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IN VITRO EMBRYO RESCUE OF F_1 PROGENIES FROM CROSSES BETWEEN SEEDLESS GRAPES AND CHINESE WILD TYPES

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Abstract

Ovules of seedless grapes × Chinese wild types were cultured by embryo rescue system through *in vitro*. The study indicated that ovules were cultured on Nitsch medium supplemented with IAA 0.5 mg/l + BA 0.5 mg/l + GA_3 0.5 mg/l. The best sampling duration for ovules inoculation was different among seedless grapes × Chinese wild types. They were 30 days in Olmo seedless grape × Heilongjiang seedling grape, 50 days (berry soft) in Olmo seedless grape × Jingxi-2 grape, 47 days (berry soft) in Olmo seedless grape × Beichun grape, and 20 days in long-bunch Sultanina × Heilongjiang seedling grape after pollination. Variation was also great when the same male parent was used with different seedless female parents and the use of different male parents with the same seedless female parents. When long-bunch Sultanina was used to cross with Chinese wild types as female parent. *In vitro* ovules cultured germination percentage (28.57%) and established plant percentage (28.57%) were higher than Olmo seedless grape as female parent. When Jingxi-2 grape was used to cross with seedless grapes as male parent, germination percentage (42.11%) and established plant percentage (36.84%) were all higher than Beichun and Heilongjiang seedling grape. The berries of OB after 35 days pollination were treated after 30 days of storage at 4°C, then ovules were cultured *in vitro* at 25°C. Development percentage (97.22%), germination percentage (37.14%), and established plant percentage (25.71%) were all the highest.

Introduction

Seedless grapefruit quality is excellent. The new seedless grape cultivar breeding is an important part of the grape breeding program. Zygotic embryos of seedless grapes cannot develop normal seeds because of abortion, so seedless grape cultivars are only taken as paternal parents in breeding new seedless grapes by conventional crossing, but the seedless progeny is only 0 to 15.9% (Pearson 1932, Loomis *et al.* 1979, Ledbetter *et al.* 1994). *In vitro* embryo rescue technology provides an attractive alternative to conventional methods of breeding for seedless grape by allowing recovery of progeny from abortive ovules of seedless grape × seedless grape (Ramming *et al.* 1982, Cain *et al.* 1983, Spiegel *et al.* 1985, Mathias *et al.* 1990, Ramming 1990). This technique could increase the proportion of seedless progenies (Burger *et al.* 2000, Notsuka *et al.* 2001, Li *et al.* 2014, Shi *et al.* 2018). During the breeding of seedless grapes, most seedless grapes susceptible to diseases are found (Alleweldt *et al.* 1988, Li *et al.* 2001). New seedless grape cultivars which are resistant to diseases need to be obtained. Goldy (1988) once obtained progenies with *V. vinifera* (2n = 38) fruit quality and *V. rotundifolia* (2n = 40) disease tolerance, but their chromosome number is different, having a crossing barrier, the seedling rate was extremely low. The wild grapes whose origin of species in China are not only resistant to diseases but also easy to cross with *V. vinifera* (Wang *et al.* 1998, Wan *et al.* 2008). With the embryo rescue technique coming, it is a feasible way to cross between the seedless grapes (*V. vinifera*) and Chinese

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wild types in order to breed new seedless grape cultivars which are resistant to diseases (Tian *et al.* 2008). It was less reported in hybridization of seedless grapes × Chinese wild types (Li *et al.* 2001). In this study, hybridization between seedless grapes and Chinese wild types are attempted to produce plants with seedless grape fruit quality and Chinese wild grape disease tolerance. The embryo rescue technique was studied by using 5 crosses as materials that were taking the seedless grapes as maternal parents. The objective of this research was to determine the feasibility of obtaining hybrids of seedless grapes × Chinese wild types. The effects of sampling durations, low temperature on ovule development and selection of parents on the germination and seedling survival of immature seeds from crosses between seedless grapes and Chinese wild types by *in vitro* embryo rescue culture were investigated. F1 progenies from seedless grapes × Chinese wild grapes were obtained.

