis	X	RFLP (1)[70]
	Aegilops longissima	$1S^l$	EST-STS (6), EST-SSR (1), SSR (2)[71]
	Secale cereale L.	T1RS·1BL	Specific marker (1)[72]
	Hordeum californicum	H3	STS (1)[73]
	Roegneria ciliaris	Sc, Yc genome	Specific marker (162)[74]
Triticum turgidum L. *var. durum*	*Thinopyrum elongatum*	1E	SSR (1)[75]
Secale cereale	*Aegilops tauschii*	2D	SSR (7)[76]
Brassica napus	*Raphanus sativus*	A–I	RAPD (18)[77]; RAPD (143)[78]

(待续表 2)

(续表 2)

Brassica oleracea var. botrytis	*Brassica nigra*	Chromosomes fragment	RAPD (65), AFLP (77)[79]
Brassica compestris ssp. *pekinensis*	*Brassica napus*	C6	SSR (26)[80]
	Brassica oleracea var. capitata L.	C1	InDel (67)[81]
Brassica napus	*Raphanobrassica*	Chromosomes fragment	Specific marker (32) [82]
	Carinata	B genome	SSR (34)[83]
Oryza sativa	*Oryza officinalis*	C genome	RFLP (192)[84]
Solanum tuberosum L.	*Solanum lycopersicum*	1–12	RFLP[85]
Nicotiana tabacum	*N. plumbaginifolia*	No.9	Specific marker (80)[86]
Gossypium hirsutum	*Gossypium australe*	1G–13G	SSR (160)[7]
Gossypium hirsutum	*Gossypium bickii*	$1G_1$–$13G_1$	Specific marker (183)[87]
Allium fistulosum	*Gossypium anomalum*	1B–13B	SSR (170)[88]; SSR (230)[89]
	Allium roylei	2R, 3R 4R, 7R	EST (2), SCAR (2), SSR (1)[90]
Allium cepa	*Allium fistulosum*	1F–8F	SSR (12), Specific marker (5)[91]
Cucumis sativus	*Cucumis hystrix*	H genome	Specific marker (12)[92]
Saccharum	*Erianthus procerus*	Chromosomes fragment	Specific marker (1)[93]

1.4.3 微阵列比较基因组杂交

Array-CGH 是基于杂交信号检测 DNA 拷贝数异常的一项技术，将等量的不同荧光标记的待测 DNA 和参照 DNA 分别杂交于分布有标准全基因组寡核苷酸探针的微阵列上，通过扫描软件计算微阵列上两种荧光信号相对强度的 $\log_2$ 比率，生成 array-CGH 数据可视化的显示图，可作为高分辨率遗传标记以实现对异源 DNA 拷贝数变异 (Copy number variation，CNV) 的鉴定和定位。该技术已被应用于大豆近等基因系 LoPro NIL 渗入基因的分析，定位了野生大豆渗入基因的具体位置，并检测出了常规方法无法鉴定的渗入基因座[94]。相比于传统的鉴定方法，array-CGH 具有更高的分辨率，高密度的杂交信号能够检测到更加细微的异源基因渗入，只需一次实验即可对样本在整个基因组上拷贝数的变化进行检测。但该方法相对于其他的鉴定方法成本较高、操作难度大，目前主要应用于人类的肿瘤发生、发育畸形等与染色体不平衡相关的研究[95]，在植物异源基因鉴定上的应用还有待开发。

1.4.4 全基因组测序

近 10 年来全基因组测序 (Whole genome sequencing，WGS) 领域在通量、速度、读取长度等方面取得了令人瞩目的进展，下一代测序技术 (Next generation sequencing，NGS) 的出现使得单碱基检测成本大幅降低。对于有参考基因组的物种，通过测序比对后在全基因组水平上扫描差异位点，无疑是最精准全面的方式。近年来基于全基因组测序数据和生物学分析手段相结合的方式实现了快速有效地捕获外源基因片段插入的位置、方向、拷贝数、侧翼序列信息，以及进一步分析转基因样本的纯合与杂合状态。外来元素检测器 (Foreign element detector，FED) 作为一种新的 Web 应用程序工具被开发出来[96]，可利用全基因组测序获得的数据来阐明外源 DNA 成分，在未知外源基因序列的情况之下也可检测成千上万的外源成分。FED 与传统染色体步移、实时定量 PCR 等技术相比，具有时间短、可重复性高、结果稳定且更容易解释等优势。以上方法目前主要在检测转基因事件与基因编辑残留外源载体组件中应用，在杂交育种方面少有涉及。

