杂交兰转录组SSR信息分析及EST-SSR标记开发应用

钟淮钦 林榕燕 林兵 吴建设 樊荣辉 叶秀仙*

(福建省农业科学院作物研究所, 福建省农业科学院花卉研究中心,
福建省特色花卉工程技术研究中心, 福州 350013)

摘要 该研究主要开发筛选适用于杂交兰的EST-SSR引物, 为杂交兰种质资源评价和遗传变异研究等提供可靠的分子标记。该研究对杂交兰进行转录组高通量测序, 挖掘SSR位点和开发EST-SSR标记, 并对不同种质的遗传多样性进行分析。结果表明, 从31 724 条杂交兰Unigene中检测出18 603个SSR位点, SSR出现频率为58.64%; SSR位点中的主导类型是单核苷酸重复, 占总SSR的65.10%, 其次是二核苷酸(23.56%)和三核苷酸(10.76%)重复; 优势重复基元为A/T、AG/CT、AT/AT和AAG/CTT, 分别占总位点的64.72%、13.74%、8.19%和2.51%。利用Primer Premier 5.0共设计了565对SSR引物, 从筛选出的64对有效扩增引物中随机选择28对引物, 对40份杂交兰种质进行多态性验证与遗传关系分析, 其中16对(占57.14%)引物表现出可重复的高多态性, 平均多态信息量(PIC)达0.789。基于扩增的多态性SSR信息, 40份种质资源可聚为4类, 聚类结果与其遗传背景基本一致。该研究印证了转录组测序获得的Unigene是SSR标记开发的有效来源, 开发的EST-SSR引物可为杂交兰及近缘种的良种鉴别、遗传图谱构建、分子标记辅助育种及功能基因挖掘等提供有价值的候选标记。

关键词 杂交兰; 转录组; EST-SSR; 多态性

Analysis on SSR Information in Transcriptome and Development of EST-SSR Markers for Hybrid *Cymbidium*

ZHONG Huaiqin, LIN Rongyan, LIN Bing, WU Jianshe, FAN Ronghui, YE Xiuxian*

(*Crop Research Institute, Fujian Academy of Agricultural Sciences, Flower Research Center, Fujian Academy of Agricultural Sciences, Fujian Engineering Research Center for Characteristic Floriculture, Fuzhou 350013, China*)

Abstract EST-SSR primers suitable for hybrid *Cymbidium* were developed and screened to provide reliable molecular markers for germplasm resource evaluation and study on genetic variation. In the present study, transcriptome data of hybrid *Cymbidium* was obtained by high-throughput sequencing technology. The SSR loci were screened and the EST-SSR markers were developed, and then the genetic diversity of different germplasm were analyzed. The results showed that a total of 18 603 SSR loci were mined from 31 724 Unigenes with a frequency of 58.64%. Mononucleotide repeat was the main type, accounted for as much as 65.10% of all SSRs, followed by dinucleotide repeat (23.56%) and trinucleotide repeat (10.76%). The dominant repeat elements were A/T, AG/CT, AT/AT and AG/CTT, accounting for 64.72%, 13.74%, 8.19% and 2.51% of the total loci, respectively. 565 pairs of SSR

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*通讯作者。Tel: 0591-87572540, E-mail: yxx7861@163.com
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*Corresponding author. Tel: +86-591-87572540, E-mail: yxx7861@163.com
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primers were found by Primer Premier 5.0 and 28 efficient pairs of primers were randomly selected from 64 valid pairs for polymorphism analysis. Thereinto, 16 pairs of primers showed clear and reproducible results indicating the high poly morphism among 40 different germplasms, with the average PIC was 0.789. Based on the amplified polymorphic SSR data, the 40 materials were divided into 4 major groups by UPGMA. The dendrogram results were accordance with the genetic backgrounds. In conclusion, the Unigenes generated from transcriptome data of hybrid *Cymbidium* can be used as an effective source to development EST-SSR markers. The SSR markers obtained in the study could be valuable candidate markers for improved varieties identification, genetic map construction, molecular marker-assisted breeding and functional gene mining of hybrid *Cymbidium* and other *Cymbidium* species.

