Embryo Rescue and Molecular Marker-Assisted Selection of Hybrid Seedless Grape

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Abstract

Seedless grapes play an important role in fresh food and dry production. New varieties breeding by hybridization with seedless varieties as female parents is the most effective way to cultivate seedless varieties. However, the embryos of Seedless varieties cannot develop normally, so it is difficult to obtain hybrid offspring as hybrid female parent. Moreover, grape is a perennial tree species with highly heterozygous genes, with long breeding cycle and low efficiency. In this study, embryo rescue technology was used to cultivate hybrid offspring by crossing with ‘Ruby Seedless’ as female parent and ‘Hongqitezao’ as male parent, so as to solve the problem that seedless varieties can not be female parent, and molecular technology was used to carry out assisted breeding research to solve the problems of long cycle and low efficiency. TP-M13-SSR technique was used to carry out authenticity breeding. SCAR marker SCF27-2000 was used to detect the seedless traits of hybrid plants, phenotypic traits was used to verify the results of molecular markers, and Seedless trait-related SSR markers VMC7F2, VrSD10 and P3_VvAGL11 was used to detect and verify the genotypes of individual plants with inconsistent detection results by the two methods. In this study, a total of 384 hybrid offspring were finally obtained, and the hybridization rate was 84.43%. A total of 163 fruit-bearing plants were identified, and the phenotypes of their seeds were identified. The coincidence rate of genotypic and phenotypic analyses was 93.88%. Additionally, 305 F₁ plants were detected using the SCF27-2000 marker, and the abortion rate was 64.92%. We speculate that the inconsistent results were caused by parthenocarpy, SCF27 marker limitation, among other factors. Overall, this study shows that embryo rescue is an effective method for breeding seedless grape cultivars, and the application of molecular markers could facilitate the early identification of hybrid traits, and improve breeding efficiency.

Introduction

Grape (Vitis vinifera L.) is grown all over the world, and has the second largest cultivation area and yield among all fruit crops (Kong 2002). The seedless trait is an important index of grape fruit. Seedless varieties fulfill the market for table grapes, which are usually consumed fresh or as raisins after drying. Therefore, the development of seedless cultivars is an important goal of grape breeding projects. In the past, because the embryos of seedless varieties could not develop normally, seedless varieties could be selected only through conventional hybridization, with very low efficiency (Zhao et al. 2004). With the application of tissue culture technology in hybrid breeding, seedless varieties can be used as female parents to realize high-efficiency seedless grape breeding (Ramming 1982, 1990). A number of seedless grape varieties have been developed to date using the tissue culture technology, including ‘Qincui seedless’, ‘Qinhong seedless’ (Wang et al. 2004), ‘Shenai’ (Jiang et al. 2014), ‘Melissa’ (Ramming et al. 1998), ‘Thorncorp’, ‘Autumncrip’, ‘Midnight’ and ‘Adora’ (Vial et al. 2005; Fan et al. 2012, 2017; Jiang 2016), among others.

With the development of molecular biology techniques, molecular markers are being increasingly used in grape breeding. The simple sequence repeat (SSR) marker TP-M13-SSR uses fluorescence to detect the PCR amplification products. Because of its good repeatability, high accuracy and simple operation procedure, the TP-M13-SSR marker has been used for grape fingerprinting and genetic relationship analysis (Ma et al. 2018; Wang et al. 2020).

