

# 葡萄无核性状分子标记在不同遗传背景下的通用性验证

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**摘要:**【目的】在自然群体和2个杂交群体中对6个葡萄无核分子标记进行通用性鉴定。【方法】以自然群体的157份种质和2个杂交群体的263份F<sub>1</sub>子代为试验材料, 鉴定6个葡萄无核分子标记的准确率。【结果】分子标记p1\_VvAGL11、p2\_VvAGL11、p3\_VvAGL11、5U\_VviAGL11、VvSD10和KASP\_VviAGL11在阳光玫瑰×红艳无核杂交群体的无核鉴定准确率分别为70.90%、63.43%、74.63%、73.13%、76.12%和75.37%; 在红地球×郑艳无核杂交群体的无核鉴定准确率分别为87.60%、64.34%、89.92%、89.15%、91.47%和89.92%。根据自然群体的鉴定结果, KASP标记KASP\_VviAGL11的A等位基因与无核表型100%相关。【结论】分子标记VvSD10和KASP\_VviAGL11无核鉴定准确率高, 综合表现最优, 在葡萄无核性状分子标记辅助育种中可以优先使用。

**关键词:** 葡萄; 无核性; 分子标记; 准确率; 通用性

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## Validation of the universality of molecular markers for seedlessness in grape under different genetic backgrounds

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**Abstract:** 【Objective】A universality assessment was conducted on six seedlessness-related molecular markers in grape that have been previously reported both domestically and internationally. This evaluation was performed using a diverse set of 420 grape germplasm accessions to comprehensively verify the effectiveness and reliability of these markers across different genetic backgrounds. The results aim to provide robust technical support for the application of molecular markers in marker-assisted selection (MAS) for the breeding of seedless grape cultivars. 【Methods】A total of 420 grapevine accessions were used in the study, comprising a natural population of 68 seedless and 89 seeded accessions, along with two F<sub>1</sub> hybrid populations. The first population, derived from the cross of Shine Muscat × Hongyan Seedless, included 134 individuals (35 seedless and 99 seeded), while the second population, from Red Globe × Zhengyan Seedless, included 129 individuals (56 seedless and 73 seeded). Six molecular markers—p1\_VvAGL11, p2\_VvAGL11, p3\_VvAGL11, 5U\_VviAGL11, VvSD10, and KASP\_VviAGL11—were selected for analysis based on previous studies both domestically and internationally. Polymerase chain reaction (PCR) was used to amplify target fragments. Capillary electrophoresis was applied to determine the allele sizes of SSR markers, while the genotypes of KASP markers were identi-

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fied using KASP genotyping technology. Chi-square ( $\chi^2$ ) independence tests ( $P < 0.05$ ) were performed using R software to assess the association between seedless phenotype and molecular marker alleles and genotypes. The false positive rate, false negative rate, seedlessness identification accuracy, and seedlessness detection rate were calculated for each marker to evaluate their performance and general applicability. **【Results】** In both  $F_1$  hybrid populations, allelic and genotypic analyses of SSR markers showed the following results. p1\_VvAGL11: The 250 bp allele showed a highly significant association with seedlessness, while the 257 bp allele was significantly associated with the seeded phenotype. Similarly, the genotype 250/250 was highly associated with the seedlessness phenotype, whereas the genotype 257/257 was significantly associated with the seeded phenotype. The 250 bp allele showed seedlessness identification accuracies of 70.90% and 87.60%, with corresponding detection rates of 46.97% and 80.30% in the two hybrid populations, respectively. p2\_VvAGL11: The 171 bp allele was moderately significantly associated with seedlessness. The genotypes 158/171 and 171/171 were highly significantly associated with the seedlessness phenotype, while the genotype 158/158 was highly significantly associated with the seeded phenotype. The 171 bp allele showed seedlessness identification accuracies of 63.43% and 64.34% and detection rates of 41.67% and 54.90%. p3\_VvAGL11: The 195 bp allele was highly significantly associated with seedlessness. The genotype 185/195 was highly significantly associated with seedlessness, while the genotype 185/185 was highly significantly associated with the seeded phenotype. The 195 bp allele showed seedlessness identification accuracies of 74.63% and 89.92% and detection rates of 50.82% and 85.25%. 5U\_VviAGL11: The 316 bp allele was highly significantly associated with the seedlessness phenotype, while the 308 bp and 318 bp alleles were highly significantly associated with the seeded phenotype. The genotypes 304/316 and 308/316 were highly associated with the seedlessness phenotype, while the genotypes 304/308, 304/318, and 308/318 were strongly associated with the seeded phenotype. The 316 bp allele showed seedlessness identification accuracies of 73.13% and 89.15% and detection rates of 49.21% and 85.00%. VvSD10: The 105 bp allele was significantly associated with the seedlessness phenotype, while the 113 bp allele was highly associated with the seeded phenotype. Genotype 105/107 was highly associated with the seedlessness phenotype, while genotypes 107/107 and 107/113 were strongly associated with the seeded phenotype. The 105 bp allele showed seedlessness identification accuracies of 76.12% and 91.47%, with corresponding detection rates of 52.54% and 88.14%, respectively. Among germplasm carrying the corresponding alleles, the proportion of seedless individuals was 63.6% for p1\_VvAGL11, 48.9% for p2\_VvAGL11, 68.0% for p3\_VvAGL11, 66.7% for 5U\_VviAGL11, and 70.3% for VvSD10. Among germplasm carrying the corresponding genotypes, the proportions were 69.8%, 63.2%, 80.1%, 72.6%, and 84.0%, respectively. The KASP\_VviAGL11 marker allele A was significantly associated with seedlessness. The genotypes A:A and A:C corresponded to the seedlessness phenotype, whereas genotype C:C corresponded to the seeded phenotype. Both false positive and false negative rates were observed in the two hybrid populations. The A allele showed seedlessness identification accuracies of 75.37% and 89.92%, detection rates of 51.67% and 85.25%, respectively. In the natural population, the A allele of KASP\_VviAGL11 was 100% associated with the seedless phenotype in diploid germplasm. **【Conclusion】** Six seedlessness-related molecular markers were validated in the two  $F_1$  hybrid populations of Shine Muscat  $\times$  Hongyan Seedless and Red Globe  $\times$  Zhengyan Seedless. Among them, VvSD10 and KASP\_VviAGL11 exhibited the highest seedlessness identification accuracies and detection rates, along with the lowest false positive and false negative rates, thus showing the best overall performance. The 5U\_VviAGL11 marker also demonstrated high accuracy and detection rates, low error rates, and provided rich genetic informa-