Keywords hybrid *Cymbidium*; transcriptome; EST-SSR; polymorphism

杂交兰(hybrid *Cymbidium*)为国兰(Chinese *Cymbidium*)和大花蕙兰(*Cymbidium hybridium*)杂交培育而成的一类兰花的特称, 兼具国兰的植株小巧、幽香典雅和大花蕙兰的花大、色艳等优异特性[1], 花期长, 有花观花、无花观叶, 观赏和经济价值高, 深受消费者青睐, 成为兰花市场的新宠, 发展潜力巨大[2-3]。目前, 国内杂交兰的研究主要集中在组培快繁[4-6]、鉴定评价[7-9]及栽培技术[10-12]等方面, 有关分子标记在杂交兰上的应用研究还较少, 仅见基于随机扩增多态性DNA(random amplified polymorphic DNA, RAPD)标记[13]和相关序列扩增多态性(sequence-related amplified polymorphism, SRAP)标记[14]的杂交兰种质资源遗传多样性分析的相关报道。现阶段, 种质资源评价利用与新品种选育等工作受到越来越多的重视, 但是基因组信息研究的缺乏使我们对其遗传信息了解较少。而目前所使用的分子标记难以满足资源评价、后代精准鉴定等育种工作的需要。

分子标记是反映生物个体或种群间基因组中某种差异的特异性DNA片段, 通过对这些片段的检测, 可直接反映出基因组间的遗传差异。微卫星序列[即简单重复序列(simple sequence repeat, SSR)]广泛分布于植物基因组中, SSR分子标记具有共显性遗传、多态性高、重复性好及分布广等独特优势, 成为目前遗传育种中应用最多的一种分子标记[15-16], 被广泛应用于DNA指纹图谱构建[17-18]、遗传多样性分析[19-21]、品种鉴定[22-23]、遗传图谱构建与数量性状位点(quantitative trait loci, QTL)定位[24-26]及分子标记辅助育种[27-28]等方面。SSR标记依据位点的来源不同, 分为基因组SSR(genomic SSR, gSSR)标记和表达序列标签SSR(expressed sequence tag SSR, EST-SSR)标记。其中EST-SSR标记来源于基因的转录区, 与功能基因紧密连锁, 可直接反映基因的表达信息, 更易分离鉴定出与重要表型性状关联的等位基因, 且具引物开发简单、信息量大、通用性高及成本低等优点[29-30], 已在多种园艺植物中开发应用[31-36]。

本研究以杂交兰转录组测序数据为基础, 对转录序列中的SSR标记的分布特征进行分析和筛选, 设计开发EST-SSR标记并验证其有效性。本研究将其应用于40份杂交兰种质资源遗传多样性的评价, 旨为杂交兰种质鉴定、遗传多样性分析、基因定位、核心种质构建及分子标记辅助育种等提供更丰富的标记资源。

1 材料与方法

1.1 转录组数据来源

转录组测序样本为杂交兰品种‘紫妍氏’及其叶色变异品系‘叶艺紫妍氏’生根组培苗、1.5寸杯苗及3.5寸杯苗的叶片, 供试材料均取自福建省农业科学院作物研究所花卉种质资源圃。叶片液氮速冻后, 委托北京百迈客生物科技有限公司采用Illumina HiSeq4000系统进行RNA-Seq转录组测序(无参), 采用Trinity进行序列组装, 去除其中的接头序列及低质量Reads, 各样品均获得Clean Data 6.09 Gb, 共获得88 734条Unigene, 选取长度1.0 Kb以上的31 724条Unigene作为EST-SSR标记开发的基础数据。

1.2 实验材料和基因组DNA提取

用于SSR引物筛选和种质遗传多样性评价的杂交兰材料共40份(表1), 采集开花株新鲜叶片, –80 °C保存备用。基因组DNA提取采用CTAB改良法进行, 获得的总DNA经1.0%琼脂糖凝胶电泳检测其完整性后, 采用Nanodrop ND-2000超微量蛋白核酸测定仪检测DNA浓度与纯度。将检测合格的基因组DNA原液稀释至20 ng/μL, 置于–20 °C条件下保存备用。