To date, great progress has been made in the investigation of the seedless traits of grape using molecular markers. In 1998, Lahogue and colleagues developed the SCP18 and SCC8 markers linked to the seedless trait gene, and proposed the seed development inhibitor (SDI) hypothesis, according to which the seedless trait is controlled by a single dominant gene and several recessive genes (Lahogue et al. 1998). Subsequently, many scholars confirmed the existence of SDI loci through quantitative locus (QTL) mapping (Cabezas et al. 2006; Costantini et al. 2008; Doligez et al. 2002; Mejía et al. 2007). The seedless trait is mainly controlled by the main SDI gene located on chromosome 18 (Cabezas et al. 2006; Costantini et al. 2008; Mejía et al. 2007). Wang et al. (2002) developed an 18-bp specific probe, GSLP-569, which can be used to detect non-nuclear genes; this marker was named as GSLP1. Mejia and Hinrichsen (2003) reported a sequence characterized amplified region (SCAR) marker, SCF27, which was associated with the seedless trait of grape. Wang et al. (2018) considered that the genes associated with SCP18 and SCC8 markers were recessive or minor genes, which could be detected in both nucleated and seedless varieties. The seedless gene probe GSLP1 could only detect seedless varieties related to ‘Thompson seedless’.

The gene marked by SCF27 is a dominant or major gene, which can be used for the identification of seedless traits. Cabezas et al. (2006) reported the SSR marker VMC7F2. QTL analysis showed that VMC7F2 is closely linked to a 198-bp allele at the SDI site, which can be used for the seedless character detection. The MADS-box gene VvAGL11 is homologous to known genes expressed in ovule and during seed development (Díaz-Riquelme et al. 2009), and is considered as a potential SDI controlling (Costantini et al. 2008; Mejía et al. 2011; Carlo Bergamini 2013). Ma et al. (2018) developed the SSR marker VVSD10 for the selection of seedless grape traits.

The objective of this study is to apply embryo rescue technology and molecular biotechnology to grape seedless hybrid breeding to solve the problems of low rate of Seedless offspring and low efficiency of seedless grape breeding.

Materials And Methods

Plant materials

Eight-year-old seedless grape variety ‘Thompson seedless’ was used as the control. Additionally, 8-year-old ‘Ruby Seedless’ and 6-year-old ‘Hongqitezao’ grape varieties were used as female and male parents, respectively, to generate 305 F₁ hybrids in 2017 (Table 1, Fig. 1).

Table 1 Characteristics of grape (Vitis vinifera L.) plants used in this study
**Plant material**  | **Role**  | **Characteristics**
---|---|---
'Thompson seedless' | Control | Seedless, yellow-green, oval
'Ruby Seedless' | Female parent | Seedless, purplish-red, oval
'Hongqitezao' | Male parent | Seeded, red-purple, rose flavor, round
305 hybrids | F₁ generation | -

**Primers**

Primer pairs for five SSR markers and one SCAR marker used in this study are listed in Table 2.

**Table 2 List of primers used in this study**

| Gene name | Forward primer (5'®3') | Reverse primer (5'®3') | Tm (°C) | Reference |
|---|---|---|---|---|
| Vchr4a | CAACTGGGATCCAAGACCTC | CAGCTTCACAGGTAACCACA | 55 | Cipriani et al. (2008) |
| Vchr18a | TTCCACCCGGTAAATATGA | CATCCAAACATCACGCTGAG | 53 | Tomic et al. (2012) |
| SCF27 | CAGGTGGGAGTAGTTATGGAATG | CAGGTGGGAGTGAAAGTTTGT | 62 | Mejia and Hinrichsen (2003) |
| VMC7F2 | AAGAAAGTTTGCAGTTTATGGTG | AAGATGACAATAGCGAGAGAA | 61 | Adam-Blondon et al. (2001) |
| VrSD10 | AGAGCTCATTTGGATTAAGAGAGTAATTGT | GGAAAAATCCATCGCTAAACAAATTAATTCTCTCTCT | 62 | Ma et al. (2018) |
| P3_VvAGL11 | CTCCCTTTCCCTCTCCCTCT | AAACCGTATCCCAATGAAG | Touch down | Bergamini et al. (2013) |

**Hybridization**

When 15% of the flower buds were open, the middle and upper robust inflorescences of vigorous plants were selected for pollen collection. The emasculation of inflorescences began 3–4 days before anthesis. The collected pollen were transferred to a 20-ml glass bottle and dried at low temperature. After emasculation, the inflorescences were rinsed with clean water. Pollination was started when mucus appeared on the stigma, and was performed once a day for 3 days.