2 各鉴定方法的比较

表 3 中总结了以上鉴定策略的特性与局限

表 3 不同鉴定方法的比较

Table 3 Comparison of different identification methods

Characteristics	Characteristics of observation	Karyotypes or banding analysis	*In situ* hybridization	Specific proteins	DNA sequences
Operability	Simple	Difficult	Difficult	Middle	Most are simple
Number of markers	Less			Less	Many
Cost	Low	Low	High	Middle	Most are low
Time	Short	Long	Long	Middle	Middle
Precision		Fragment	≥200 bp	≥400 bp	≥1 bp
Alien chromosome identification	Have or not	Karyotype, number, band type, translocation fragment, pairing, origin	Number, translocation fragment, origin	Alien gene, origin, homology analysis	Alien gene, origin, homology analysis, insertion position
Applicable species	With easily distinguishable traits	With representative karyotypes and bands	Common	Abundant enzyme system	Common (part requires known genome)
Unique advantages	Rapid screening in large number of materials	Intuitive chromosomal evidence	Intuitive hybrid signal	Detect changes in protein levels	High throughput, detecting in large quantities
Limitations	Inaccurate, affected by the environment	Limited material, low resolution	Identification of related species is difficult	Each enzyme requires a specific staining technique	Some markers are difficult to develop for species with no genomic information

性。性状标记易实现田间大范围的初步筛选，但准确性低。染色体核型/带型分析是最直观的鉴定技术，但取材受限于细胞分裂旺盛的分生组织，且染色体制片复杂，在近缘物种之间难以找到遗传差异，对于染色体辨识度高的物种较为适用，如白菜[97]、甘蓝[98]、黄瓜[99]等。原位杂交在各物种上的应用则具普遍性，但其成本高且探针的获取有难度。基于特异蛋白 (生化标记) 的鉴定方法依赖于已知蛋白的基因定位，标记数量有限，主要用于揭示染色体间的同源性,适用于酶系统数量多的物种,如中间偃麦草[100]、洋葱[6]。基于 DNA 序列的鉴定方法具效率高、操作简单等优点，可在材料生长发育早期实现批量检测。基于高质量的参考基因组的鉴定方法如 array-CGH、重测序比对等，利用 NGS 技术的优势，可在整个基因组水平上检测成千上万的外源成分。高密度的分子标记如 InDel、SNP 等，是实现数量性状基因座定位 (Quantitative trait locus，QTL)、标记辅助选择、全基因组选择育种的重要手段[101]，大大加快了植物育种的步伐。该方法足够精细却难以实现外源染色体条数的鉴定，因此还需其他方法辅助。

以上方法的综合分析可进一步剖析基因、蛋白、性状之间的相互关系，如：分子标记对于某一性状或蛋白的追踪；生化标记、分子标记遗传图谱的构建是实现重要性状 QTL 定位的有效手段。Lee 等[102]利用两个不同黑腐病抗性的甘蓝亲本构建了高密度的 SNP 连锁图谱，检测到抗黑腐病性状相关 QTL。因此，各鉴定方法的结合分析相比于单一的鉴定方法更加全面精准。

3 组合法的应用

怎样快速高效地鉴定出异源材料 (主要针对异源附加/代换/易位系) 仍是目前的一大难题。在选择鉴定方法时要考虑物种间染色体的特性(数

量、形态) 及研究者们的不同需求 (耗时、成本)。相比于单一的鉴定方法，组合法更加全面可靠，这在许多研究中均有提及，但具体的参考组合方案还未明确。本文选取了近 30 年来外源基因组成分鉴定方法相关文献 137 篇进行了统计分析 (图 2)，对其使用的鉴定方法进行了整理分析。统计得出图 2 所示 10 种组合，主要集中在性状标记、染色体分析 (核/带型分析、原位杂交)、分子标记这三者的组合。

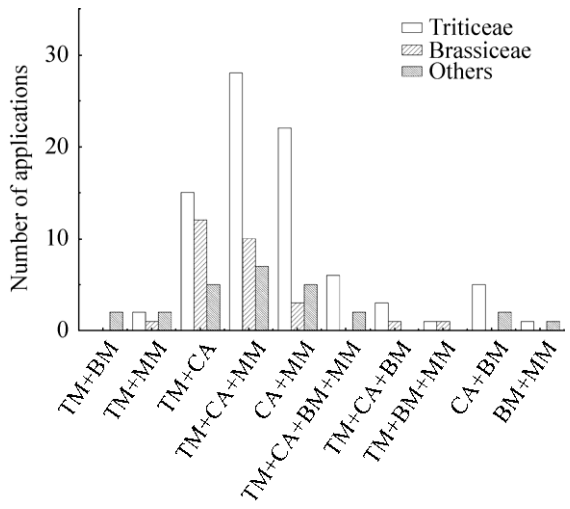

图 2　不同鉴定方法组合的比较

Fig. 2　Comparison of different combinations of identification methods. TM: trait marker; CA: chromosome analysis; BM: biochemical marker; MM: molecular marker.