表1 杂交兰供试材料

Table 1 Experimental material of hybrid *Cymbidium*

序号 No.	名称或编号 Name or code	主要观赏性状 Main ornamental characters
C1	*Cym.* ‘K18-1’	Yellow flowers, leaves with claw art, slightly fragrance
C2	*Cym.* ‘Liye Dafeng’	Bluish yellow flowers, slightly fragrance
C3	*Cym.* ‘Green Jade’	Yellow-green flowers, slightly fragrance
C4	*Cym.* ‘K32-1’	Pink petals with longitudinal lines, lips with red spots, leaves with claw art, light fragrance
C5	*Cym.* ‘Golden Beauty’	Golden yellow flowers, lotus petals, aroma
C6	*Cym.* ‘Yufeng’	Bluish yellow flowers, slightly fragrance
C7	*Cym.* ‘Purple Element’	Reddish brown petals, delicate fragrance
C8	*Cym.* ‘Eighteen Princess’	Yellow-green petals, white lips with pink spots, faint scent
C9	*Cym.* ‘K37’	Rose-bengal petals, white lips with purplish red patch, slightly fragrance
C10	*Cym.* ‘Huayun Danxia’	Crimson flowers with rouge red spots, slightly fragrance
C11	*Cym.* ‘K40’	Light pink petals, white lips with rose-bengal spots, lotus petals
C12	*Cym.* ‘Ballet Dance’	Orange-red petals with purplish red stripes, delicate fragrance
C13	*Cym.* ‘K21-1’	Reddish brown petals with dark red stripes, leaves with gold edge, delicate fragrance
C14	*Cym.* ‘Red Beauty’	Dark red petals, yellow lips, slightly fragrance
C15	*Cym.* ‘K36’	Orange-red petals with carmine stripes, aroma
C16	*Cym.* ‘K21-2’	Reddish brown petals with dark red stripes,leaves with silver edge, delicate fragrance
C17	*Cym.* ‘Huayun Hongxia’	Bright red flowers with carmine spots, slightly fragrance
C18	*Cym.* ‘K18’	Yellow flower, slightly fragrance
C19	*Cym.* ‘Summer Perfume’	Bluish yellow flowers with dark purplish red spots, aroma
C20	*Cym.* ‘Miss Taipei’	Pink petals with purplish red stripes, lips with deep red spots, delicate fragrance
C21	*Cym.*‘Miss Korea’	Pink petals with longitudinal lines, lips with red spots, light fragrance
C22	*Cym.* Golden Elf ‘Sundust’	Yellow flower, delicate fragrance
C23	*Cym.* ‘Drunk Beauty’	Orange petals with red spots at base, aroma
C24	*Cym.* ‘Orient Express’	Orange-red petals with purplish red stripes, lips with red spots, delicate fragrance
C25	*Cym.* ‘K20’	Yellow-green flower, slightly fragrance
C26	Cym. ‘K33’	Yellow flower, lotus petals, leaves with claw art, slightly fragrance
C27	*Cym.* ‘Shuangyi Jinlong’	Yellow flower, slightly fragrance
C28	*Cym.* ‘K39’	Red petals with purplish red stripes, leaves with gold line art, delicate fragrance
C29	*Cym.* ‘K12’	Bluish yellow petals, lips with red spots, aroma
C30	Cym. ‘K26’	Yellow-green flower, delicate fragrance
C31	*Cym.* ‘K13’	Emerald green petals, white lips with red spots, lotus petals, delicate fragrance
C32	*Cym.* ‘Hong Zuanshi’	Red petals with white edge, white lips with red spots, slightly fragrance
C33	*Cym.* ‘Hong Guifei’	Pink petals with purplish red stripes, white lips with purplish red spots, delicate fragrance
C34	*Cym.* ‘Korea Peach’	Copper red petals with longitudinal lines, yellow lips with dark red spots, light fragrance
C35	*Cym.* ‘K38’	Beige petals with purplish red stripes, lips with bright red spots, aroma
C36	*Cym.* ‘K44’	Yellow flower, delicate fragrance, leaves with claw art
C37	*Cym.* ‘Teipei Express’	Beige petals with purplish red stripes, lips with bright red spots, aroma
C38	*Cym.* ‘K45’	Yellow flower, delicate fragrance
C39	*Cym.* ‘K42’	Yellow-green petals, light pink lips with red spots, aroma
C40	*Cym.* ‘K48’	Khaki petals with red spots, delicate fragrance

1.3 转录组SSR位点的鉴别与引物设计

对杂交兰转录组测序获得的31 724条长度≥1.0 Kb的Unigene, 使用位点挖掘工具MISA软件进行SSR位点搜索, 搜索标准为: 二、三、四、五、六核苷酸的重复数目至少为6、5、5、5、5次。利用Primer Premier 5.0软件设计PCR扩增引物, 引物主要参数为: 长度为18~22 bp, 退火温度为48~60 °C, 上下游引物退火温度差*T*m≤3 °C; GC含量为40%~60%, 预期扩增产物大小为150~300 bp, 无二级结构和二聚体。设计完成后在NCBI数据库中对引物进行BLAST验证。

1.4 EST-SSR PCR扩增

PCR反应体系为20 μL, 其中含有20 ng/μL的DNA模板2.5 μL、10 μmol/L的上下游引物各1.0 μL、10× 缓冲液(含Mg^{2+}) 2.0 μL、2.5 mmol/L的dNTPs 2.0 μL、5 U/μL的Taq聚合酶0.2 μL、ddH_2O 11.3 μL。PCR 扩增程序为: 94 ℃预变性 4 min; 94 ℃变性30 s, 48~54 ℃退火30 s, 72 ℃延伸45 s, 共35个循环; 最后72 ℃延伸10 min; 4 ℃保存。PCR扩增产物先采用2.5%琼脂糖凝胶电泳进行初步检测, 舍去无条带或是效果不理想的引物, 然后将条带清晰、有目标片段的产物利用全自动核酸蛋白分析仪(LabChip GX Touch 24)进行分析。