**Ovule culture**

Young hybrid fruits were picked approximately 55 days after pollination, and placed in a 500-ml glass bottle. The fruits were washed with tap water for 20 min, soaked in 75% ethanol for approximately 1 min, and washed once with sterile water. Then, the fruits were disinfected with 0.5% (w/v) NaClO for 20 min, and washed twice with sterile water. Subsequently, the ovules were peeled, and 25–30 peeled ovules were placed in a 100-ml plastic bottle containing solid-liquid double-layer Emershad and Ramming (ER) medium (pH 6.0) supplemented with 500 mg/L CH₃, 1.21 g/L cysteine, 60 g/L sucrose, 3 g/L activated carbon and 0 or 7 g/L agar.

**Embryo culture**

After ovule culture for 8 weeks, young embryos were peeled, and embryo germination culture was started in 100-ml transparent glass test tubes containing Woody Plant (WP) medium supplemented with 20 g/L sucrose, 3 g/L activated carbon and 7 g/L agar. One young embryo was placed in each bottle.

**Subculture**

After the young embryo developed into healthy seedlings, the stem segments were cut and placed in 100-ml transparent plastic bottles (2–3 stem segments per bottle) containing half-strength Murashige and Skoog (1/2 MS) medium supplemented with 0.35 mg/L IBA, 20 g/L sucrose and 7 g/L agar. The stem fragments were cultured under LED light.

**Transplantation of embryo rescue-derived seedlings**

After the stem segments developed into seedlings, the robust seedlings were transplanted into the coconut bran: rotten leaf soil: vermiculite (2:1:1) mix under sterile conditions. The substrate and water used for transplanting seedlings were disinfected. After transplanting, the seedlings were covered with a transparent cover to maintain moderate humidity. Finally, the seedlings were transferred to the greenhouse for cultivation.


Breeding of seedless grape

Grape varieties ‘Ruby Seedless’ and ‘Hongqitezao’ were crossed in mid-May 2017. The ovules were stripped in mid-July 2017, and young embryos were stripped in late October 2017. Test-tube seedlings were subcultured and propagated in early December, and transplanted in mid-January 2018. In April, the survived seedlings were transferred to the greenhouse. Hybrid seedlings were harvested in mid-November and planted in the facility greenhouse in February 2019. Some results will be obtained in 2020, while the majority of results will be obtained in 2021.

Identification of authentic hybrids

Using the genomic DNA of ‘Ruby Seedless’ and ‘Hongqitezao’, two pairs of SSR-specific primers, Vchr4a-166/173bp and Vchr18a-159/172bp (Table 2), were selected from a collection of 16 primer pairs of SSR labeled primers, and used to identify authentic 305 hybrid offspring via the TP-M13-SSR technology. PCR was performed under the following conditions: initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 40 s, annealing at 55°C or 53°C for 40 s and extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The PCR products were determined via capillary electrophoresis by Beijing Junweinuo Technology Co., Ltd.

Marker assisted selection of seedless traits

The seedless trait of 305 F1 hybrids was screened using the seedless molecular marker SCF27-2000bp (Table 2), and the individual plants with the 2,000-bp PCR product were identified. PCR was performed under the following conditions: 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 55°C or 51°C for 30 s, and 72°C for 1.5 min, and finally 72°C for 10 min. Amplification products were separated by electrophoresis on 1.8% agarose gels, and photographed.

Identification of seedless traits and genotyping of seedless loci

A total of 163 hybrid progenies were used to monitor seed development and nucleation. The genotypes of some individual plants were determined using SSR markers such as VMC7F2, VrSD10 and P3_VvAGL11 (Table 2). PCR reactions were carried out according to the corresponding references, and PCR products were determined via capillary electrophoresis by Beijing Junweinuo Technology Co., Ltd.

