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

Yong Wang (✉ wangyong3711100@163.com)

Research Institute of Grape and Melon of Xinjiang Uygur Autonomous Region, Shanshan <https://orcid.org/0000-0001-9673-7549>

Yuling Li

Research Institute of Grape and Melon of Xinjiang Uygur Autonomous Region, Shanshan

Feng Sun

Research Institute of Grape and Melon of Xinjiang Uygur Autonomous Region, Shanshan <https://orcid.org/0000-0002-7571-5487>

Guohong Wu

Research Institute of Grape and Melon of Xinjiang Uygur Region, Shanshan

Aizezi Sulaiman

Research Institute of Grape and Melon of Xinjiang Uygur Autonomous Region, Shanshan

Shijian Bai

Research Institute of Grape and Melon of Xinjiang Uygur Autonomous Region, Shanshan

Zhaorong Hua

Research Institute of Grape and Melon of Xinjiang Uygur Autonomous Region, Shanshan

Pingfeng Guo

Research Institute of Grape and Melon of Xinjiang Uygur Autonomous Region, Shanshan

Research Article

Keywords: Grapevine, seedless breeding, embryo rescue, molecular marker

Posted Date: December 28th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1185496/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

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 can not 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', 'Autumnncrisp', '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 *VvAGL 11* 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	F ₁ 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')	T _m (°C)	Reference
Vchr4a	CAACTGGGATCCAAGACCTC	CAGCTTCACAGGTAACCACA	55	Cipriani et al. (2008)
Vchr18a	TTCCACCCGGTAAATATGA	CATCAAACATCACGCTGAG	53	Tomic et al. (2012)
SCF27	CAGGTGGGAGTAGTGGAATG	CAGGTGGGAGTAAGATTTGT	62	Mejia and Hinrichsen (2003)
VMC7F2	AAGAAAGTTTGCAGTTTATGGTG	AAGATGACAATAGCGAGAGAGAA	61	Adam-Blondon et al. (2001)
VrSD10	AGAGCTCATTTGGATTAAGAGCGAGTAATTATATTGT	GGAAAAATCCATCGCTAACAAAGTATTAATTCTCTTCA	62	Ma et al. (2018)
P3_VvAGL11	CTCCCTTTCCCTCTCCCTCT	AAACGCGTATCCCAATGAAG	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 CH, 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 Junweiniu 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 Junweiniu 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

Gene name	Maternal genotype	Paternal genotype	Genotype of F ₁ hybrids							
			Hybrid type		Inbreeding type			Pseudo-hybrid type		
Vchr4a	173/173	180/189	173/180	173/189	173/173			173/185		
			132	126	42			5		
			84.59%		13.77%			1.64%		
Vchr18a	160/172	152/152	152/160	152/172	160/172	160/160	172/172	143/172	155/160	
			125	132	24	8	12	2	2	
			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
VMC7F2	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 VMC7F2 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. Plants containing this fragment may show 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 fragments 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 VMC7F2 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 parthenocarp 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 9).

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’ × ‘Pirovan075’ 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’ × ‘Huozhouheiyu’, ‘Huozhouheiyu’ × ‘Zaoyan’ and ‘Huozhouheiyu’ × ‘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)_n]. 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.

(2013), 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 Korpasetal 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

Funding This research was supported by the Natural Science Foundation of Xinjiang Uygur Autonomous Region (2020D01A98) and by China Agriculture Research System of MOF and MARA(CARS-29).

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.

References

1. Adam-Blondo AF, Lahogue-Esnault F, Bouquet A, Boursiquot JM (2001) Usefulness of two SCAR markers for marker-assisted selection of seedless grapevine cultivars. *Vitis* 40(3):147-155
2. Akcrt M, Cakir A, Shidfar M, Celikol BP, Soylemezoglu G (2012) Using SCC8, SCF27 and VMC7f2 markers in grapevine breeding for seedlessness via marker assisted selection. *Genet Mol Res* 11(3):2288-2294
3. Bergamini C, Cardone MF, Anaclerio A, Perniola R, Pichierri A, Genghi R, Alba V, Forleo LR, Caputo AR, Montemurro C, Blanco A, Antonacci D (2013) Validation assay of p3-VvAGLII marker in a wide range of genetic background for early selection of stenospermocarpy in *Vitis vinifera* L. *Mol Biotechnol* 54(3):1021-1030
4. Cabezas JA, Cervera MT, Ruiz-Garcia L, Carreno J, Martinez-Zapater JM (2006) A genetic analysis of seed and berry weight in grapevine. *Genome* 49:1572-1585
5. Cipriani G, Marrazzo MT, Di Gaspero G, Pfeiffer A, Morgante M, Testolin R (2008) A set of microsatellite markers with long core repeat optimized for grape (*Vitis* spp.) genotyping. *BMC Plant Biol* 8(1): 127.
6. Costantini L, Battiana J, Lamaj F, Fanizza G, Grando MS (2008) Berry and phenology-related traits in grapevine (*Vitis vinifera* L.): from quantitative trait loci to underlying genes. *BMC Plant Biol* 8(1):38

