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Original scientific article

F_1 PROGENY OF 'RUBY SEEDLESS'× 'HONGQITEZAO' DEVELOPED AND SELECTED USING EMBRYO-RESCUE AND MOLECLAR MARKERS

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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 technique 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 cannot be female parent; and molecular markers 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 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. The results showed that a total of 384 hybrid offspring were finally obtained, and the hybridization rate was 84.43%. The seed phenotypes of 163 fruit-bearing plants were identified, and the coincidence rate of genotypic and phenotypic analyses was 95.71%. Additionally, 305 F_1 plants were detected using the SCF27 marker, and the abortion rate was 64.92%. This study shows that embryo rescue is an effective method for breeding seedless grape cultivars, and the application of

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molecular markers could facilitate the early identification of hybrid traits, and improve breeding efficiency.

Keywords: Grapevine, seedless breeding, F_1 progeny, embryo rescue, molecular marker

INTRODUCTION

Grape (*Vitis vinifera* L.) is grown all over the world, and has the second largest cultivation area and yield among all fruit crops. 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. 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; LI *et al*., 2020).

With the development of molecular biology techniques, molecular markers are being increasingly used in grape breeding. The simple sequence repeat (SSR) marker simple sequence repeat with tailed primer M13 (TP-M13-SSR) uses fluorescence to detect the PCR amplification products (SCHUELKE *et al*., 2000). 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.

To date, great progress has been made in the investigation of the seedless trait 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. Subsequently, many scholars confirmed the existence of *SDI* loci through quantitative locus (QTL) mapping (DOLIGEZ *et al*., 2002; CABEZAS *et al*., 2006; MEJIA *et al*., 2007; COSTANTINI *et al*., 2008). The seedless trait is mainly controlled by the main *SDI* gene located on chromosome 18 (LAHOGUE *et al*., 1998). WANG *et al*., (2002) developed an 18-bp specific probe, which can be used to detect seedless genes named GSLP1. SCF27 is a sequence characterized amplified region (SCAR) marker for genotyping the seedless trait of grape varieties and amplified 2,000 bp target band (MEJIA *et al*., 2003). Many additional studies (AKKURT *et al*., 2012; LI *et al*., 2015, 2018) affirmed its value in the identification of the seedless trait of grapes. 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 seeded 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. 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). In 2011, P3_VvAGL11 was developed for seedless trait-related SSR marker by *MEJIA et al*., and

proproved to be a very effective marker for detecting grape seedless trait by using 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 F_1 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 (MA *et al*., 2018) is a seedless trait-related SSR marker developed by China. BENNICI *et al*. (2019) conducted the marker efficiency validation analysis was performed on four molecular markers linked to seedlessness of SCF27, VMC7F2, p3_VvAGL11, VvVn16 in table grapes in different genetic backgrounds, considered VMC7F2 and p3_VvAGL11 were the most efficient markers.

The objective of this study is to apply embryo rescue technique 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 ‘Ruby Seedless’ and six-year-old ‘Hongqitezao’ grape varieties were used as female and male parents, respectively, to generate 305 F_1 hybrids, and of these, 163 plants had results in 2020 and 2021. Additionally, Eight-year-old seedless grape variety ‘Thompson seedless’ was used as the control. ‘Ruby Seedless’ is seedless, purplish-red, oval. ‘Hongqitezao’ is seeded, red-purple, rose flavor, round. The F_1 plants were numbered for 'RH'. ‘Thompson seedless’ is seedless, yellow-green, oval.

Hybridization

Grape varieties ‘Ruby Seedless’ and ‘Hongqitezao’ were crossed in mid-May 2017. When 15% of the flower buds were open, the middle and upper robust inflorescences of vigorous plants were selected for pollen collection. The collected pollen were transferred to a 20-ml glass bottle and dried at low temperature. The emasculation of inflorescences began 3–4 days before anthesis（Fig.1 a）. 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

Immature hybrid fruits were picked approximately 55 days after pollination in mid-July (Fig.1 b), 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 Casein acids Hydrolysate (CH), 1.21 g/L cysteine, 60 g/L sucrose, 3 g/L activated carbon and 0 or 7 g/L agar (Fig. 1c).

Embryo culture

After ovule culture for 8 weeks, young embryos were peeled (Fig. 1d) in October 2017, and embryo germination culture was started in 100-ml transparent glass test tubes containing

Woody Plant (WP) medium supplemented with 0.2 mg/L 6-Benzylaminopurine (6-BA), 20 g/L sucrose, 3 g/L activated carbon and 7 g/L agar. One young embryo was placed in each bottle (Fig. 1c).