1.5 数据分析

SSR发生频率为含有SSR的Unigene数目与总Unigene数目的比值, SSR分布频率为SSR位点数与总Unigene数目的比值。统计并记录电泳图谱中每一样品扩增所产生的DNA条带数, 计算总条带数、多态性条带数、各引物的多态性信息量(polymorphism information content, PIC)及条带大小等。对记录的DNA条带构建原始数据矩阵, 有扩增条带的标记为1, 无条带的则记为0。应用NTSYS-pc2.10e软件按系统聚类法对其进行聚类绘图。

2 结果与分析

2.1 杂交兰转录组数据组装结果分析

杂交兰叶片转录组数据组装后共得到88 734条Unigene, 其总长度为94 452 074.00 bp, 平均长度为1 064.44 bp, N50长度为1 627.00 bp, 组装完整性较高。由图1可见, Unigene长度(length, L)在200≤L<500 bp之间的有31 047条, 所占比例为34.99%; 长度在500≤L<1 000 bp之间的有25 961条, 所占比例为29.26%; 长度在1 000≤L<2 000 bp之间的有19 373条, 所占比例为21.83%; 长度≥2 000 bp的有12 351条, 所占比例为13.92%。

2.2 杂交兰转录组SSR位点的数量与分布

用MISA软件对1.0 Kb以上的31 724条Unigene序列进行SSR位点搜索, 共搜寻到18 603个SSR位点, 分布于13 637条Unigene中, 发生频率为42.99%。其中, 只含有1个SSR位点的Unigene序列有9 735条, 含有2个及以上SSR位点的序列仅有3 902条, SSR分布频率为58.64%。杂交兰转录组SSR类型主要以单核苷酸、二核苷酸和三核苷酸重复为主, 分别占SSR位点总量的65.10%、23.56%和10.76%; 而四、五和六核苷酸重复类型较少, 仅占SSR位点总量的0.58%(表2)。

由表2可见, 杂交兰转录组SSR重复基元的重复次数为5~35次, 其中5~10次重复的有11 601个, 占总SSR的62.36%; 其次为11~20次重复, 有6 839个, 占总SSR的36.76%; 20次重复以上的有163个, 仅占0.88%。由图2可见, 杂交兰转录组SSR的长度集中在10~60 bp之间, 其中长度在10~14 bp之间的最多, 有14 620个, 占总数的78.59%; 其次为15~20 bp, 有3 157个, 占总数的16.97%; 而长度20 bp以上的比例低, 仅占4.44%。

2.3 杂交兰转录组SSR类型和分布特征

由表3可见, 杂交兰18 603个SSR位点共包含129种重复基序, 单核苷酸到六核苷酸重复分别有4、12、59、42、6、6种重复基序。从分布频率来看, 单核苷酸重复基序以A/T为主, 占单核苷酸SSR的99.41%, 占总SSR的64.72%; 二核苷酸重复基序

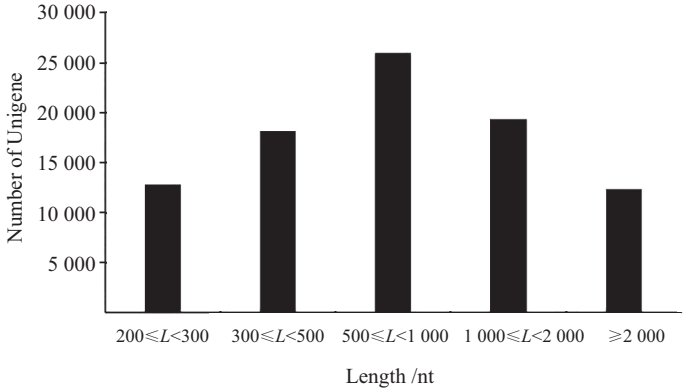


图1 杂交兰转录组Unigene长度分布

Fig.1 Length distribution of all assembled Unigene in the transcriptome of hybrid *Cymbidium*