Results

Breeding of seedless grape via embryo rescue

In this study, 7,400 ovules were obtained from the ‘Ruby Seedless’ × ‘Hongqitezao’ cross. A total of 1,296 embryos were obtained using the embryo rescue technology, resulting in 454 seedlings, of which 384 survived after transplanting. At present, 163 seedlings have been obtained. The embryo stripping and germination rates were 17.51% and 35.03%, respectively, while the seedling rate was 84.58%. The fruiting rate in the third year after planting was 42.45% (Table 3).

Table 3 Statistics of ‘Ruby Seedless’ × ‘Hongqitezao’ hybrid offspring

| Creation time | Breeding mode | No. of ovules | No. of naked embryos | Embryo stripping rate | No. of test-tube seedlings | No. of survived seedlings | No. of results |
|---------------|---------------|---------------|----------------------|-----------------------|-----------------------------|---------------------------|---------------|
| 2017          | Hybridization | 7,400         | 1,296                | 17.51%                | 454                         | 384                       | 163           |

Verification of F1 hybrids

In this study, 305 robust individual plants were selected from a collection of 384 hybrid seedlings, and the genotype of these hybrids was verified using primers for SSR markers Vchr4a and Vchr18a, which can amplify distinct bands from ‘Ruby Seedless’ and ‘Hongqitezao’. As shown in Table 5, Vchr4a-specific primers amplified three types of hybrid type, inbreeding type, pseudo-hybrid type and four genotypes of 173/180, 173/189, 173/173, 173/185. Among these types, the hybrid type included two genotypes (173/180 and 173/189); the inbreeding type included one genotype (173/173); and the pseudo-hybrid type, i.e., derived by intervention from foreign pollen, included one genotype (173/185). The results revealed 84.59% hybridization rate, 13.77% inbreeding rate and 1.64% foreign pollen intervention rate. The Vchr18a-specific primers amplified three types and seven genotypes: two genotypes (152/160 and 152/172) for the hybrid type; three genotypes (160/172, 160/160 and 172/172) for the inbreeding type; and two genotypes (143/172 and 155/160) for the pseudo-hybrid type. The hybridization, inbreeding and foreign pollen intervention rates were 84.26%, 14.43% and 1.31%, respectively. The average hybridization inbreeding and foreign pollen intervention rates were 84.43%, 14.10% and 1.48%, respectively (Table 4).

Table 4 Verification of the authenticity of 305 ‘Ruby Seedless’ × ‘Hongqitezao’ F1 hybrids

| Verification of F1 hybrids | | | | | | | |
|---------------------------|---|---|---|---|---|---|---|
|                           |   |   |   |   |   |   |   |


| Gene name | Maternal genotype | Paternal genotype | Genotype of F₁ hybrids |
|-----------|-------------------|-------------------|------------------------|
|           |                   |                   | Hybrid type | Inbreeding type | Pseudo-hybrid type |
| Vchr4a    | 173/173           | 180/189           | 173/180     | 173/189         | 173/185             |
|           |                   |                   | 132         | 126             | 42                  |
|           |                   |                   | 84.59%      | 13.77%          | 1.64%               |
| Vchr18a   | 160/172           | 152/152           | 152/172     | 160/172         | 143/172 155/160     |
|           |                   |                   | 125         | 132             | 24                  |
|           |                   |                   | 84.26%      | 14.43%          | 1.31%               |
| Average value |                   |                   | 84.43%      | 14.10%          | 1.48%               |

**Verification of SCAR marker SCF27-2000**

**Table 5** Detection of 163 fruit-bearing individual plants and determination of the seed developmental phenotype using the seedless trait-specific SCAR marker SCF27

| Determination method | No. of test samples | No. of seedless plants/plant | Coincidence rate (%) | No. of plants with seeds | Coincidence rate (%) |
|----------------------|---------------------|------------------------------|----------------------|--------------------------|----------------------|
| SCF27 marker         | 163                 | 98                           | 93.88                | 65                       | 80.00                |
| Phenotypic evaluation| 92                  |                              |                      | 52                       |                      |

