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The study of triploid progenies crossed between different ploidy grapes

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The cross between different ploidy grape was one of the effective ways to obtain new seedless cultivars, in this study, through testing the changes of the ovule weight and observing its anatomical structure, the period and mode of ovule abortion in crosses between diploid and tetraploid grape cultivars were studied, reciprocal cross was also conducted between them, the results indicated that the proper sampling period is one of the most important factors of the grape in ovule culture, unlike the seedless cultivars, the embryo abortion did not occur at the same time for most seeded cultivars crossed with different ploidy plants, the ovule abortion was only individual behavior. The period of ovule abortion was correlated with the mature period of the female parent. The abortive embryo of early-maturing cultivars occurred at five weeks after pollination, then the mid-maturing ones and late-maturing ones were at six and nine weeks after pollination comparatively. The reciprocal crossing results showed that this crossing form has great mating obstruction and the situation was more serious when tetraploid cultivars were used as female parent. Few seeds from 2X×4X had good germinability, while those from 4X×2X had lost their germinability and no seedlings were obtained, the highest germination rate and seedling survivals rate were obtained when the ovule was excised 60 days after pollination, it is easier to harvest hybrid progeny when using diploid as female parent.

Key words: *Vitis vinifera*, ovule culture, hybridization, polyploidy, reciprocal cross.

INTRODUCTION

Seedless grapes are usually preferred by customers for fresh consumption, 'Thompson Seedless' producing small fruit is a major seedless cultivar in the world, 'Red globe', 'Kyoho' and 'Italia' which produce large sized berries, are major seeded cultivars in USA, Japan and Italy, respectively; large size seedless berries are produced in China mainly with the aid of twice treatments. Seedless table grape demand is increasing in every parts of the globe, so the breeding of seedless grape has become the research focus in different programs, in order to improve the efficiency to get seedless grape, ovule rescue method have been developed (Spiegel-Roy et al.,

1985; Emershad et al., 1989; Valdez et al., 1997; Ponce et al., 2000). This method allows obtaining plants from crossed between diploid and tetraploid cultivars (Li et al., 1998; Pan et al., 1998; Yamashita et al., 1998; Xu et al., 2001; Zhao and Guo, 2004). With the technique of embryo rescue culture, several seedless cultivars were released from crossing between diploid and tetraploid, such as 'Honey seedless' (Mattsunoto et al., 2003) and 'Champion Seedless' (Zhao et al., 2000) and 'Summer black' (Bessho et al., 2000). There are many factors in the inducement of the embryo, in this study, we focused on the effects of crossing parents, reciprocal crossing, media type and sampling time on the germination and seedling survival rate, after more than ten years of study, the whole inducing system was established.

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Abbreviations: MS, Murashige and Skoog; NN, Nitsch and Nitsch; 6-BA, 6-benzylaminopurine; NAA, naphthaleneacetic acid; IAA, indole-3-acetic acid; GA₃, gibberellic acid.

MATERIALS AND METHODS

Hybridization

The experiment was carried out in Beijing Institute of Forestry and

Table 1. Cross combinations.

Cross	Female parent	Male parent
1	Muscat Hamburg 2X	Jingyou 4X
2	Agate early 2X	Fenghou 4X
3	Delaware 2X	Zizhenxiang 4X
4	Muscat Hamburg 2X	Delaware 4X
5	Muscat Hamburg 2X	Fenghou 4X
6	Kai ji 2X	Muscat Hamburg 4X
7	Zizhenxiang 4X	Delaware 2X
8	Jingyou 4X	Muscat Hamburg 2X
9	Fenghou 4X	Agate early 2X
10	Delaware 4X	Muscat Hamburg 2X

Pomology from 1999 to 2003, the cross was set as follows, shown in Table 1. All plants grown in the grape germplasm collection field, located in Beijing, the vines were 4 years old with a planting density of 1.5 m × 0.8 m, one rachis was chosen from the well developed shoot, 40 to 80 flowers were kept on the tested rachis according to the female parents. Emasculation was conducted 3 to 4 days before anthesis, after being emasculated, inflorescence were immediately rinsed with distilled water. The rachis were then encased in paper bags, the pollen were collected at the beginning of flowering and kept at room temperature with desiccation, then artificial pollination was conducted using a hairbrush twice when the stigmas begin to excrete secretion. 20 to 25 rachises were pollinated for each cross.