小麦族外源基因组成分的鉴定主要采用 TM+CA+MM 组合，这与小麦族基因组学与分子遗传育种研究的快速发展有着紧密的关系，且 RFLP、SSR 等标记遗传图谱的建立加速了异源基因的准确定位。该组合法的可行性在普通小麦的外源紫色芽鞘基因的研究中有很好的体现。起初，研究者们以普通小麦-中间偃麦草部分二倍体及其衍生的附加系、易位系为材料，以胚芽鞘颜色、C-显带分析将外源的紫色芽鞘基因定位于中间偃麦草 7J 染色体上。现今，Li 等[103]进一步通过颜色的评估、mFISH、基于 PCR 的地标唯一基因 (PCR-based landmark unique gene，PLUG) 和内含子靶向标记 (Intron targeting，IT) 对 7J 易位系进行了分析且构建了 7J 染色体的物理图谱，精准地将外源紫色芽鞘基因定位于 7JS 的 0.35–0.63 之间。

芸薹族物种的鉴定多采用 TM+CA 组合法，该类植物大多有着易于分类的形态学标记且染色体数目较少，核型辨识度高。张雅琨等[104]以基生叶形状、花器官大小、开花时间等性状与核型分析对大白菜-结球甘蓝 4 号染色体单体异附加系自交后代的外源遗传物质进行了分析，得出了外源染色体的分离比且初步判断该染色体上携带影响开花时间的相关基因。

综上，组合法的应用不仅提升了鉴定效率且推进了外源染色体至外源基因的研究进程。由图 2 的统计分析可看出，不同物种常用的组合方式也有所差异，这主要取决于人们的研究需求以及物种特性。对于类似小麦[105]、棉花[106]等具高质量基因组信息的物种采用 CA+MM 可实现高效、准确地鉴定外源基因组成分。如样品数目较多且具性状标记如颜色、抗病性等，可以进一步结合性状标记进行初步的大规模筛选来缩短鉴定时间。对于性状标记明显且染色体核型辨识度高的物种，如芸薹族物种，适合采用 TM+CA 组合法进行鉴定。此外，对于基因组信息较缺乏的一些野生种采用该组合也可以经济高效地得到一个初步的鉴定结果。Narain 等[107]通过对栽培稻-野生水稻附加系的 TM+CA 组合法鉴定确定了不育、卷叶等性状标记与各附加染色体的对应关系。若进一步结合分子标记，还可以实现异源易位片段的鉴定、目的基因的定位、染色体同源性的分析等。

4　总结与展望

4.1　标记开发方法的创新

在 NGS 的推动下，许多新型分子标记陆续应用于外源基因的鉴定工作中，如用于区分直系同源基因与分析染色体同源关系的分子标记

PLUG[108]、用于鉴定小麦 Dv 染色体片段的内含子靶向标记 IT[109]等。但对于缺乏基因组信息的物种，依然存在分子标记开发困难的问题。基于某些物种间基因的共线性原则，通过已知序列物种去开发另一未测序物种的分子标记的方法被广泛应用。Ishikawa 等[110]基于水稻与小麦的同源基因，开发了可用于检测小麦内含子多态性的 PLUG 标记。此外，基于转录组测序开发 SSR 分子标记也逐渐成为一种常规有效的方法，近年来已应用于小麦[111]、萝卜[112]、鹰嘴豆[113]等多个物种分子标记的开发。Singh 等[59]基于普通小麦、百萨偃麦草转录组序列差异分析，开发了可用于检测百萨偃麦草渐渗系的 EST-STS 标记。简化基因组测序技术如测序基因分型 (Genotyping by sequencing，GBS)[114-115]，在成本和操作难度上展现了一定的优越性，可同时执行 SNP 的发现与基因分型，仅关注重要区域 SNP 和小的插入/缺失，这对于缺乏参考基因组序列的物种研究尤其有利。

4.2 新型鉴定方法的加入

目前，渐渗系异源基因的检测还需更多鉴定方法的加入，利用 array-CGH 实现对异源渗入基因的鉴定和定位展现了一定的可行性[116]。SSH 是用于富集差异表达基因的一种快速有效的方法，也是植物特异表达基因分离、抗性相关基因筛选的重要手段。该方法检测效率高，一次 SSH 反应可同时分离上百至上千个差异表达的基因片段，适用于大规模的基因表达分析。后续可对该方法进一步改进以实现对差异 DNA 的富集，可能会有助于外源基因组成分的快速鉴定。针对目前分子标记存在难以鉴定染色体条数的问题，可以考虑结合实时荧光定量 PCR 的方式，通过特异标记扩增的相对剂量来评估外源染色体的具体条数。Wen 等[117]基于染色体相对剂量的不平衡开发了一种可检测新增染色体的方法 (SSR-qPCR)，通过对单个植株各个染色体上分子标记扩增产物的相对剂量的检测以识别增加的染色体身份。DNA 显微镜[118]的发明使得单核苷酸测序与显微镜完美整合，可以直观快速地识别基因序列的差异，将其引入外源渗入基因的鉴定工作中，可能会有助于快速获取更详细的差异基因信息。

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