以AG/CT、AT/AT为主, 占二核苷酸SSR的58.33%和34.78%, 占总SSR的13.74%和8.19%。三核苷酸重复基序以AAG/CTT、AAT/ATT、AAC/GTT和ATC/ATG占优势, 分别占三核苷酸SSR的23.33%、18.83%、16.33%和12.84%; 四核苷酸重复基序中以AAAT/ATTT为主, 占四核苷酸SSR的41.94%; 五核苷酸和六核苷酸重复基序仅为6个, 出现频率低。

2.4 杂交兰EST-SSR的有效性及多态性

利用Primer Premier 5.0软件设计了565对EST-SSR引物, 随机挑选出105对不同重复单元(二、三、四、五核苷酸)的引物, 对3份杂交兰种质的DNA进行SSR-PCR扩增检测。结果表明, 98对引物可扩增

表2 杂交兰转录组SSR的类型、数量及分布频率

Table 2 The type, number and distribution frequency of SSRs explored from the transcriptome dataset in hybrid *Cymbidium*

重复类型 Repeat type	重复次数 Repeat number								总计 Total	比例/% Ratio /%
	5	6	7	8	9	10	11~20	>20		
Mono-nucleotide	0	0	0	0	0	5 271	6 677	163	12 111	65.10
Di-nucleotide	0	1 065	770	750	992	645	160	0	4 382	23.56
Tri-nucleotide	1 115	547	318	18	2	0	2	0	2 002	10.76
Tetra-nucleotide	80	12	0	1	0	0	0	0	93	0.50
Penta-nucleotide	6	1	0	0	0	0	0	0	7	0.04
Hexa-nucleotide	5	1	0	0	1	1	0	0	8	0.04
Total	1 206	1 626	1 088	769	995	5 917	6 839	163	18 603	
Ratio	6.48%	8.74%	5.85%	4.13%	5.35%	31.81%	36.76%	0.88%		