The molecular marker SCF27-2000, linked to the seedless trait-controlling gene of grape, was used to identify the three grape varieties, ‘Thompson seedless’ (seedless control), ‘Ruby Seedless’ and ‘Hongqitezao’ (Fig. 4). The results showed that a 2,000-bp band was amplified from the seedless varieties ‘Thompson seedless’ and ‘Ruby Seedless’ but not from the seeded variety ‘Hongqitezao’. This shows that the SCF27-2000 marker is suitable for the detection of the seedless trait in hybrid populations of ‘Ruby Seedless’ × ‘Hongqitezao’. The results of 163 individual plants tested using the SCF27-2000 marker are shown in Table 6. Among the 163 individual plants, 98 carried the target bands, of which 92 were abortive; thus, the rate of coincidence between genotypic and phenotypic detection methods was 93.88% (Fig. 4, Table 5). Of the 65 plants that did not carry the target band of the marker, only 52 were seeds; thus, the coincidence rate was 80.00% (Fig. 4, Table 5).

**Detection of the seedless trait in 305 F₁ hybrids using the SCAR marker SCF27-2000**

The seedless trait of 305 F₁ hybrids was tested using the SCAR marker SCF27-2000. The results showed that 198 plants carried the 2,000-bp target band of 2000bp, accounting for 64.92% of the total samples (Table 6).

**Table 6** Genotyping of 305 F₁ plants using the SCAR marker SCF27-2000

| Gene name | Target band size (bp) | No. of test samples | Proportion of single plants in the target strip(%) |
|-----------|-----------------------|---------------------|-----------------------------------------------|
| SCF27     | 2,000                 | 305                 | 64.92                                         |

**Genotypic detection of the seedless trait loci in hybrid plants using SSR markers**

**Table 7** Genotypes detected using three SSR markers in three grape varieties

| SSR marker | Control genotype | Maternal genotype | Paternal genotype |
|------------|------------------|-------------------|-------------------|
| VMCF2      | 190/192          | 190/192           | 192/192           |
| VrSD10     | 97/99            | 97/99             | 99/99             |
| P3_VvAGL11 | 178/188          | 178/188           | 178/178           |

The seedless trait-related SSR marker VMCF2 detected 190/192 genotype loci in the control seedless variety ‘Thompson seedless’ and in the female parent variety ‘Ruby Seedless’, while 192/192 genotype loci were detected in ‘Hongqitezao’. This indicates that the 190 fragment at this site is related to embryo abortion. The seedless trait-related SSR markers VrSD10 and P3_VvAGL11 detected 97/99 and 178/188 genotypes in ‘Thompson seedless’ and ‘Ruby Seedless’, respectively, while 99/99 and 178/178 genotypes were detected in ‘Hongqitezao’, indicating that the 97 and 188 segments at the corresponding loci are related to embryo abortion. Plants containing the 97-bp and 188-bp fragments may display embryo abortion (Table 7).

The VMCF2 marker amplified a total of five genotypes, including four 190/190 genotypes, one 192/192 genotype and one 190/192 genotype, all of which were phenotypically seeded, indicating sampling errors or identification problems. The 192/192 genotype carries a 2,000-bp fragment, which indicates that the SCF27 marker has some limitations. The VrSD10 marker detected three 97/97 genotypes, one 99/99 genotype, two 97/99 genotypes
and five 97 genotypes, while the P3_VvAGL11 marker detected three 188/188 genotypes, one 178/178 genotype, two 178/188 genotypes and five 188
genotypes. The results obtained using VrSD10 and P3_VvAGL11 markers were identical to those obtained using the VMC7F2 marker (Table 8).