tion. In contrast, p2\_VvAGL11 had the lowest accuracy and detection rate. These results indicate that certain molecular markers, particularly VvSD10 and KASP\_VviAGL11, have strong potential for use in marker-assisted selection in seedless grape breeding.

**Key words:** Grape; Seedlessness; Molecular marker; Accuracy; Generality

葡萄是世界上最重要的果树之一,广泛应用于鲜食、酿酒、制汁与制干等<sup>[1]</sup>。无核葡萄由于更适合鲜食和加工<sup>[2]</sup>,深受消费者和生产者喜爱,因此无核已成为国内外葡萄育种工作的重要目标之一<sup>[3-4]</sup>。葡萄的无核性状通常需要在植株成年后进行鉴定,这限制了无核葡萄新品种的快速选育<sup>[5]</sup>。在幼苗期对无核葡萄进行精确高效的分子标记辅助选择对提高葡萄育种效率和降低育种成本至关重要<sup>[6-7]</sup>。因此,筛选准确率高、成本低且适用于多种遗传背景 of 无核分子标记对加快无核葡萄育种进程具有重要意义。

目前已经开发出多种类型的葡萄无核分子标记,其中 SSR 分子标记和 KASP 分子标记的鉴定效率较高。Mejía 等<sup>[5]</sup>通过 QTL 定位分析,发现 MADS-box 基因 *VviAGL11* 对应先前被描述为主要无核位点的 SDI (seed development inhibitor), 根据启动子调控区开发了 SSR 分子标记 p1\_VvAGL11、p2\_VvAGL11 和 p3\_VvAGL11; 基于数量性状基因座 (quantitative trait loci, QTL) 的定位结果, p3\_VvAGL11 位于 *VviAGL11* 基因的 5'UTR 处,含有 (GAGA)*n* 重复序列,且该标记鉴定到的 198 bp 等位基因与无核性状密切相关<sup>[6]</sup>。此外,Ocarez 等<sup>[8]</sup>在 p3\_VvAGL11 相同的基因组区域开发了另一个 SSR

标记 5U\_VviAGL11, 并发现该标记鉴定到的 319 bp 等位基因与无核性状显著相关。马亚茹等<sup>[9]</sup>定位到了与无核性状相关联的 QTL (Chr18: 26835846~26960426), 并基于此开发了无核性状的 SSR 分子标记 VvSD10。Royo 等<sup>[10]</sup>发现 *VviAGL11* 基因的外显子区域 Chr18:26889437(A/C) 存在单碱基突变,致使第 197 位精氨酸替换为亮氨酸,该突变位点与无核结实完全相关。Wang 等<sup>[11]</sup>根据 *VviAGL11* 基因的突变位点 Chr18:26889437(A/C) 开发了 KASP\_VviAGL11 和 VviAGL11\_410, 并在 101 个葡萄品种和 3 个 F<sub>1</sub> 代杂交群体的 81 个子代中进行了验证。Xi 等<sup>[12]</sup>根据已验证的 *VviAGL11* 基因 SNP 位点 Chr18:26889437(A/C) 与有核/无核表型的关联, 开发并验证了与无核相关的 KASP 分子标记。