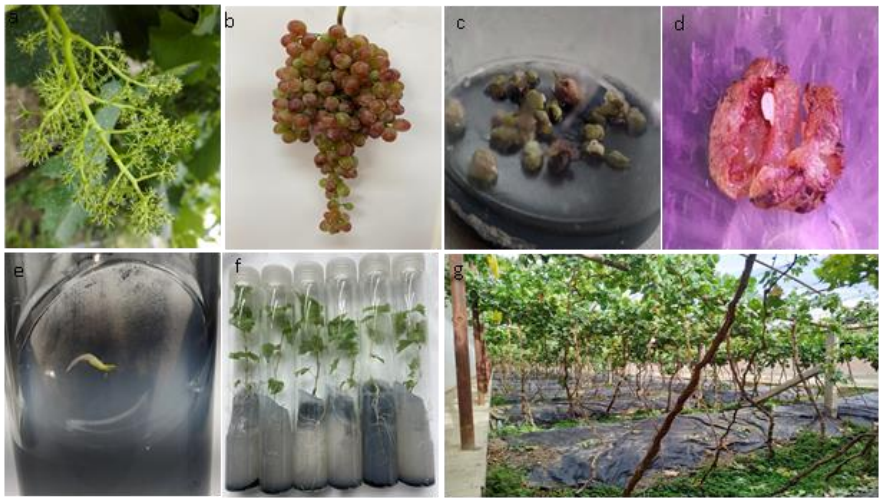

Fig. 1 Generation of hybrid grape plants via embryo rescue. (a) The inflorescence removed the stamens. (b) Hybrid fruit. (c) Ovule culture. (d) Stripping naked embryo. (e) Immature embryo germination. (f) Test tube seedling. (g) Embryo rescue-derived F_1 hybrids.

Subculture

After the young embryo developed into healthy seedlings (Fig. 1f), 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 3-Indolebutyric acid (IBA), 20 g/L sucrose and 7 g/L agar. The stem fragments were cultured under LED light. Test-tube seedlings were sub-cultured and propagated in December 2017, and transplanted in mid-January 2018.

Transplantation of embryo rescue-derived seedlings

In January, 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. 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 (Fig. 1g).

Using the genomic DNA of ‘Ruby Seedless’ and ‘Hongqitezao’, two pairs of SSR-specific primers, Vchr4a-166/173 bp and Vchr18a-159/172 bp (Table 1), 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℃ for 4 min, followed by 35 cycles of denaturation at 94℃ for 40 s, annealing at 55℃ or 53℃ for 40 s and extension at 72℃ for 1 min, and a final extension at 72℃ for 10 min. The PCR reaction system was conducted with 20 μl reaction volumes containing 10 μL of 2× Taq Master Mix, 0.8 μL of SSR double primer mixture (10 μM/mL), 2.0 μL of DNA template (20 ng/μL), 7.2 μL of dd H_2O. 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 F_1 hybrids was screened using the seedless molecular marker SCF27 (Table 1), and the individual plants with the 2,000-bp PCR product were identified. PCR was performed under the following conditions: 94℃ for 5 min, followed by 35 cycles of 94℃ for 30 s, 55℃ or 51℃ for 30 s, and 72℃ for 1.5 min, and finally 72℃ for 10 min. The PCR reaction system was conducted with 25 μl reaction volumes containing 12.5 μL of 2× Taq Master Mix , 1 μL of primer (10 μM / mL), 2.0 μL of DNA template (20 ng/μL), 9.5 μL of dd H_2O. Amplification products were separated by electrophoresis on 1.8% agarose gels, and photographed.

Table 1. List of primers used in this study

Gene name	Forward primer (5'→3')	Reverse primer (5'→3')	Tm (℃)	Reference
Vchr4a	CAACTGGGATCCAAGACCTC	CAGCTTCACAGGTAACCACA	55	Cipriani *et al*., (2008)
Vchr18a	TTCCCACCCGGTAAATATGA	CATCCAAACATCACGCTGAG	53	Tomic *et al*., (2012)
SCF27	CAGGTGGGAGTAGTGGAATG	CAGGTGGGAGTAAGATTTGT	62	Mejia and Hinrichsen (2003)
VMC7F2	AAGAAAGTTTGCAGTTTATGGTG	AAGATGACAATAGCGAGAGAGAA	61	Mejla *et al*., (2011)
VrSD10	AGAGCTCATTTGGATTAAGAGCG AGTAATTATATTGT	GGAAAAATCCATCGCTAACAAA GTATTAATTCTCTTCA	62	Ma *et al*., (2018)
P3_VvAGL11	CTCCCTTTCCCTCTCCCTCT	AAACGCGTATCCCAATGAAG	Touch down	Bergamini *et al*., (2013)