Fruits were collected at different times after pollination, from 5 weeks after pollination, 30 berries from each cross were excised once every week, the number of ovule in 10 berries was recorded and the color, shape and the structure of endosperm were observed, the other 20 berries were then surface sterilized with 70% ethanol for 1 min, followed by 0.1% HgCl₂ for 10 min and rinsed three times with sterilized water, the ovules were dissected so as not to damage ovular tissue and then transferred to appropriate media, the experiments were set with 20 ovules per treatment with three replications, all the plantlets were grown at the temperature of 25°C under a 16 h photoperiod with light intensity of 2000 to 3000Lx. After 8 to 12 weeks of ovule culture, germinated ovules were counted; the germination rate is defined as the number of germinated ovule/the number of inoculated ovule.

Culture mode

Two culture mode were set, (1) the ovule were transferred to media for continuing development, there were two types of media No. 1 and 2; after 40 days the embryo were excised and placed on the germination media, the seedlings were put on rooting media after germination; (2) the immature seeds were removed 60 days after pollination, the embryo were directly excised from the well developed ovule and transferred to the No. 3 media.

Media

Development media: MS (Murashige and Skoog, 1962) or NN (Nitch and Nitsch,) media with 0.1% activated carbon, 30 g/l sucrose, supplemented with IAA, NAA, GA₃ and 6-BA on different concentration, media No.1: MS+GA₃0.4 mg/l+IAA1.7 mg/l; media No.2: MS+6-BA1.0 mg/l+GA₃0.4 mg/l+NAA0.5 mg/l; media No. 3: NN+6-BA1.0 mg/l+GA₃0.4 mg/l+IAA0.5 mg/l; germination media: MS +6-BA 0.2 mg/l.

rooting media: 1/2 MS + IAA 0.2 mg/l.

RESULTS

Effect of sampling time on ovule germination rate

Sampling time is a very important factor in the embryo rescue procedure. Table 2 shows the average germination rate of different sampling time. The germinated ovule was obtained from July 7 (45 days after pollination) to August 7 (75 days after pollination). The highest rate was gotten from the sampling time from July 17 to 27 (55 to 65 days after pollination) when diploid cultivars were used as female parents, except for 'Kai ji', which is the extremely late maturing cultivars, the highest rate was from August 7 (55 to 65 days after pollination). This may be related to the fruit development of different cultivars; the seedlings were only obtained when Delaware 4X and Zizhenxiang were used as female parents and the highest rate was from August 7 (70 days after pollination), which indicated that the parental genotype highly affected the germination rate.

Effect of culture mode and media on ovule germination rate

The results showed that the ovule developed fast and better in the culture mode II, the maximum rate reached 36.67% and the average rate was 17.34%; for the mode I, the rate was 8.34 and 3.10%, respectively (Table 3).

For the culture mode I, the germination rate was very different from No.1 and 2 media. The average rate for all combination were 4.12 and 2.18%, respectively; when using diploid cultivars as female parents, the maximum rate reached 16.67% and average rate was 6.27% in No.1 media, while the rate was 1.77 and 3.33%, respectively in No. 2 media obviously lower than No. 1 media. Additionally, no seedling was obtained from No. 2 media, when using 'Kai ji' and 'Delaware' as female parent.

But when using tetraploid cultivars as female parent in No. 1 media, the seedling was obtained only in one cross (Zizhenxiang×Delaware), the germination rate was 4.04% and the average rate was only 1.01%. In No. 2 media, the maximum rate reached 10.27% and average rate was 3.35%.

The effect of reciprocal crossing on ovule germination rate

Almost all embryo derived from each combination when diploid were used as female parents seedling survived from all the cross combination; the average germination rate was 17.34%, but this rate declined to 2.18% when the female parent was tetraploid. The reciprocal crossing

Table 2. Effect of sampling time on ovule germination rate.

Cross	Sampling time (Month/Day)					
	6/28	7/7	7/17	7/27	8/7	8/17
*Delaware×Zizhenxiang		0.00	2.28%	0.00	2.50%	0.00
*Agate early×Fenghou	1.12	0.00	4.17%	4.29%		
*Muscat Hamburg×Jingyou	4.76%	0.00	8.75%	4.03%	0.00	0.00
*Muscat Hamburg×Fenghou			8.00%	2.94%	2.08%	
*Kai ji× 4X Muscat Hamburg			0.00	0.00	10.53%	
*Muscat Hamburg×4X Delaware			28.85%	9.08%	8.33%	
**Zizhenxiang× Delaware	0.00	0.00	7.69%	13.33%	20.83%	10.53%
**Fenghou×Agate early	0.00	0.00	0.00	0.00	0.00	
**Jingyou×Muscat Hamburg	0.00	0.00	0.00	0.00	0.00	0.00
**4X Delaware×Muscat Hamburg		5.00%	0.00	0.00	13.33%	

Cross column means female parent× male parent;* means 2X×4X; ** means 4X×2X.