目前无核分子标记的准确性大多在自然群体中进行验证<sup>[13-15]</sup>, 尽管已有多个与无核性状相关的分子标记被成功用于特定杂交群体, 但验证效率与亲本关联度较高, 在不同遗传背景下的鉴定效率尚不明晰, 因此需要在其他遗传背景中测试其稳定性与适用性<sup>[16-17]</sup>。笔者在本研究中基于 6 个无核性状分子标记(图 1), 在自然群体和杂交群体中对他们的适用范围及准确性进行通用性鉴定, 筛选出准确率高且检测方法简单的分子标记, 以期为分子标记辅助

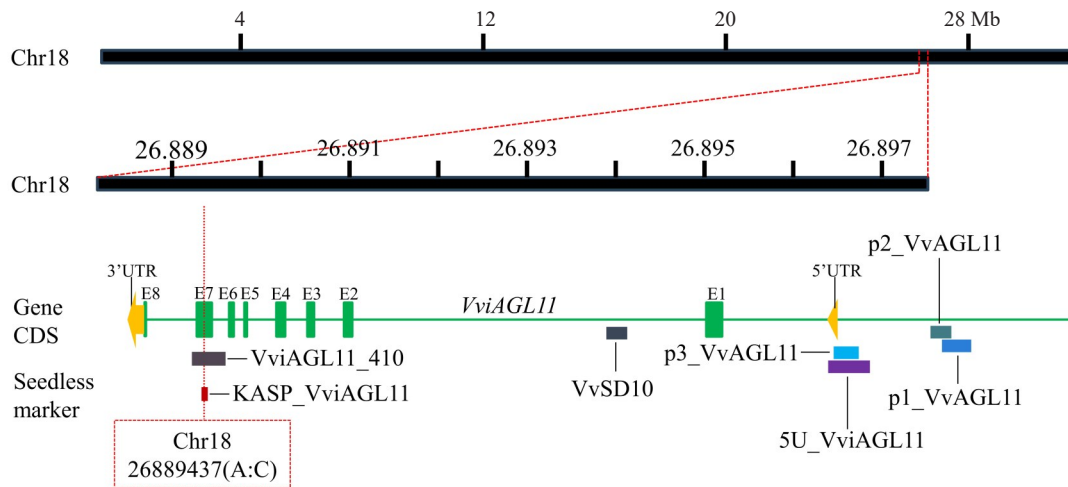


图 1 常用无核分子标记在葡萄第 18 号染色体上的位置 (PN 40024 12X)  
 Fig. 1 Genomic locations of commonly used seedlessness related molecular markers on chromosome 18 of grape (PN 40024 12X)

无核葡萄育种提供技术支持。

## 1 材料和方法

### 1.1 材料

试验材料共420份,包括68份无核种质和89份有核种质组成的自然群体,保存于中国农业科学院郑州果树研究所国家葡萄种质资源圃;35份无核种质和99份有核种质组成的阳光玫瑰×红艳无核F<sub>1</sub>代杂交群体保存于中国农业科学院郑州果树研究所基地;56份无核种质和73份有核种质组成的红地球×郑艳无核F<sub>1</sub>代杂交群体保存于中国农业科学院郑州果树研究所基地。

### 1.2 无核性状调查

在葡萄果实成熟期调查种子性状,种子性状分类标准参照《葡萄种质资源描述规范和数据标准》<sup>[18]</sup>,无核、软核与残核的单株均属于无核,种子充分发育的单株属于有核。

### 1.3 葡萄DNA提取

取420份葡萄材料的健康幼嫩叶片,液氮研磨,

采用CTAB植物基因组DNA快速提取试剂盒(北京艾德莱生物科技有限公司)提取叶片基因组DNA。利用1%琼脂糖凝胶电泳和NanoDrop1000(Thermo Scientific)检测DNA纯度和浓度,然后将DNA浓度稀释到工作液质量浓度(约50 ng·μL<sup>-1</sup>),保存于-20℃,用于后续的PCR扩增。

### 1.4 SSR分子标记PCR扩增条件及检测

选用5个SSR分子标记(表1)对263份试验材料进行准确率验证。含有FAM(5'-fluorescein-CE phosphoramidite)和HEX(5'-hexachloro fluorescein-CE phosphoramidite)2种荧光探针的SSR引物由北京擎科生物科技股份有限公司合成。SSR分子标记的PCR反应体系为25 μL:2×Taq Master Mix 12.5 μL,正、反向引物各0.5 μL(10 μmol·L<sup>-1</sup>),1 μL DNA模板(50 ng·μL<sup>-1</sup>),ddH<sub>2</sub>O补至25 μL。反应程序:94℃预变性3 min;94℃变性10 s,适宜T<sub>m</sub>值退火10 s,72℃延伸10 s,共35个循环;72℃延伸5 min,扩增产物在4℃保存。具体退火温度根据引物设计进行筛选。