Identification of seedless traits and genotyping of seedless loci

A total of 163 hybrid progenies were used to monitor seed development and nucleation. The seed types of them had been comprehensive identification based on seed size and embryo, divided into three types: seedless, rudimental seed and ligneous. The genotypes of some individual plants were determined using SSR markers such as VMC7F2, VrSD10 and P3_VvAGL11, PCR reactions were carried out according to the corresponding references (Table 1). 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 technique, 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 2).

Table 2. 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

Identification of the hybridization authenticity of F_1 hybrid seedlings

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 3, 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.

Table 3. Verification of the authenticity of 305 F_1 hybrids of 'Ruby Seedless' × 'Hongqitezao'

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%	

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.

Verification of SCAR marker SCF27

The molecular marker SCF27, linked to the seedless trait-controlling gene of grape, was used to identify the three grape varieties, 'Ruby Seedless', 'Hongqitezao' and 'Thompson seedless' (seedless control) (Fig. 2). The results showed that a 2,000-bp band was amplified from the seedless varieties 'Ruby Seedless' and 'Thompson seedless', but not from the seeded variety 'Hongqitezao'. This shows that the SCF27 marker is suitable for the detection of the seedless trait in hybrid populations of 'Ruby Seedless' × 'Hongqitezao'.

In this study, seed phenotypes of parental material, control and 163 fruit-bearing F_1 progeny were identified (Table 4). The results showed that parent 'Ruby Seedless', control 'Thompson seedless' and 103 F_1 progeny for SL type, parent 'Hongqitezao' and 58 F_1 progeny for LS type and 2 F_1 progeny for RL type. The hybrid seed type ratio was matched against theoretical values ($X^2=11.34>X^2_{0.05}=3.84$) in X^2 detection, indicating that grape seedless traits should be controlled by multiple genes.

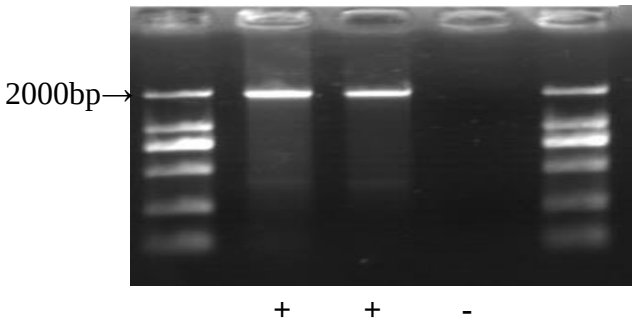

Fig. 2 Detection of three varieties by the seedless trait-specific SCAR marker SCF27. M, Marker; 1, 'Thompson seedless'; 2, 'Ruby Seedless'; 3, 'Hongqitezao'

Table 4. They are reported seeds phenotype, SCF27 marker detection results and the coincidence r-ate of seed type and marker detection results of 'Ruby seedless ' × 'Hongqitezao' hybrid offspring（SL=seedless,RL=rudimental seed, LS=ligneous）

Seeds phenotype	Distribution of F1 generations	SCF27 bp		Coincidence rate (%)
		+	-	
SL	103	102	1	99.03
RL	2	2	0	100.00
LS	58	6	52	89.66
Total	163	110	53	95.71
X^2	11.34	19.93		

Note：*mean $X^2 < X^2_{0.05} = 3.84$,that observation times and theoretical frequency match.

The seedless trait of 163 fruit-bearing F_1 progeny had been detected by SCF27 marker,

showing 110 F_1 progeny were positive (+), 53 F_1 progeny were negative (-), and 102 were positive (+) and 1 was negative (-) in LS type, and 2 were positive (+) in RL type, and 6 were positive (+) and 52 were negative (-) in RL type. The types of F_1 progeny by SCF27 were also inconsistent with theoretical values ($X^2=19.93>X^2_{0.05}=3.84$) in X^2 detection, which is consistent with the results of seed type detection. The coincidence rate of seed phenotypes and the results of SCF27 marker detection were 95.71%, including 99.03% for F_1 progeny with SL type, 100% for F_1 progeny with RL type, and 89.66% for LS type (Table 4). This shows that the significance of SCF27 markers in the detection of seedless traits in F1 progeny, especially for the detection of abortive hybrid single strain.