Table 8 Genotypes detected using three pairs of seedless trait-related SSR markers in six individual plants carrying the 2,000-bp insertion and displaying inconsistent phenotype

| Gene name | Genotypes | quantitative distribution | Genotypes | quantitative distribution | Genotypes | quantitative distribution |
|-----------|-----------|---------------------------|-----------|---------------------------|-----------|---------------------------|
| VMC7F2    | 190/190   | 4                         | 192/192   | 1                         | 190/192   | 1                         |
| VrSD10    | 97/97     | 3                         | 99/99     | 1                         | 97/99     | 2                         |
| P3_VvAGL11| 178/178   | 1                         | 188/188   | 3                         | 178/188   | 2                         |

The VMC7F2 marker detected two 190/190 genotypes, three 192/192 genotypes and eight 190/192 genotypes, which was amplified by three pairs of
primers for seedless traits on 13 individual plants that did not carry 2,000-bp fragment but showed the abortion phenotype. Ten individual plants carrying
the 190 genotype exhibited abortion, indicating that the phenotype was accurate, but the genotype determined using the SCF27 marker was incorrect.

Three individual plants with the 192/192 genotype showed abortion, which may be caused by the failure of normal pollination and fertilization, resulting
in parthenocarpy and consequently misjudgment on behalf of the staff. The VrSD10 marker detected two 97/97 genotypes, three 99/99 genotypes, eight
97/99 genotypes and ten 97 genotypes. P3_VvAGL11 amplified two 188/188 genotypes, three 178/178 genotypes, eight 178/188 genotypes and ten 188
genotypes. The results obtained using VrSD10 and P3_VvAGL11 markers were identical to those obtained using the VMC7F2 marker (Table 8).

Table 9 Genotypes detected using three pairs of seedless trait-related SSR markers in 13 individual plants carrying the 2,000-bp insertion and displaying inconsistent phenotype

| Gene name | Genotypes | quantitative distribution | Genotypes | quantitative distribution | Genotypes | quantitative distribution |
|-----------|-----------|---------------------------|-----------|---------------------------|-----------|---------------------------|
| VMC7F2    | 190/190   | 2                         | 192/192   | 3                         | 190/192   | 8                         |
| VrSD10    | 97/97     | 2                         | 99/99     | 3                         | 97/99     | 8                         |
| P3_VvAGL11| 178/178   | 3                         | 188/188   | 2                         | 178/188   | 8                         |

Discussion

The salvageability of grape embryo is determined by the grape genotype (Ramming et al. 2000; Valdez 2005; Tian and Wang 2008; Li et al. 2014, 2015,
2018). The larger the residual nucleus, the easier the embryo rescue (Pommer et al. 1995). ‘Ruby Seedless’ is a seedless variety with a large residual
nucleus developed from the ‘Emperor’ × ‘Pirovan-075’ cross in California, USA. In this study, ‘Ruby Seedless’ was used as a female parent to cross with
‘Hongqitezao’, and the embryo rescue rate was 17.51%, consistent with the results of Li et al. (2019) and Zhao et al. (2005).

Hybridization between two seedless grape varieties or between a seeded grape variety (as male parent) and a seedless grape variety (as female parent) is
an effective way to create new seedless grape varieties. Raming et al. (2000) used this approach to achieve a seedless ratio of 45–82%. In the current
study, we used the seedless × seeded cross to achieve a seedless rate of 64.92%.

Most grape varieties are monoecious. The key to hybridization is to remove stamens. A proper time should be selected for emasculation. Stigma may be
damaged if emasculation is conducted too early, and self-pollination may occur if the flower is emasculated too late. In this study, some problems were
detected in the emasculation of ‘Ruby Seedless’ ×‘Hongqitezao’ combination, as approximately 14.10% of the offspring were self-pollinated. Sealing
treatment after pollination is also important to ensure high hybridization efficiency.