PCR扩增产物通过琼脂糖凝胶电泳确定模板浓

表1 SSR分子标记对应引物名称及信息

Table 1 SSR molecular marker correspond to primer name and its information

引物名称 Primer name	引物序列(5'-3') Primer sequence (5'-3')	退火温度 Annealing temperature/°C	产物大小 Fragment size/bp	参考文献 Reference
p1_VvAGL11	F: GACACGCGAATTTAATAATCCA R: TTTATGAAACCCGTATTGGTG	50	245	[5]
p2_VvAGL11	F: TGTACACCAATACGGGTTTCAT R: GTTTGCTGGATTCCGATGT	53	171	[5]
p3_VvAGL11	F: CTCCTTTCCCTCTCCCTCT R: AAACGCGTATCCCAATGAAG	55	194	[5]
5U_VviAGL11	F: CGCCATTCTCTCTCGCTAT R: GTGCAAAAACGCGTATCCCA	55	319	[8]
VvSD10	F: AGAGTCAATTTGGATTAAGAGCGAGTAATTATATTGT R: GGAAAAATCCATCGCTAACAAAGTATTAATTCTCTTCA	56	111	[9]

度,稀释至工作浓度后采用荧光毛细管电泳仪(ABI 3730 xL遗传分析仪)进行验证;在毛细管电泳数据分析中,当某一峰的ht(peak height)值大于400 RFU(relative fluorescence units)或大于主峰ht值的20%时,认定其为有效峰。

### 1.5 KASP分子标记PCR扩增条件及基因型验证

KASP标记引物包括正向引物Fa和Fb,Fa引物用5'-荧光素-CE亚磷酰胺(FAM)染料特异性识别A等位基因;Fb引物则使用5'-六氯荧光素-CE亚磷酰胺(HEX)染料特异性识别C等位基因。将正向引物

Fa、Fb和反向通用引物R按1:1:3的比例混合制备KASP引物混合物。反应体系包括2 μL模板DNA,0.5 μL KASP引物混合物,5 μL KASP 2×PCR Mix,2.5 μL ddH<sub>2</sub>O,总体积为10 μL。在Light Cycler 480(LC480)上的PCR反应程序为:95℃预变性10 min;95℃变性15 s,61~55℃退火延伸60 s,10个循环(每循环降低0.6℃);95℃变性15 s,55℃退火延伸60 s,32个循环;37℃荧光读数1 min。反应结束后,根据检测到的不同荧光信号来判断样本的基因型情况(表2)。

表 2 KASP 分子标记对应引物名称及信息

Table 2 KASP molecular marker correspond to primer name and its information

引物名称 Primer name	引物序列(5'-3') Primer sequence (5'-3')	等位基因 Allele	参考文献 Reference
KASP_VviAGL11	Fa: <u>GAAGGTGACCAAGTTCATGCTATCATATTGGGCTGAAAGAAATTGA</u> Fb: <u>GAAGGTCTGGAGTCAACGGATTTCATATTGGGCTGAAAGAAATTGC</u> R: TATCAACACATGAGTTCAATGCCATCCAG	A C	[11]

注:单下划线的序列是 FAM 标记,双下划线的序列是 HEX 标记。

Note: Sequences with a single underline are labeled with FAM; sequences with a double underline are labeled with HEX.

为了验证 KASP\_VviAGL11 标记的基因型,采用常规 PCR 技术扩增位于 Chr18: 26889269~26889678 区域片段长度为 410 bp 的 DNA 序列,然后进行桑格测序,以确认 Chr18:26889437 位置的样本基因型与 KASP\_VviAGL11 标记检测到的基因型一致。VviAGL11\_410 的正向引物序列为 5'-GGC-TACTTGGTGATTATGTGCTCT-3',反向引物序列为 5'-GAAGGCACAACAGTTGATACCGATC-3'<sup>[11]</sup>。PCR 反应体系为 25  $\mu$ L: 2 $\times$ Phanta Max Master Mix 12.5  $\mu$ L, 正、反向引物各 1  $\mu$ L (10  $\mu$ mol $\cdot$ L<sup>-1</sup>), 2  $\mu$ L DNA 模板 (50 ng $\cdot$  $\mu$ L<sup>-1</sup>), ddH<sub>2</sub>O 补至 25  $\mu$ L。反应程序: 95  $^{\circ}$ C 预变性 3 min; 94  $^{\circ}$ C 变性 15 s, 适宜 Tm 值退火 15 s, 72  $^{\circ}$ C 延伸 30 s, 共 35 个循环; 72  $^{\circ}$ C 延伸 5 min, 扩增产物在 4  $^{\circ}$ C 保存。具体退火温度根据引物设计进行筛选。引物合成及测序由生工(上海)生物技术有限公司完成。