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

The seedless trait-related SSR marker VMC7F2, VrSD10, P3_VvAGLS11 detected 190/192, 97/99, 178/188 genotypes loci in the control seedless variety 'Thompson seedless' and in the female parent variety 'Ruby Seedless' with positive by SCF27 markers, while 192/192, 99/99, 178/178 genotype loci were detected in the male parent 'Hongqitezao' with negative by SCF27 markers. This indicates that 190/192, 97/99, 178/188 are all seedless heterozygous dominant genotypes, and 190 bp, 97 bp, 188 bp fragment at this site are related to embryo abortion. Plants containing this fragment may show embryo abortion. 192/192, 99/99, 178/178 are seedy homozygous recessive genotypes, and the plants with this genotypes are seedy (Table 5).

Table 5. They are reported genotypes detected using three pairs of seedless trait-related SSR markers in seven individual plant whose seeds phenotype were inconsistent with results of the seedless trait molecular marker SCF27 test（SL=seedless, RL=rudimental seed, LS=ligneous）

Name	Seeds phenotype	SCF27	Genotype		
			VMC7F2	VrSD10	P3_VvAGLS11
Thompson seedless	SL	+	190/192	97/99	178/188
Ruby Seedless	SL	+	190/192	97/99	178/188
Hongqitezao	LS	-	192/192	99/99	178/178
RH168	LS	+	190/190	97/97	188/188
RH289	LS	+	190/190	97/97	188/188
RH14	LS	+	192/192	99/99	178/178
RH228	LS	+	192/192	99/99	178/178
RH248	LS	+	190/192	97/99	178/188
RH67	LS	-	190/192	97/99	178/188
RH179	SL	-	192/192	99/99	178/178

The genotypes of seven plants, whose seeds phenotype were inconsistent with results of the seedless trait molecular marker SCF27, were tested by VMC7F2, VrSD10, P3_VvAGLS11 (Table 5). Four plants of LS type were tested positive by the seedless trait marker SCF27,and their locus genotypes were homozygous by three seedless trait SSR markers VMC7F2, VrSD10,

P3_VvAGLS11, among RH168, RH289 were homozygous dominant genes for respectively 190/190, 97/97, 188/188, and RH14 and RH228 were homozygous negative genes for respectively 192/192, 99/ 99, 178/178. This suggests that the seedless trait marker SCF27 may malfunction in detecting plants where the seedless trait locus is homozygous for genotype. One plant of LS type (RH248) was positive, and its locus genotype was dominant heterozygous genes for 190/192, 97/99, 178/188, this was possibly operate miss. One plant of LS type (RH67) was negative, and its locus genotype was dominant heterozygous genes for 190/192, 97/99, 178/188, this may also be caused by operate miss. Another plant of SL type (RH179) was negative, and its locus genotype was negative homozygous genes for 192/192, 99/99, 178/178, which may be due to the failure of normal pollination and fertilization.

Detection of the seedless trait in 305 F_1 hybrids using the SCAR marker SCF27

The seedless trait of 305 F_1 hybrids was tested using the SCAR marker SCF27. The results showed that 198 plants carried the 2,000-bp target band of 2000 bp, accounting for 64.92% of the total samples (Table 6).

Table 6. Genotyping of 305 F_1 plants using the SCAR marker SCF27

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

DISCUSSION

The salvageability of grape embryo is determined by the grape genotype (RAMMING *et al*., 2000; VALDEZ, 2005; 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%. 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. In the current study, we used the seedless × seeded cross to achieve a seedless rate of 64.92% detected by marker SCF27. The study found that 'Hongqitezao' is a variety with a denuclearization tendency, which also played a role in the seedless F_1 generation cultivation of this combination. However, the locus genotypes detected by VMC7F2, VrSD10, and and P3_VvAGLS11 were recessive homozygous, and there may be other genes affecting the nucleability of the variety.

This study reported hybrid authenticity was identified for 305 F_1 generations using SSR markers, Vchr4a and Vchr18a with parent-specific complementary bands. The authors believed that the majority of true hybrids in the hybrid population, there was a certain proportion of selfing hybrids, and a small number of false hybrids appeared. This shows that there is a high hybrid affinity between 'Ruby Seedless' × 'Hongqitezao', but in hybridization process, 'Ruby Seedless'may have closed flower powder phenomenon, and there are other pollen intervention. It is of great significance to carry out the authenticity identification.