Materials and Methods

Olmo seedless grapes have high sugar content. Long-bunch Sultanina berry is seedless, very big and long. Heilongjiang seedling grape, Jiangxi-2 grape and Beichun grape are all Chinese wild type. Heilongjiang seedling grape is resistant to ripe rot and cold. Jiangxi-2 grape is better resistant to downy. Beichun grape is better resistant to cold. Crosses: (1) OH: Olmo seedless grape × Heilongjiang seedling grape; (2) OJ: Olmo seedless grape × Jiangxi-2 grape; (3) OB: Olmo seedless grape × Beichun grape; (4) LH: long-bunch Sultanina × Heilongjiang seedling grape. Ovules of F_1 progenies from crosses between these seedless grapes × Chinese wild grapes were collected during different time points (days). Sampling dates (days) of OH were 30, 35, 40, 45 and 50 days after pollination. Sampling dates (days) of OJ were 20, 25, 30, 35, 40, 45, 50 and 50 days (soft). Sampling dates (days) of OB were 26, 30, 35, 39, 43, 47d and 47days (soft) after pollination. Sampling dates of (days) LH were 20, 24, 28, 32 and 36 days after pollination. Then all the cross-ovules were cultured *in vitro* on Nitsch medium + IAA 0.5 mg/l + BA 0.5 mg/l + GA_3 0.5 mg/l. Low temperature was treated. (1) T1: The berries of Olmo seedless grape × Beichun grape after 35 days pollination were treated after 30 days of storage at 4°C, then ovules were cultured *in vitro* at 25°C, (2) T2: *In vitro* ovules were treated at 4°C/30 days and then cultured at 25°C, (3) T3: After ovules were cultured *in vitro* 30 days, *in vitro* ovules were treated at 4°C /30 days and then cultured at 25°C, (4) CK: *In vitro* ovules were cultured at 25°C all the time.

Results and Discussion

Ovule germination and plant development from 5 crosses of seedless grapes × Chinese wild types were obtained by *in vitro* embryo rescue (Fig. 1 A-C). Table 1 showed that the best sample date of Olmo seedless grape × Heilongjiang seedling grape was 30 days after pollination. The development percentage was 79.31% and germination percentage was 23.91%. The best sample date of Olmo seedless grape × Jiangxi-2 grape was 50 days (soft) after pollination. The development percentage was 90.48 and germination percentage was 42.11. The best sample date of Olmo seedless grape × Beichun grape was 47 days (soft) after pollination. The development percentage was 92.11 and germination percentage was 42.86. The best sample date of Olmo seedless grape as female parent was 50 days and their berries have become soft. The best sample date of long-bunch Sultanina × Heilongjiang seedling grape was 20 days after pollination. The development percentage (70) was higher and germination percentage was 28.57. It is the pre-condition and key to successful embryo rescue to determine the optimum period of ovule sampling and inoculation (Celso *et al.* 1995, Guo *et al.* 2007). If the sample is taken too early, the embryo development degree is not complete, the embryo rescue efficiency is low, and the seedling rate is low. If the sample is taken too late, the embryo has been aborted, which also results in a low

seedling rate. Therefore, sampling should be appropriate and timely. Striem *et al.* (1992) mainly observed the embryo abortion period from the perspective of cytology as one of the criteria for determining the best sampling period. On the other hand, after ovule sampling and inoculation, the optimal sampling period was determined by the number of developmental embryos obtained from the ovules *in vitro*, which was also the threshold period of embryo abortion (Midani *et al.* 2002). Li *et al.* (2001) used the weight changes of seedless grape berries and ovules as an assistant to determine the optimal embryo rescue sampling period. Xu *et al.* (2001) summarized that the sampling period of embryo rescue could be determined according to the maturation period of the female parent. This study concluded that sample times (days) of *in vitro* embryo rescue culture of F_1 progenies from crosses between seedless grapes and Chinese wild grapes were different.

Table 1. The effect of sampling dates on *in vitro* ovules cultured.