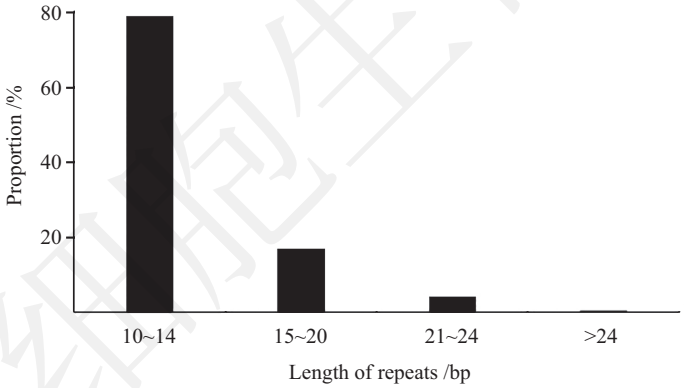


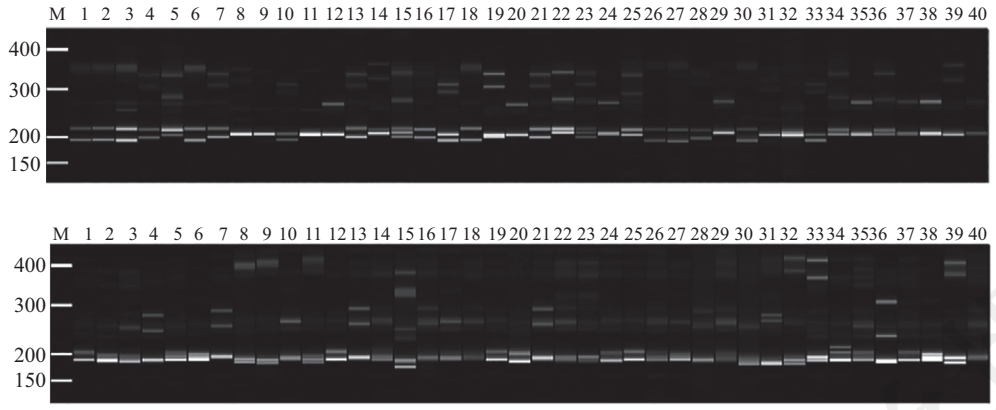
图2 杂交兰转录组SSR重复长度分布

Fig.2 The length distribution of repeats in SSR of hybrid *Cymbidium*

表3 杂交兰转录组SSR的主要重复基元

Table 3 The main SSR motifs in hybrid *Cymbidium* transcriptome

重复类型 Repeat type	类型种数 Type number	主要重复基序及其个数 Main repeat motif and its' number
Mono-nucleotide	4	A/T (12 039)
Di-nucleotide	12	AG/CT (2 556)、AT/AT (1 524)、AC/GT (290)
Tri-nucleotide	59	AAG/CTT (467)、AAT/ATT (377)、AAC/GTT (327)、ATC/ATG (257)、CCG/CGG (184)、AGG/CCT (182)、AGC/GCT (137)
Tetra-nucleotide	42	ATTT/AAAT (39)、TATG/CATA (10)、AAGA/TCTT (9)、AAAC/GTTT (6)
Penta-nucleotide	6	AAAAG/CTTTT (2)、TAAAA/TTTTA (1)、GAATC/GATTC (1)、CTTCT/AGAAG (1)、GAAAT/ATTTC (1)、TGAGA/TCTCA (1)
Hexa-nucleotide	6	TCAACA/TGTTGA (2)、ACAGAC/GTCTGT (2)、CGAGGA/TCCTCG (1)、TGGAGG/CCTCCA (1)、CACCGC/GCGGTG (1)、TCAGCC/GGCTGA (1)



M: DNA marker; 1~40分别为表1所列的40份杂交兰资源。

M: DNA marker; 1-40 represent 40 DNA samples of hybrid *Cymbidium* in table 1.

图3 引物CYM547(上)和CYM287(下)在40份杂交兰资源中的扩增图

Fig.3 Profile of amplification in 40 hybrid *Cymbidium* germplasms by primer CYM547 (up) and CYM287 (down)

出条带, 其中能扩增出预期产物大小的引物共64对, 有效扩增率为60.95%。

从64对有效扩增引物中随机选取28对EST-SSR引物对40份杂交兰种质资源进行扩增及多态性评价, 其中有16对引物可检测出多态性位点(表4), 占有效扩增引物的57.14%, 图3为引物CYM547和CYM287的扩增情况。16对引物共扩增出193个条带, 其中多态性片段176个, 各引物产生的多态性片段数在5~19个之间, 每对引物平均产生11个多态性条带。16对EST-SSR标记的PIC为0.644~0.902, 平均值为0.789, 16个标记均为高度多态性位点(PIC≥0.500)。表明这16对新开发的引物具有较高的质量和多态信息, 可用于杂交兰遗传资源的评价。

对基于16对引物扩增的多态性EST-SSR进行聚类分析, 结果显示, 40份杂交兰种质间的遗传距离变化在0.17~0.90之间, 其中‘华韵玉凤’与‘立叶大凤’亲缘关系最近。在遗传距离0.75处, 40份种质被分成四大类群(图4), 第I类群包含20份种质, 主要为黄色系与红色系种质; 第II类群包含14份种质, 主要为黄色系、橘红色、粉红色种质; 第III类群只有‘黄金美人’1个种质, 表现为金黄色素花、花大、浓香; 第IV类群包含5份种质, 表现为植株矮小、花瓣肥厚、花期为1~2月。

当以遗传距离0.68为阈值, 第I类群种质被分为4个亚类, ‘金龙爪’、‘立叶大凤’、‘华韵玉凤’、‘绿翡翠’和‘黄金龙’5个黄绿色、微香种质聚为第1亚类; ‘红贵妃’单独为第2亚类; 第3亚类由红色系‘韩国小姐’、‘紫妍氏’及其叶艺变异种质与姐妹品种‘福韵丹霞’、‘福韵红霞’等10个种质组成; 第4亚类由‘金凤冠’、‘双艺金龙’、‘青龙冠’和‘爪艺金龙’4个叶艺品种组成。第II类群种质被分为4个亚类, ‘黄金小神童’及2个叶片变异种质聚为第1亚类; ‘金玉满堂’、‘醉美人’和‘韩国桃花’3个橘红色种质聚为第2亚类; ‘芭蕾舞’、‘台北小姐’、‘东方快车’、‘小快车’、‘台北快车’、‘夏日彩虹’和‘金边凤凰’7个种质聚为第3亚类; ‘大果’单独为第4亚类。

3 讨论

种质资源是良种选育的原始材料, 是遗传多样性和物种多样性的基础, 对种质资源的鉴定、评价显得尤为重要。随着测序技术的快速发展, 大量的转录组数据为EST-SSR的开发提供了丰富的资源[37], EST-SSR标记直接或间接地加速了分子标记辅助育种的进展[38], 而杂交兰EST-SSR分子标记的研究还未见相关报道。本研究在杂交兰中共挖掘了88 734条Unigene, 从31 724条长度大于1.0 Kb的Unigene中搜索, 发现其中13 637条Unigene序列中含有18 603个SSR位点, 出现频率为58.64%, 显著高于莲雾的55.03%[32]、厚朴的25.31%[39]、萝卜的23.79%[40]、刺梨的20.37%[41]、茄子的18.32%[42]、紫楠的15.89%[43]和腊梅的14.25%[35]。SSR位点的出现频率差异可能与物种基因组差异、转录组数据中Unigene数量及长度、SSR搜索标准及挖掘工具等有关[38], 若忽略EST数据量及来源等对分布频率、出现频率差异的影响[41], 这种差异可能反映了物种间真实的SSR信息差异, 表明杂交兰转录组SSR位点分布较为密集,