MEJIA and HINRICHSEN (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%. The authors used the SCF27 marker to detect 163 fruit-bearing F_1 progeny of the 'Ruby Seedless' × 'Hongqitezao' cross, with a coincidence rate of 95.71%, believing that the marker was suitable for the seedless traits of this combination. However, due to the different seed types of the plants, the accuracy of the test results varies, and the accuracy of the plants with the abortive type is higher. Genotypes of seedless trait-associated loci also had some effect on the test results, with SCF27 marker failing on some plants carrying homozygous genotypes.

The fragments amplified by SSR markers were different from previous studies, such as VMC7F2, Literature reports (KARAAGAC *et al*., 2012) the gene fragments of seedless traits is 198 bp, but the result of this paper is 190 bp. VrSD10's 111bp (MA *et al*., 2018), but the result is 197 bp. P3_VvAGLS11's 194 bp (LI *et al*., 2018), but the result is 188bp. All genotypes of the three markers amplified on the test materials were completely consistent, and the associated seedless trait genes were dominant. In 163 fruit-bearing F_1 progeny the genotypes of RH67, RH248, RH168, RH289, and RH179 as determined by the three markers did not match the seed phenotype at a ratio of 3.07%. The identification of RH179 may be caused by human factors, RH67, RH248, RH168, RH289 carry dominant seedless genes, but the phenotype is ligneous, a phenomenon that is unexplained.

CONCLUSION

The results of this study confirmed that Embryo rescue hybrid breeding technology realizes seedless hybrid breeding in a seedless cultivar × seedy cultivar. The true hybrid rate and seedless ratio of F_1 progeny of the combination 'Ruby Seedless' × 'Hongqitezao' are very high.

SCF27 Marker is a very cost-effective technique for detecting seedless traits in this combination, but has some limitations.

VMC7F2, VrSD10, and P3_VvAGLS11 are all dominant markers of grape seedless traits and may be the same or similar to the seedless trait genes linked to them.

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F1 POTOMSTVO "RUBY BEZ SEMENA" × "HONGQITEZAO" RAZVIJENO I ODABRANO UPOTREBOM MARKERA ZA SPASAVANJE EMBRIONA I MOLEKULARNIH MARKERA

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Izvod

Grožđe bez semena igra važnu ulogu u proizvodnji sveže i suve hrane. Stvaranje novih sorti hibridizacijom sa sortama bez semena kao ženskim roditeljima najefikasniji je način oplemenjivanja sorti bez semena. Ipak, embrioni sorti bez semena ne mogu se normalno razviti tako da je teško dobiti hibridno potomstvo od hibridnog ženskog roditelja. Pored toga, vinova loza je višegodišnja vrsta drveća s visokoheterozigotnim genima s dugim ciklusom razmnožavanja i niskom efikasnošću. U ovoj studiji, tehnika spašavanja embriona korišćena je za dobijanje hibridnog potomstva ukrštavanjem "Ruby bez semena" kao ženskog roditelja i "Hongqitezao" kao muškog, čime se rešava problem da sorte bez semena ne mogu biti ženski roditelj; tehnologija molekularnih markera korišćena je da bi se rešili problemi dugog ciklusa oplemenjivanja i niske efikasnosti. TP-M13-SSR tehnika je korišćena za obavljanje autentničkog oplemenjivanja. SCAR marker SCF27-2000 korišćen je za otkrivanje svojstava hibridnih biljaka bez semena, fenotipska svojstva korišćena su za proveru rezultata molekularnih markera, a SSR markeri vezani uz svojstva bez semena VMC7F2, VrSD10 i P3_VvAGL11 korišćeni su za otkrivanje i proveru genotipova pojedinih biljaka s nekonzistentnim rezultatima dobijenih na ova dva načina. Rezultati su pokazali da je ukupno dobijeno 384 hibirdnih potomaka, te je stopa hibridiziracije 84.43%. Identifikovani su fenotipovi semena 163 plodonosne biljke, a koincidencija fenotipskih i genotipskih analiza bila je 95.71%. Dodatno, 305 F1 biljaka otkriveno je upotrebom SCF27-2000 markera dok je stopa prekida bila 64.92%. Ova studija pokazuje da je spašavanje embriona efikasan način za oplemenjivanje kultivara grožđa bez semena, a primena molekularnih markera mogla bi da olakša rano identifikovanje hibridnih svojstava i poboljša efikasnost oplemenjivanja.

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