Crosses	Sampling dates (days)	No. ovules cultured	Development of ovules		Germination of ovules	
			Number	Development (%)	Number	Germination (%)
OH	30	58	46	79.31	11	23.91
	35	30	17	56.67	1	5.89
	40	48	33	68.75	0	0.00
	45	30	13	43.33	1	7.69
	50	46	12	26.09	0	0.00
OJ	20	30	10	33.33	1	10.00
	25	53	25	47.17	0	0.00
	30	48	30	62.50	0	0.00
	35	60	39	65.00	0	0.00
	40	72	50	69.44	12	24.00
	45	60	45	75.00	9	20.00
	50 (soft berry)	42	38	90.48	16	42.11
OB	26	32	26	81.25	0	0.00
	30	46	36	78.26	0	0.00
	35	36	35	97.22	13	37.14
	39	32	28	87.50	4	14.29
	43	45	40	88.89	11	27.50
	47	34	32	94.12	12	37.50
	47 (soft berry)	38	35	92.11	15	42.86
LH	20	50	35	70.00	10	28.57
	24	53	29	54.71	6	20.69
	28	44	27	61.36	5	18.52
	32	48	32	66.67	2	6.25
	36	48	35	72.92	1	2.86

Female parent genotypes in hybrid combinations play a key role in embryogenesis, development and seedling formation. Centennial seedless, Crimson seedless and so on are not suitable for seedless grape hybridization as female parents (Pommer *et al.* 1995, Sahijram *et al.* 2004). When Delight, Olmo seedless, Thompson seedless and so on were female parents, higher

embryo rescue seedling rate could be obtained (Emershad *et al.* 1984, Li *et al.* 2001). Goldy *et al.* (1989) concluded that when the ovules of 10 seedless grapes were cultured *in vitro*, embryo germination rate varied from 0 ("Reliance") to 45 ("Venus"), indicating that the germination rate of embryos varied with the genotype of the female parent. Table 2 showed that when Heilongjiang seedling grape was male parent, *in vitro* ovules cultured germination percentage (28.57) and established plant percentage (28.57) of long-bunch Sultanina × Heilongjiang seedling grape were all higher. The male genotype also had a significant effect on the fruit setting rate and embryo rescue seedling survival rate (Gray *et al.* 1990, Ebadi *et al.* 2004, 2009). In this study, long-bunch Sultanina as female parent was better than Olmo seedless. Jingxi-2 grape as male parent was the best among three male parents. Table 3 showed that Olmo seedless grape crossed with Heilongjiang seedling grape (30d), Jingxi-2 grape (50d, soft), and Beichun grape (47d, soft) resulted in different germination rates. When Jingxi-2 grape was male parent, germination percentage (42.11) and established plant percentage (36.84) were all higher.