### 1.6 数据处理

根据 Vihinen<sup>[19]</sup>、Baldi 等<sup>[20]</sup>和 马亚茹等<sup>[9]</sup>等计算 6 项指标: 假阳性 (false positive, FP)、假阴性 (false negative, FN)、真阳性 (true positive, TP)、真阴性 (true negative, TN)、准确率 (accuracy) 和无核检测率 (detection rate of seedlessness, NDR)。假阳性表示样本携带无核等位基因,但不表现无核表型;假阴性表示不携带无核等位基因,却表现无核表型。准确率/%=(TP+TN)/(TP+FP+TN+FN), 无核检测

率/%=TP/(TP+FP)。为评估表型与分子标记数据之间的关联,利用 R 软件对分子标记的等位基因及基因型进行卡方 ( $\chi^2$ ) 独立性检验 ( $P<0.05$ )。

## 2 结果与分析

### 2.1 SSR 分子标记的等位基因检测及基因型分析

使用 5 个 SSR 分子标记对 2 个杂交群体的 F<sub>1</sub> 代单株进行鉴定。结果(表 3)显示, 5 个分子标记检测到的等位基因大小为 101~318 bp; 表现最好的等位基因如表 4、表 5 所示, 且无核等位基因均从无核父本遗传。分子标记 p1\_VvAGL11、p2\_VvAGL11、p3\_VvAGL11、5U\_VviAGL11、VvSD10 在携带相应等位基因的种质中, 无核种质所占比例分别为 63.6%、48.9%、68.0%、66.7% 和 70.3%。

5 个 SSR 分子标记中, 5U\_VviAGL11 检测到的基因型数量最多, 每个标记表现最好的基因型如表 6、表 7 所示。分子标记 p1\_VvAGL11、p2\_VvAGL11、p3\_VvAGL11、5U\_VviAGL11、VvSD10 在携带相应基因型的种质中, 无核种质所占比例分别为 69.8%、63.2%、80.1%、72.6% 和 84.0%。

### 2.2 KASP 分子标记的等位基因检测及基因型分析

通过 KASP 标记对自然群体和 2 个杂交群体的 F<sub>1</sub> 代单株进行检测, KASP\_VviAGL11 检测到的 A:A 和 A:C 为无核基因型, C:C 为有核基因型。KASP\_VviAGL11 标记在阳光玫瑰 $\times$ 红艳无核杂交

表 3 5 个分子标记在 4 个父母本中的基因型

Table 3 Genotypes of five molecular markers in four parental lines

分子标记 Molecular marker	阳光玫瑰 Shine Muscat	红艳无核 Hongyan Seedless	红地球 Red Globe	郑艳无核 Zhengyan Seedless
p1_VvAGL11	250/257	250/257	250/257	250/257
p2_VvAGL11	158/171	158/171	158/171	158/171
p3_VvAGL11	185/187	185/195	185/185	185/195
5U_VviAGL11	298/304	308/316	304/308	316/318
VvSD10	101/107	105/107	107/107	105/113

表4 SSR标记在阳光玫瑰×红艳无核杂交后代的等位基因分布与无核种质相关性

Table 4 Allele distribution of SSR markers in the Shine Muscat × Hongyan Seedless hybrid progeny and their association with seedless germplasm

SSR 标记 SSR marker	等位基因 Allele/bp	频率 Frequency	表型 Phenotype		相关性 Correlation	
			—	+	$\chi^2$	P
p1_VvAGL11	250	0.49	31	35	31.366	2.137E-08**
p2_VvAGL11	171	0.63	35	49	2.405	0.121
p3_VvAGL11	195	0.46	31	30	16.746	4.300E-05**
5U_VviAGL11	316	0.47	31	32	23.261	1.000E-06**
VvSD10	105	0.44	31	28	18.164	2.000E-05**

注:—表示无核;+表示有核;\*\*表示极显著相关( $P<0.01$ )。下同。

Note:— means seedless;+ means seeded;\*\* means extremely significant correlation ( $P<0.01$ ). The same below.

表5 SSR标记在红地球×郑艳无核杂交后代的等位基因分布与无核种质相关性

Table 5 Allele distribution of SSR markers in the Red Globe × Zhengyan Seedless hybrid progeny and their association with seedless germplasm

SSR 标记 SSR marker	等位基因 Allele/bp	频率 Frequency	表型 Phenotype		相关性 Correlation	
			—	+	$\chi^2$	P
p1_VvAGL11	250	0.51	53	13	73.473	1.020E-17**
p2_VvAGL11	171	0.79	56	46	16.964	3.800E-05**
p3_VvAGL11	195	0.47	52	9	29.547	5.457E-08**
5U_VviAGL11	316	0.47	51	9	56.198	6.552E-14**
VvSD10	105	0.46	52	7	57.673	3.096E-14**

表6 SSR标记在阳光玫瑰×红艳无核杂交后代的基因型分布与无核种质相关性

Table 6 Genotype distribution of SSR markers in the Shine Muscat × Hongyan Seedless hybrid progeny and their association with seedless germplasm