表4 16对SSR引物及其扩增情况

Table 4 16 pairs of primers developed from hybrid *Cymbidium* and their amplification

引物编号 Primer No.	引物序列(5′→3′) Primer sequence (5′→3′)	重复基序 Repeat motifs	预期长度/bp Expected length /bp	总条带 Total bands	多态性条带 Polymorphism bands	多态率/% Polymorphism percentage /%	多态性信息量 PIC
CYM45	F: TGG CAC TAG TCA ACC GTC AG R: GCC AGT TAC TTT CCT TAG GCG	$(AT)_8$	257	14	14	100.00	0.896
CYM65	F: CCG AGC ACT GAA ACA TGA GA R: CAC ATG TGC TTG CTA CCG AC	$(CA)_6$	268	8	8	100.00	0.808
CYM193	F: CAT GGT TCA TGT GGG TGG TA R: GCC AAC AGA GAA ATT TGG GA	$(TC)_6$	274	9	6	66.67	0.753
CYM207	F: GGA AAC TAG TGA ACC AGA CGG R: CGC GAT GCC ATT TCT TAT TC	$(TG)_8$	242	19	19	100.00	0.891
CYM247	F: ATA GCA GAC AGG ATG GTG CC R: CTC CAC CTC CTC TGC TTC AC	$(AAG)_5$	266	7	5	71.43	0.598
CYM269	F: CCC AGT GTT CTC CTG GTC AT R: ATT CTC CGT ACC GTC TGG TG	$(ACC)_5$	220	18	18	100.00	0.804
CYM 278	F: AGG CAC ATA GGA GAG CCT GA R: CTG AGC AGG AAC TTG AAG CC	$(AGA)_6$	269	14	14	100.00	0.845
CYM287	F: CAT CAA CGC GGT GTA TGA AC R: CCG AGA TTT GAG TGT CGG AT	$(AGC)_8$	198	18	17	94.44	0.889
CYM354	F: CTT CAT TCC CGG CTA AAT CA R: GAA ACT TCG CTC AAA AAC GC	$(CCA)_6$	251	12	11	91.67	0.821
CYM373	F: GGG GCT AAT GAT GCA AGG TA R: ATT TCT CCA CCT TTG GGG TT	$(CGA)_7$	227	8	7	87.50	0.733
CYM423	F: AGC TCT CCC CCA TTT CAG AT R: GTC CAT CGT CGT CGA ACT CT	$(GAA)_6$	214	12	10	83.33	0.772
CYM541	F: GAA TTG GAA CAA TGA ACG CA R: AAG CAG GTG TTT CCA GCA CT	$(TTA)_6$	212	11	10	90.91	0.834
CYM504	F: TCA CAA TAC AGG CAG AAG CG R: GGA ACT TCA TGA CAG CCC AT	$(TCA)_6$	238	10	8	80.00	0.644
CYM547	F: CGC GTT CAT ACT CCG ATA CA R: TAA TTT AGA AGG AGG CGG GG	$(TTC)_7$	203	15	15	100.00	0.902
CYM555	F: GCT GCT CGT GCT ACA AAA CA R: CTA CGG TTT GTT GGC CGT AT	$(CAGA)_6$	187	11	9	81.82	0.767
CYM562	F: GAA GCA AGA GCA GTG GAA GG R: TCG AAG ATT TGG ATT CCT GC	$(GAA TC)_5$	235	7	5	71.43	0.716

是遗传资源评价、鉴定的理想标记之一。

前人研究表明, 大多数物种的SSR类型以二、三核苷酸重复为主, 主导重复基序因物种而异[44-45]。在本研究中, 杂交兰以单核苷酸重复(65.10%)为主, 其次是二核苷酸(23.56%)、三核苷酸(10.76%)重复, 而四、五和六核苷酸重复数量极少, 仅占SSR总量的0.58%, 与莲雾[32]、中国南瓜[46]、芙蓉李[47]等相似, 与腊梅[35]、黄秋葵[36]、紫楠[43]等不同。这可能与不同物种表达程度、进化水平或突变频率等因素的差异有关, 重复基元类型碱基数越少的物种, 其进化水平或突变频率相对越高[48-49], 可见, 杂交兰属于进化程度较高的物种。杂交兰转录组中单核苷酸重复主要为A/T, 与莲雾[32]、芙蓉李[47]、烟草[50]等一致; 二核苷酸重复以AG/CT、AT/AT为主, 与蝴蝶兰[51]、蓝靛果忍冬[52]、龙眼[53]等一致; 三核苷酸重复最丰富的为AAG/CTT, 这与双子叶植物中三核苷酸优势重复基元应为AAG/CCT的观点相符[54]。