SCF27 is a SCAR marker for genotyping the seedless trait of grape varieties. The amplified target band size was 2,000 bp. Mejia and Hinrichsen et al.
(2003) used the SCF27 marker to identify 127 hybrid offspring of the 'Ruby Seedless' × 'Thompson seedless' cross, and showed that the correlation
between the identified seedless rate and the actual seedless rate measured in the field was 81%. Wang et al. (2018) used the SCF27 marker to detect 116
hybrid progenies of three crosses, 'Ruby Seedless' × 'Houzhouhuiyu', 'Houzhouhuiyu' × 'Zaoyan' and 'Houzhouhuiyu' × 'Flame Seedless', and the
coincidence rate between molecular detection results and field identification results was 96.4%. Many additional studies (Akkurt et al. 2012; Li et al. 2015,
2018) affirmed its value in the identification of the seedless trait of grapes, and the results of the current study are consistent with those of previous
studies.

VMC7F2 (Pellerone et al., 2001) is an SSR marker located on grape chromosome 18, and is used for screening the seedless trait of grape cultivars. Previous
studies confirmed that VMC7F2 has a high application value, with high accuracy and stability in the process of marker-assisted selection of the
seedless trait of grape (Pellerone et al., 2001; Cabezas et al., 2006; Akkurt et al., 2012; Bergamini et al., 2013).

P3_VvAGL11 (Mejia et al. 2011) is another seedless trait-related SSR marker, and has a tetranucleotide repeat unit [(GAGA)]. Mejia et al. believed that
P3_VvAGL11 is the most effective marker for detecting the seedless character of grape, and used it on the 'Ruby seedless' × 'Sultanina' hybrid offspring
for three consecutive years. The efficacy of this marker in detecting the seedless trait of grape in the F1 generation was confirmed by Bergamini et al.
who studied 475 seeded × seedless combinations. This conclusion was further strengthened by the study of Conner et al. (2018). VvSD10 (Mayaru et al. 2018) is a seedless trait-related SSR marker developed by China.

In the current study, the genotypes of 18 F1 plants with seedless trait loci were detected by using the three markers whose molecular detection results were inconsistent with the field identification results. The results showed that the genotypes detected by the three markers were almost the same, suggesting that it was related with the genes linked to the three markers. One of the nineteen F1 plants carried a 2,000-bp band labeled with SCF27, and its genotypes at the three loci were 192/192, 99/99 and 178/178, respectively. Plants with these genotypes should be seeded, which indicates that SCF27 has limitations in detecting homozygous nucleated genotypes, consistent with the conclusions of Adamblondon et al. (2001) and Korpas et al. (2009).

**Conclusion**

The results of this study confirmed that embryo rescue is an effective method for breeding seedless grape varieties. The molecular marker technology can help realize the early identification of hybrid traits, reduce the number of ineffective plants, and improve the breeding efficiency.

**Abbreviations**

- CH Casein acids Hydrolysate
- ER Emershad and Ramming medium
- IAA Indole-3-acetic acid
- MS Murashige and Skoog (1962) medium
- SCAR Sequence characterized amplified region
- TP-M13-SSR Simple sequence repeat with tailed primer M13
- WP Woody Plant medium

**Declarations**

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**Conflict of interest** The authors declare that they have no conflict of interest.

**Author Contributions** All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Yong Wang, Yuling Li and Feng Sun. The first draft of the manuscript was written by Yong Wang and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**Data Availability** The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

**Human and animal rights** No human or animal material was used. The research conducted complied with all institutional and national guidelines.

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Figures
Figure 1

Photographs of the fruit of parental grape varieties. (a)'Ruby Seedless'. (b)'Hongqitezao'

Figure 2

Photographs of the seed or residual nucleus of parental grape varieties. (a) 'Ruby Seedless' residual nucleus. (b)'Hongqitezao' seed

Figure 3
Generation of hybrid grape plants via embryo rescue. (a) Young hybrid fruit. (B) Hybrid fruit. (c) Ovule culture. (d) Stripping naked embryo. (e) Immature embryo germination. (f) Test tube seedling. (g) Embryo rescue-derived $F_1$ hybrids.

Figure 4

Detection of three varieties by the seedless trait-specific SCAR marker SCF27. M, Marker; 1, 'Thompson seedless'; 2, 'Ruby Seedless'; 3, 'Hongqitezao'