SSR 标记 SSR marker	基因型 Genotype	频率 Frequency	表型 Phenotype		相关性 Correlation	
			—	+	$\chi^2$	P
p1_VvAGL11	250/250	0.42	30	26	37.571	8.814E-10**
p2_VvAGL11	158/171	0.47	35	28	53.389	2.736E-13**
p3_VvAGL11	185/195	0.20	19	8	34.311	4.697E-09**
5U_VviAGL11	304/316	0.25	19	14	22.450	2.000E-06**
VvSD10	105/107	0.19	18	7	33.526	7.032E-09**

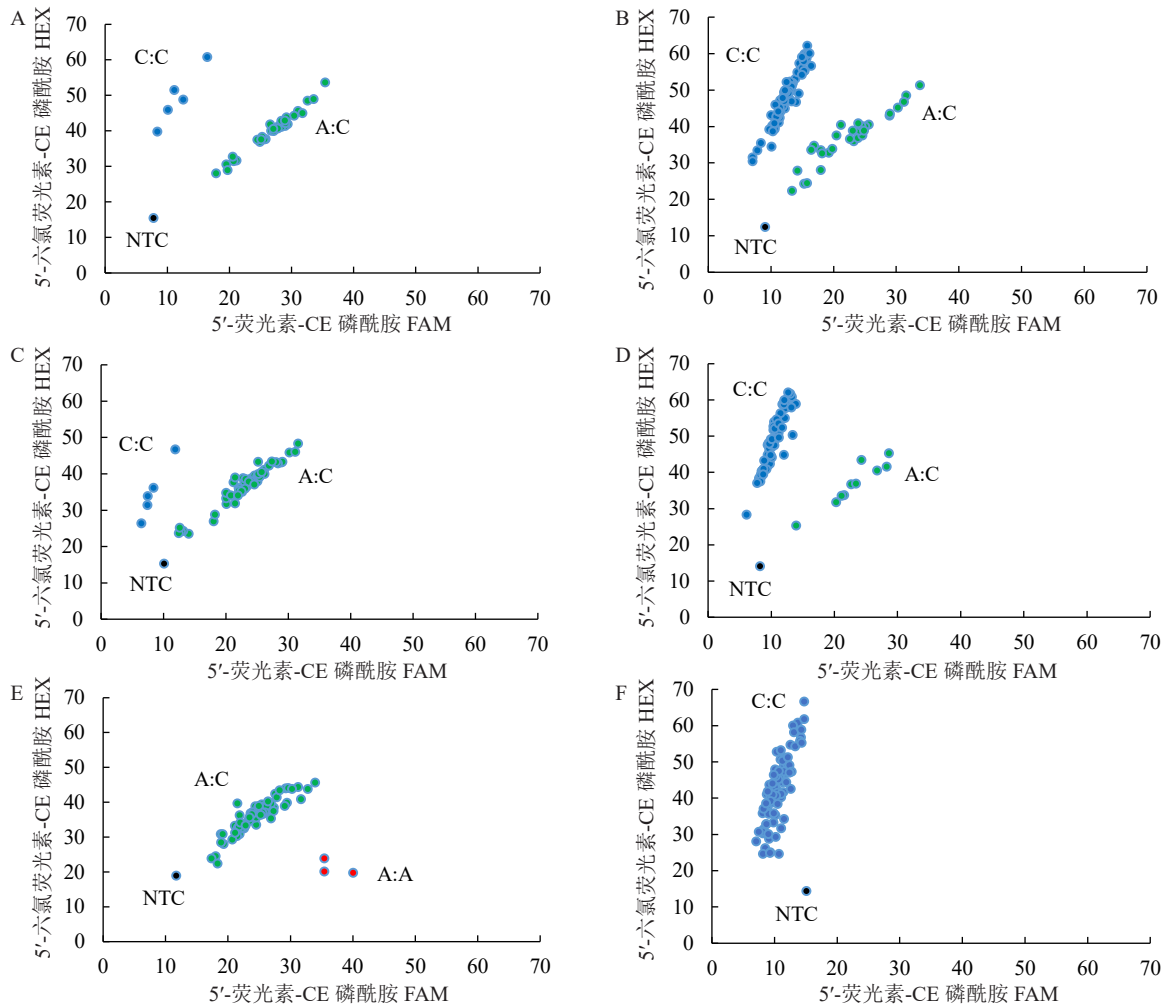
表7 SSR标记在红地球×郑艳无核杂交后代的基因型分布与无核种质相关性

Table 7 Genotype distribution of SSR markers in the Red Globe × Zhengyan Seedless hybrid progeny and their association with seedless germplasm

SSR 标记 SSR marker	基因型 Genotype	频率 Frequency	表型 Phenotype		相关性 Correlation	
			—	+	$\chi^2$	P
p1_VvAGL11	250/250	0.47	51	9	78.981	6.272E-19**
p2_VvAGL11	171/171	0.40	37	14	29.151	6.694E-08**
p3_VvAGL11	185/195	0.47	52	9	29.300	6.199E-08**
5U_VviAGL11	308/316	0.31	34	6	40.821	1.668E-10**
VvSD10	105/107	0.43	50	6	84.776	3.342E-20**