本研究中, 随机挑选的SSR引物中能扩增出预期片段的比例为60.95%, 无扩增产物或扩增出大于预期长度的片段的引物, 可能是两引物之间存在大的内含子, 或是供试材料中存在复等位基因, 或是引物特异性不高, 扩增出与引物同源的序列。随机

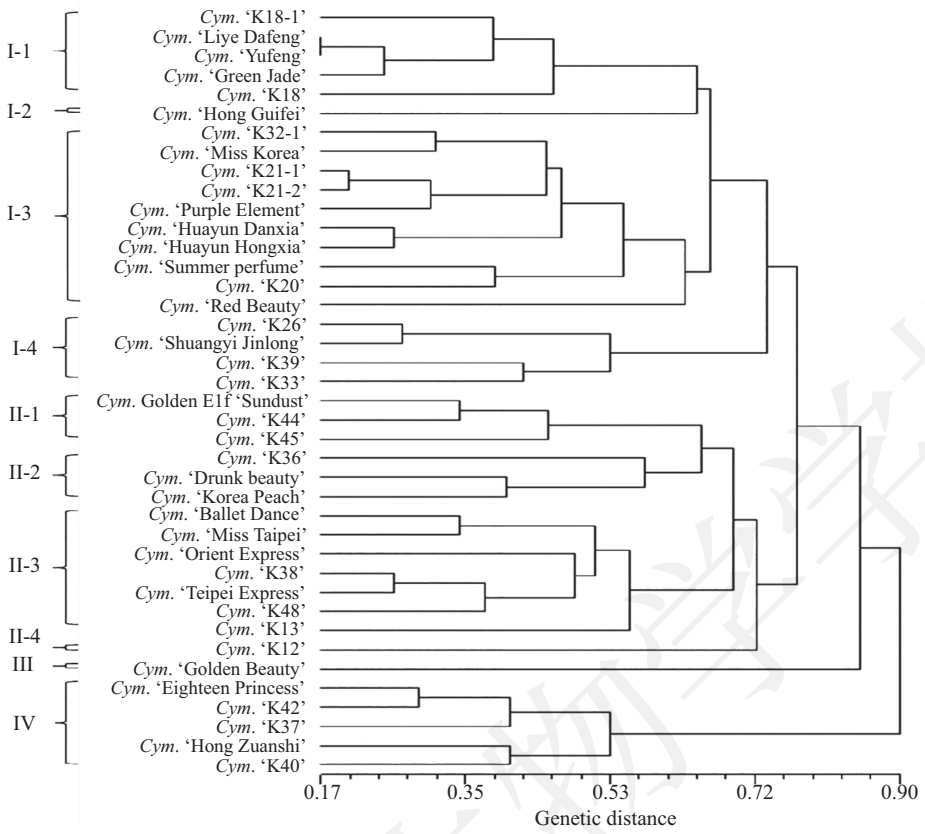


图4 供试杂交兰种质资源的UPGMA聚类图

Fig.4 Dendrogram for hybrid *Cymbidium* germplasms by UPGMA

选取28对EST-SSR引物对40份种质进行多态性评价, 其中16对引物具有多态性, 占有效扩增引物的57.14%, 低于毛竹的92.0%[55]、龙眼的76.2%[53]、莲雾的71.4%[32], 但高于蝴蝶兰的56.25%[51]、刺梨的52.17%[41]、紫楠的30.04%[43]。16对引物PIC平均值为0.789, 均属于高PIC等级, 表明开发出的EST-SSR多态性程度相对较高。利用UPGMA对40份杂交兰种质进行聚类分析, 40份材料被分为四大类; 以遗传距离0.68为阈值, 第I类群、第II类群均可被分为4小类, 聚类结果与传统植物学分类吻合, 亲本来源、花朵颜色、花瓣质地、花期等相近的种质聚为一类, 如'黄金小神童'与其2个叶片变异种质聚为1个亚类, '金龙爪'、'立叶大凤'、'华韵玉凤'、'绿翡翠'和'黄金龙'5个黄绿色种质聚为1个亚类。较ISSR、SRAP等标记的分析结果, EST-SSR标记更为准确地反映了杂交兰种质资源之间的差异, 可进一步应用于杂交兰等兰属植物的遗传多样性和分子辅助育种研究。

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