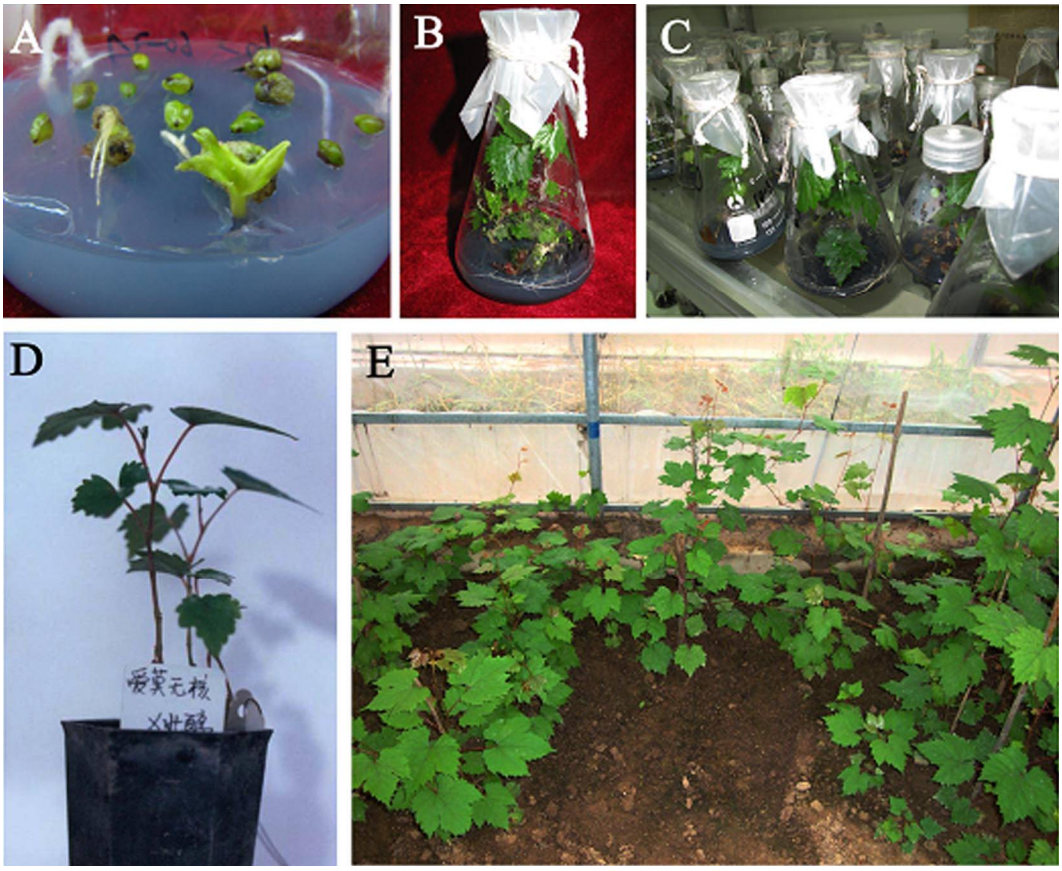

Fig. 1. Seedlings by seedless grape embryo rescue: A. Ovules cultured from seedless grapes × Chinese wild grapes after inoculated, B-C. Sterile seedlings from seedless grape embryo rescue, D. transplanted plantlets in pots after acclimatization and E. Seedless grape hybrid plants established in soil.

The promotion effect of low temperature on embryo development and germination has been widely studied. Table 4 showed the berries of Olmo seedless grape × Beichun grape after 35 days pollination were treated after 30 days of storage at 4°C, then ovules were cultured *in vitro* at 25°C.

Development percentage (97.22), germination percentage (37.14), and established plant percentage (25.71) were all the highest. Development percentage (93.33), germination percentage (35.71), and established plant percentage (21.43) of CK (*in vitro* ovules were cultured at 25°C all the time) were higher. After ovules were cultured *in vitro* 30 days, *in vitro* ovules were treated at 4°C/30 days and then cultured at 25°C, this treatment effect was the worst. Germination percentage was only 6.67. Agüero *et al.* (1996) concluded that low temperature treatment has a good effect on the germination of seedless grape embryo rescue. Tang *et al.* (2011) concluded that the ovules were inhibited by the low temperature treatment during the ovule development stage. In this study, young fruits were treated by low temperature before *in vitro* ovules cultured. It showed that the low temperature treatment had a certain promoting effect on embryo development.

Table 2. The effect of female parent on ovules cultured *in vitro*.

Crosses	No. ovules cultured	Development percentage	Germination percentage	Established plant percentage
OH (30 days)	58	79.31	23.91	10.87
LH (20 days)	50	70.00	28.57	28.57

Table 3. The effect of male parent on *in vitro* ovules cultured.

Crosses	No. ovules cultured	Development percentage	Germination percentage	Established plant percentage
OJ (50 days, soft)	42	90.48	42.11	36.84
OB (47 days, soft)	38	92.11	42.86	25.71
OH (30 days)	58	79.31	23.91	10.87

Table 4. The effect of different sequences of low temperature on ovules cultured *in vitro*.

Treatment	No. ovules cultured	Development percentage	Germination percentage	Established plant percentage
CK	30	93.33	35.71	21.43
T1	36	97.22	37.14	25.71
T2	48	62.50	6.67	0.00
T3	42	71.43	20.00	6.67

For transplantation, fit seedlings were selected. One plantlet was transplanted into each pot with proper matrix (Fig. 1D). Moisture was supplied. Seedlings were acclimated under constant temperature (25°C), high humidity, and strong light (3000 LX) in the seedling hardening room for 60 days. Acclimated seedlings were transferred to the greenhouse (approximate temperature: 25°C/day to 15°C/night) on April 15 (Fig. 1E) and planted in the field the next spring.

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