7. Diaz-Riquelme J, Ljivietky D, Martinez-Zapater JM, Carmona ML (2009) Genome-wide analysis of MIKCC-type MADS Box genes in grapevine. *Plant Physiol* 149(1):354-369
8. Doligez A, Bouquet A, Danglot Y, Lahogue F, Riaz S, Meredith CP, Edwards KJ, This P (2002) Genetic mapping of grapevine (*Vitis vinifera* L.) applied to the detection of QTLs for seedless and berry weight. *Theor Appl Genet* 105:780-795
9. Fan XC, Zhang Y, Jiang JF, Sun HS, Li M, Liu CH (2012) Progress in breeding of fresh grape varieties in recent 20 years. *Sino-overseas Grapevine & Wine* 2012(2): 53-59 (in Chinese)
10. Jiang Ai L, Xi XJ, Cheng JS, Tian YH. Breeding of a new early-ripening grape variety-Shen Ai. *J Fruit Sci* 31(2):335-336 (in Chinese)
11. Korpas A, Baranek M, Pidra M, Hradili KJ (2009) Behaviour of two SCAR markers for seedlessness within Central European varieties of grapevine. *Vitis*, 48(1):33-42
12. Kong QS, Liu CH, Pan X, Liu SJ (2002) Current situation, trend, problems and countermeasures of fresh grape development at home and abroad. *China Agricultural Information Express*, 2002(7):3-7 (in Chinese)
13. Li G, Ji W, Wang G, Zhang J, Wang Y (2014) An improved embryo-rescue protocol for hybrid progeny from seedless *Vitis vinifera* grapes × wild Chinese *Vitis* species. *Vitro Cell Dev Biol - Plant* 50:110-120
14. Li Z, Li T, Wang Y, Xu Y (2015) Breeding new seedless grapes using in ovule embryo rescue and marker-assisted selection. *Vitro Cell Dev Biol - Plant* 51:241-248
15. Li T, Li Z, Yin X, Guo Y, Wang Y, Xu Y (2018) Improved in vitro *Vitis vinifera* L. embryo development of F1 progeny of Delight × Ruby seedless using putrescine and marker-assisted selection. *Vitro Cell Dev Biol - Plant* 54:291-301. <https://doi.org/10.1007/s11627-018-9895-0>
16. Ma YR, Feng JC, Liu CH, Fan XC, Sun HS, Jiang JF, Zhang Y (2018) Development and application of SSR new molecular marker for seedless traits in grape. *Sci Agric Sin* 51(13):2622-2630 (in Chinese)
17. Mejia N, Hinrichsen P (2003) New highly assertive scar marker potentially useful to assist selection for seedlessness in table grape breeding. *Acta Hortic* 603:559-564
18. Mejia N, Gebauer M, Munoz L, Hewstone N, Munoz C, Hinrichsen P (2007) Identification of QTLs for seedlessness, berry size, and ripening date in a seedless × seedless table grape progeny. *AJEV* 58(4): 499-507
19. Boursiquot JM, Le CL, Ramos R, de los ÁMM, Houel C, Casanueva X, Guerrero M, Soto B, Mejía N, Hinrichsen P, Adam-Blondon AF (2011) Molecular, genetic and transcriptional evidence for a role of VvAGLII in stenospermocarpic seedlessness in grapevine. *BMC Plant Biol* 11 (1):57
20. Pommer CV, Ramming DW, Emershad RL (1995) Influence of grape genotype, ripening season, seed trace size, and culture date on in ovule embryo development and plant formation. *Bragantia* 54:237-249
21. Ramming DW, Emershad RL (1982) In – ovule embryo culture of seeded and seedless *Vitis Vinifera* L. (Abst.) [J]. *HortScience* 17(3):487.
22. Ramming DW, Ledbetter CA, Tarailo R (1990) Hybridization of seedless grapes. *Vitis*, Special issue:439-444
23. Ramming DW, Emershad RL, Tarailn R (2000) A stenospermocarpic, seedless *Vitis vinifera* × *Vitis rotundifolia* hybrid developed by embryo rescue. *HortSci* 35:732-734
24. Scheulke M (2000) An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology* 18:233-234.
25. Li SS, Li ZY, Zhao YN, Zhao J, Luo QW, Wang YJ (2020) New disease-resistant, seedless grapes are developed using embryo. *3 Biotech* 10(4):1-12
26. Pellerone FI, Kj E, Thomas MR (2001) Grapevine microsatellite repeats: isolation, characterisation and use for genotyping of grape germplasm from Southern Italy. *Vitis* 40:179-186
27. Tomic L, Stajner N, Jovanovic CT, Cvetkovic M, Javornik B (2012) Identity and genetic relatedness of Bosnia and Herzegovina grapevine germplasm. *Sci Hortic* 143:122-126
28. Valdez JG (2005) Immature embryo rescue of grapevine (*Vitis vinifera* L.) after an extended period of seed trace culture. *Vitis* 44:17-23.
29. Vial PM, Crisosto CH, Crisosto GM (2005) Early harvest delays berry skin browning of 'Princess' table grapes. *Calif Agric (Berkeley)* 59(2):103-108
30. Wang Y, Li YL, Wu GH, Luo QW, Li C, Bai SJ, Zheng HY (2018) Seedless trait identification of grape (*Vitis vinifera*) by using SCAR marker SCF27. *Mol Plant Breed* 16(4):1197-1207 (in Chinese)
31. Wang Y, Sun F, Sulaiman Aizezi, Li YL, Wu GH, Luo QW (2020) Construction of fingerprint of 19 self bred grape varieties (lines). *Xinjiang Agricultural Sciences*, 57(12):2238-2249 (in Chinese)
32. Wang YJ, Lamikanra O (2002) Application and synthesis on the DNA probe for detecting seedless genes in grapevine. *J N W Agric Univ* 30(3):42-46
33. Zhao MZ, Su JL, Qian YM, Wang ZW, Diao MN (2005) Technology of ovule culture for Ruby Seedless grape cultivar. *J Fruit Sci* 22(2):66-168 (in Chinese)
34. Zhao YL, Cheng GZ, Wang HH (2004) Breeding of seedless grape and application of embryo rescue technology. *Bulletin of Biology* 39(9):15-16 (in Chinese)

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 F₁ 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'