群体中的35份无核种质(图2-A)和99份有核种质(图2-B)中分别扩增出31个A:C基因型和70个C:C基因型;在红地球×郑艳无核杂交群体中的56份无

核种质(图2-C)和73份有核种质(图2-D)中分别扩增出52个A:C基因型和64个C:C基因型。在由157份二倍体葡萄种质组成的自然群体中,68份无核种



C:C(蓝点)表示有核基因型;A:A(红点)表示无核基因型;A:C(绿点)表示无核基因型;NTC(黑点)表示阴性对照(未添加 DNA 样品)。  
C:C (blue dots) indicates the seeded genotype; A:A (red dots) indicates the seedless genotype; A:C (green dots) indicates the seedless genotype;  
NTC (black dots) indicates the negative control (no DNA samples added).

图 2 420 份不同遗传背景葡萄种质有核/无核性状 KASP\_VviAGL11 标记基因型

Fig. 2 KASP\_VviAGL11 marker genotyping of seeded and seedless traits in 420 grape germplasm resources with diverse genetic backgrounds

质(图2-E)均检测到A等位基因,基因型为A:C和A:A;89份有核种质(图2-F)均检测到C等位基因,基因型为C:C。

### 2.3 不同类型分子标记准确率对比

对6个SSR和KASP类型无核分子标记进行验证,由表8、表9可知,VvSD10的无核鉴定准确率和

表 8 分子标记在阳光玫瑰×红艳无核杂交群体中的验证结果

Table 8 Validation results of molecular markers in the Shine Muscat × Hongyan Seedless hybrid population

分子标记 Molecular marker	等位基因 Allele/bp	假阳性 FPR/%	假阴性 FNR/%	无核检测率 NDR/%	准确率 Accuracy/%
VvSD10	105	20.90	2.99	52.54	76.12
KASP_VviAGL11	A	21.64	2.99	51.67	75.37
p3_VvAGL11	195	22.39	2.99	50.82	74.63
5U_VviAGL11	316	23.88	2.99	49.21	73.13
p1-VvAGL11	250	26.12	2.99	46.97	70.90
p2-VvAGL11	171	36.57	0.00	41.67	63.43

注:A 为单碱基突变位点。下同。

Note: A indicates a single nucleotide mutation site. The same below.

表9 分子标记在红地球×郑艳无核杂交群体中的验证结果

Table 9 Validation results of molecular markers in the Red Globe × Zhengyan Seedless hybrid population

分子标记 Molecular marker	等位基因 Allele/bp	假阳性 FPR/%	假阴性 FNR/%	无核检测率 NDR/%	准确率 Accuracy/%
VvSD10	105	5.43	3.10	88.14	91.47
KASP_VviAGL11	A	6.98	3.10	85.25	89.92
p3_VvAGL11	195	6.98	3.10	85.25	89.92
5U_VviAGL11	316	6.98	3.88	85.00	89.15
p1_VvAGL11	250	10.08	2.33	80.30	87.60
p2_VvAGL11	171	35.66	0.00	54.90	64.34

无核检测率最高,KASP\_VviAGL11、p3\_VvAGL11和5U\_VviAGL11均有较高的无核鉴定准确率和无核检测率,同时也有较低的假阳性率和假阴性率。p2\_VvAGL11的无核鉴定准确率和无核检测率最低。

### 3 讨论

SSR标记是一种基于特异性引物PCR扩增的分子标记,由少数核苷酸重复单元组成,具有高多态性、多等位基因和共显性遗传等优势,因而在遗传研究和分子育种中得到广泛应用<sup>[21-22]</sup>。安栋梁<sup>[14]</sup>通过对p1\_VvAGL11、p2\_VvAGL11和p3\_VvAGL11等3个标记的分析发现,p3\_VvAGL11表现最佳。Ben-nici等<sup>[23]</sup>在16个杂交组合获得的97个F<sub>1</sub>单株中发现,p3\_VvAGL11检测到的194 bp等位基因与无核性状关联性较强。张泉等<sup>[15]</sup>在自然群体中对p1\_VvAGL11、p2\_VvAGL11和p3\_VvAGL11鉴定后发现,p1\_VvAGL11表现出更高的准确性,而p3\_VvAGL11则表现出更高的无核检测率。在本研究中,p1\_VvAGL11、p2\_VvAGL11和p3\_VvAGL11在阳光玫瑰×红艳无核杂交群体中的无核鉴定准确率分别达到了70.9%、63.43%和74.63%;在红地球×郑艳无核杂交群体中的鉴定准确率分别达到了87.60%、64.34%和89.92%。相比之下,p3\_VvAGL11有较高的无核鉴定准确率和无核检测率,与前人研究结果基本一致。p1\_VvAGL11和p2\_VvAGL11标记在无核性状鉴定中的准确率较低且基因型分离比例不符合孟德尔遗传分离规律;推测其原因可能在于这两个标记位于启动子区域,该区域序列变异较为丰富<sup>[5]</sup>,从而影响引物结合效率,在PCR过程中产生一定程度的等位基因扩增偏好性,进而引起峰型判读误差。因此,这两个标记不适用于本研究群体的无核性状鉴定。在进行相关试验操作过