Table 3. Effect of culture mode and media on ovule germination rate.

Cross	Culture mode I			Culture mode II
	No. 1 media	No. 2 media	Average	No. 3 media
*Delaware×Zizhenxiang	6.04%	0.00	3.02%	25.00%
*Agate early×Fenghou	2.63%	3.13%	2.88%	5.00%
*Muscat Hamburg×Jingyou	3.23%	1.39%	2.31%	9.17%
*Muscat Hamburg×Fenghou	1.39%	3.33%	2.36%	15.00%
*Kai ji× 4X Muscat Hamburg	16.67%	0.00	8.34%	
*Muscat Hamburg×4X Delaware	7.64%	2.78%	5.21%	36.67%
**Zizhenxiang× Delaware	4.04%	3.13%	3.58%	16.53%
**Fenghou×Agate early	0.00	0.00	0.00	
**Jingyou×Muscat Hamburg	0.00	0.00	0.00	
**4X Delaware×Muscat Hamburg	0.00	10.27%	5.14%	
2X×4X	6.27%	1.77%	4.02%	18.17%
4X×2X	1.01%	3.35%	2.18%	16.50%
Average	3.68%	2.56%	3.10%	17.34%

Cross column means female parent× male parent;* means 2X×4X; ** means 4X×2X.

data showed that the highest rate was 36.67%, got from the combination of Muscat hamburg×4X Delaware, the rate was only 10.27% in the reciprocal crossing, the rate were 25.00 and 16.53% in the Delaware×Zizhenxiang, 5% and 0 in the Agate early×Fenghou, 9.17% and 0 in the Muscat hamburg×Jingyou. The ovule fertility of tetraploid was low and the seed obtained was less than diploid, so this indicated that it is easier to harvest hybrid progeny when using diploid as female parent. And also, the ovules with immature embryos were the same weight or heavier than the mature ones.

The abortion time and rate of ovules in different combination

The ovules were dissected and observed at different

developing time to study the abortion time and growth pattern. From the chart, we can get that the seed growth of the seedless variety showed the variation of low- high then low, the weight began to decrease at about 6 to 7 weeks after pollination (Figure 1). Till the ripen time, the weight always at the low level indicated that the seed abortion occurred at the critical time; so the rescue time should be before this while the seeded varieties did not show any variation tendency like the seedless variety which indicated that the seed abortion did not occur simultaneously, but at different time, so the behavior was individual.

The observation of the dissected seeds showed that the abortion time was highly related to the ripen time of the female parents (Table 4). The sampling time could be determined by the female parents; the best time for the early maturing cultivar (such as Agate early) was 5 weeks