程中,仪器或试验操作会造成扩增片段长度出现偏差<sup>[24]</sup>,因此在鉴定SSR标记扩增片段长度时,应加入标准样品进行校对,以降低试验误差并确保结果的可靠性。陈豆豆等<sup>[16]</sup>在自然群体和红地球×黎明无核F<sub>1</sub>代杂交群体验证了分子标记5U\_VviAGL11的有效性;鉴定结果显示,无核准确率及无核检测率较高,且都有较低的假阳性率和假阴性率。笔者在本研究中对2个杂交群体中的验证结果表明,分子标记5U\_VviAGL11均有较高的无核鉴定准确率和无核检测率,与前人的研究结果基本一致。马亚茹等<sup>[9]</sup>根据红地球×森田尼无核F<sub>1</sub>代杂交群体开发的分子标记VvSD10位于无核主效基因*VviAGL11*内部,等位基因111 bp与无核性状密切相关。张泉等<sup>[15]</sup>在208个自然群体中对VvSD10的研究结果显示,鉴定到的105 bp等位基因与无核性状相关,无核鉴定准确率和无核检测率分别为61.60%和63.03%。在本研究的鉴定结果中,VvSD10检测到的105 bp等位基因在2个杂交群体中均表现出了最高的无核鉴定准确率和无核检测率。

单核苷酸多态性(simple nucleotide polymorphisms, SNP)技术因为灵活、高效和经济逐渐成为研究热点<sup>[25]</sup>,KASP技术也更简单、高效、经济和精确<sup>[26-27]</sup>。Wang等<sup>[11]</sup>根据A:C杂合位点开发的KASP\_VviAGL11在自然群体和3个F<sub>1</sub>代杂交群体的81个子代中均检测到与无核性状密切相关的等位基因A的准确率为100%。在本研究中,KASP\_VviAGL11在2个杂交群体中的准确率均非100%;因遗传背景不同,在2个杂交群体中的无核鉴定准确率和无核检测率有较大差异。对于KASP\_VviAGL11标记鉴定出的假阳性和假阴性样本,均通过VviAGL11\_410标记进行了验证,发现A:C杂合位点的基因型与KASP\_VviAGL11标记的鉴定结果一致。研究表明,*VviAGL11*基因18号染色体

上导致第 197 位精氨酸替换为亮氨酸的 A:C 突变位点,在红地球×森田尼无核 F<sub>1</sub> 代中有核与无核群体中突变等位基因 A 的频率差异不显著,无法有效区分有核与无核性状<sup>[3]</sup>。此外,部分无核性状是由其他次要基因引起的<sup>[8]</sup>,这最终导致标记之间的准确性存在差异。在所检测的无核种质中,意外地检测到 3 种纯合 A:A 基因型。根据遗传规律,纯合 A:A 基因型无核材料的获得特别困难并且具有重要的研究价值,当与纯合的 A:A 基因型无核材料杂交时可以大大提高无核后代的比率<sup>[11]</sup>。

在以往的研究中,无核性状分子标记的准确率往往通过整合多个杂交群体的数据来进行总体评估<sup>[11,23]</sup>,而本研究则进一步比较了同一分子标记在不同杂交群体中的独立鉴定效果。结果显示,根据同一个基因开发的分子标记在不同遗传背景中的鉴定效果有较大的差异。这 2 个杂交群体的无核性都来源于无核白<sup>[28]</sup>,但是 KASP\_VviAGL11 标记鉴定出的 A:C 基因型和 C:C 基因型在 2 个杂交群体中的分离比并不相同且偏离表型分离比。其中在阳光玫瑰×红艳无核杂交群体中的基因型分离比为 7:9,表型分离比为 1:3;在红地球×郑艳无核杂交群体中的分离比为 1:1,表型分离比为 7:9,这也暗示了无核白中可能存在多个无核性状相关基因<sup>[8]</sup>。因此不同杂交群体之间的遗传背景差异可能导致 *VviAGL11* 的遗传效应以及与其他无核相关基因的互作效应存在差异,从而影响分子标记在不同遗传背景下的鉴定准确率。

## 4 结 论

通过自然群体和阳光玫瑰×红艳无核、红地球×郑艳无核 2 个 F<sub>1</sub> 代杂交群体对 6 个无核分子标记进行验证,*VvSD10* 和 *KASP\_VviAGL11* 的无核鉴定准确率及无核检测率最高,综合表现最优,可作为辅助葡萄无核育种过程中的可靠标记。

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