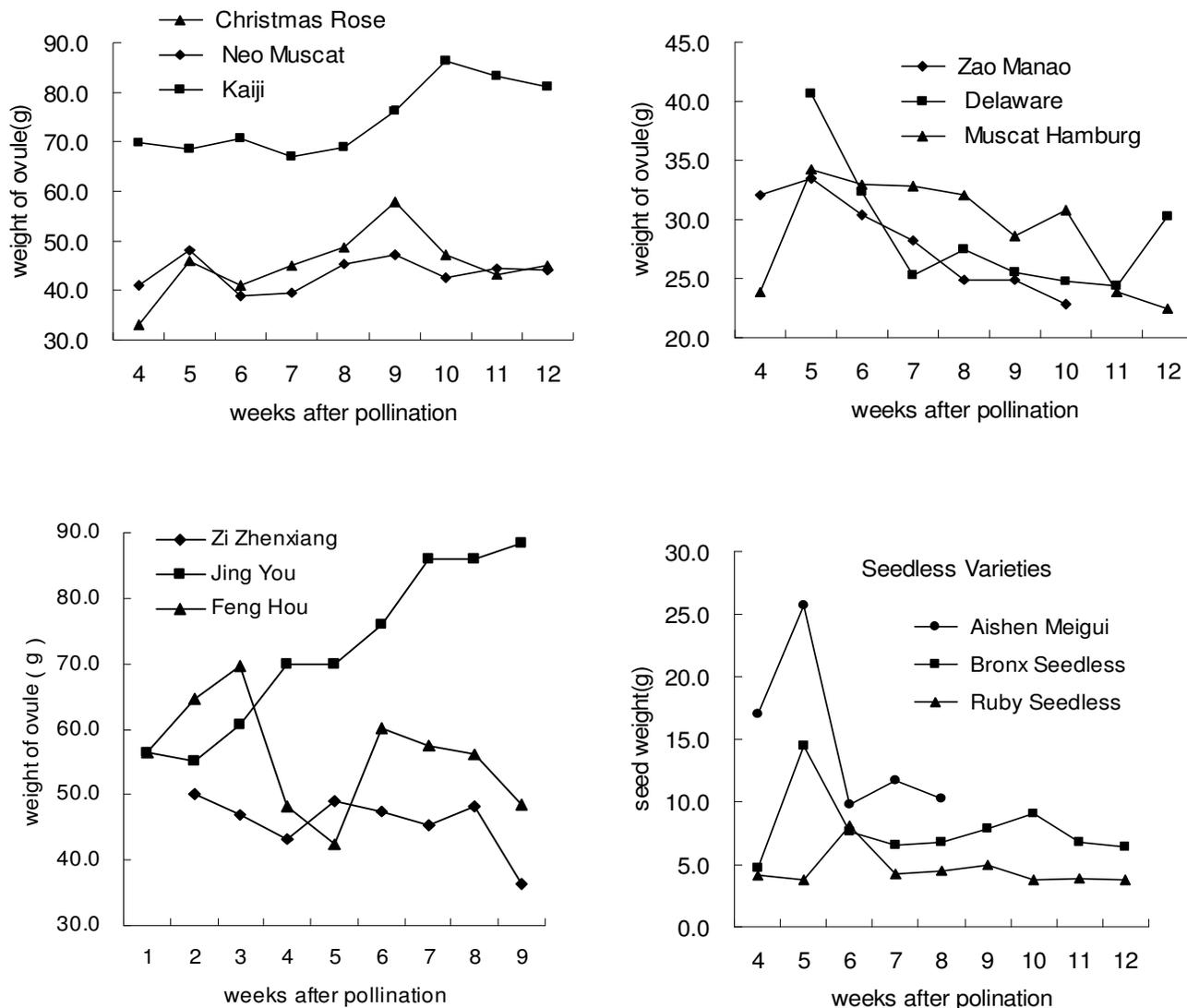


Figure 1. Changes of ovule weight from different hybridization.

after pollination, 6 weeks for the middle maturing cultivars (such as Muscat Hamburg and Jingyou) and 9 weeks for the late (such as Kai ji and Fenghou). The results from Table 5 indicated that the abortion was individual and gradually, after abortion until maturing, some normal ovule can still be obtained in 28 to 64 days after pollination, and for example, the seeds can be obtained from Muscat Hamburg in the first 11 weeks after pollination, the phenomenon that seeds can be harvested when crossing between diploid and tetraploid also proved this.

DISCUSSION

According to the experiment and the results obtained, the embryo rescue of the progeny between diploid and tetraploid consists of three phases, which were further

development of embryo, shoot induction and subculture. In the first phase, the media was basal MS plus plant hormone, and then in the second phase was MS without hormone. The germination was inhibited by 6-BA, the media in third phase depend on the genotype.

According to Johnston's hypothesis of endosperm balance number, the endosperm will develop healthy into a seed when the gametes ratio of the parents was 2:1, otherwise the pairing of the chromosome will be in disorder, the endosperm ceased developing in the procedure, which causes the abortion (Johnston et al., 1980). Differences in embryo and plant recovery between sample times and among crosses showed that embryo and plant development are independent events with different response stimuli during ovule culture, the results could be due to the differences in genetic compatibilities between parental germplasm. Totally speaking, the media components were very different when the female parent

Table 4. The ovule abortion time and rate of different cross.

Female parent cultivar	Beginning time of abortion (week after pollination)	Abortion rate (%)
Agate early	5	45.56
Muscat Hamburg	6	50.00
Kai ji	9	6.67
Jingyou	6	42.86
Fenghou	7	12.50
Delaware	7	23.45

Table 5. The rate of normal ovules after pollination with different hybridization.

Cross combination	Sampling time (week after pollination)						
	4	5	6	7	8	9	11
Muscat Hamburg×Jingyou	100.00	100.00	50.00%	36.84%	34.44%	37.50%	28.57%
Agate early×Fenghou	100.00	54.54%	/	/	38.09%	11.11%	/
Kaiji×4X Muscat Hamburg	100.00	100.00	100.00	100.00	100.00	93.33%	/
Jingyou×Muscat Hamburg	100.00	100.00	57.14%	25.00%	23.08%	9.09%	/
Fenghou×Agate early	100.00	100.00	100.00	87.50%	54.54%	16.67%	/

is diploid or tetraploid. It is easier to remove the ovule when the abortion time was late.

As a very promising fruit, grape has received so much attention from the breeders, many programs and agencies have developed new cultivars that require less management and better qualities, the embryo rescue method is already widely used in obtaining seedless grape. The new achievements in this area showed that the biotechnology is efficient in grape improvement.

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