Ovules Culture and Plant Formation of Hybrid Progeny of Seedless Grape

Embryokulturen und Regeneration bei Kreuzungen samenloser Reben

Qi Guimei[1] and Ding Hanfeng[2]

Abstract

Ovules of seedless grapes (Vitis spp.)fertilized by controlled pollination increased in size during berry development. Hybrid bunches picked at 30,35,40,45,50 and 55days after pollination. Ovules were dissected and then cultured in media for 60 to 70 days. Developing ovules were placed in germinative medium. After embryos germinated for 10 days. They were transferred in plant medium to promote them formed normal plants. The result indicated that the most appropriatest medium was Nitsch with GA 30.2m l/l, IAA 1.5mg/l, ZT1.0 mg/l.The suitable sampling period was after pollination 35 to 50 days. The germinate and the plant rate of 'perlette ×' Flame seedless' were the highest in the five crosses, which were 70.2% and 24.8%.

Chemical name used: Gibberellin (GA3), 3-Inddylacticacid (IAA), 6-Benzylaminopurine (6BA), Trans-zeatin (ZT).

Keywords:

ovule culture, embryo germination, grape.

Introduction

In recent years, seedless grapes are welcome by more and more people, breeding of high qualitative seedless grapes is one of the important aims. Before 1990s, using standard breeding techniques, seedless cultivars can only be used as pollen parents (Olmi,1936).However, with ovule culture it is now possible to use seedless vines as females (Cain et al.,1983;Emershad and Ramming,1984;-Gray et al.,1987; Spiegel-Roy et al., 1985; Gray DJ et al., 1990). This approach dramatically increases potential germplasm combinations and allows heretofore impossible crosses between seedless cultivars. Recently, there were more researches on vitro ovule culture and embryo germination in our country (Dong Xiaolin, 1990; Zhang Hongming, Zhang li, Meng Xinfa et. al., 1991-1992; Tao Jianmin et al. 1997), but it was not reported on obtaining a new cultivar of seedless grape by ovule culture. Since 1996, we have been cultured hybrid ovules of seedless seedless grapes, ovule culture derived plants have been established in vineyard for progeny tests. About fifteen out of 100 plants produced fruit in 2000 and eight of these were seedless. For comparison, conventional breeding methods using seeded females and pollen from seedless cultivars yield only 10% to 15% seedless progeny (Loomis and Weinberger,1979).

[1] Shangdong Vine and Wine-making Institute, 250100, Jinan ,P.R. China
[2] Shandong Academy of Agricultural Sciences, 250100, Jinan, P.R.China

Material and methods

Flowers on 4-year-old vines grown at the experimental vineyard of Shandong Vine and Wine-making Institute. Experimental vine were emasculated immediately before anthesis, and pollinated by placing fresh or stored pollen directly onto stigmas. Emasculated and pollinated clusters were enclosed in paper bags to exclude random pollination, and then allowed to develop. Berries were harvested at 30,35,40,45,50 and 55days after pollination, and surface-disinfected for 10 min in 0.1 % $HgCl_2$ containing a drop of surfactant and rinsed three times in sterile deionized water.

Ovules were so dissected as not to damage ovular tissue and then culture. 4-6 ovules per 50ml trigond bottle containing 25 ml of autoclaved medium. Media composition were given in Table 1. Ovules were cultured on 4 different ovules-culture media(Table 1) at 24 °C under 16-hr cool-white fluorescent illumination (2000 lx)for 60-70 days. Well-developmental ovules were transferred on embryo germination medium for 10-20 days, and then well-germinative embryo were place on plant medium to induce them to form normal plant under the aforementioned conditions. After 35 days, resulting plants were placed in Ø10cm potting with sealed clear plastic covers and moved to a greenhouse. After new growth was evident, covers were unsealed. Individual plants were placed in 1-liter pots. Well-developed pot-plants were eventually established in vineyard for progeny tests.

The experiments were designed with specific crosses, various culture-media and sample times to evaluate treatment effects on the recovery and development of ovules and plants.

Table 1 Media used with various composition

Cultural Method		Composition		
		Basic medium	Growth regulators (mgl^{-1})	Additional (substances $g\ l^{-1}$)
Ovule culture	No.1	B_5	$GA_3$0.4+IAA1.7	Sucrose 20
	No.2	B_5	$GA_3$0.2+IAA1.5+ZT1.0	Agar powder 5
	No.3	Nitsch	$GA_3$0.4+IAA1.7	PH 5.8
	No.4	Nitsch	$GA_3$0.2+IAA1.5+ZT1.0	
Embryo germination	No.5	1/2MS	IAA.0.4+BA0.6+NAA0.2	
Plant culture	No.6	1/2MS	IAA0.4	

Results and Discussion

During ovules were cultured, more and more ovules became green or brown, the green ovules size were evident. The result showed that ovules already began developing. The green ovules was treated as growing embryo in the statistics.

Effect of various media on ovule development.

The test was designed with four kinds of ovules-culture media to evaluate treat-

ment effects (Table 1).The percentage of ovule (after 45 days pollination) culture in the 4 media listed in Table 2, There was significant difference among various media. The developmental rate of ovule of Perlette × Flame seedless' was the highest which was 81.3%, and developmental rate of ovule of 'Himlod × Guifei rose' was the lowest, which was 21.9%. To sum up, the percentage of ovule of all crosses was higher in No.2 and No.4 , then No.1 and No.3. The results showed : it is possible that trans zeatin(ZT) can promote ovule development.

Table 2 The percentage of embryo development in various media %

Cross	Media			
	No.1	No.2	No.3	No.4
Himlod × Guifei rose	30.5b	44.2a	21.9c	46.5a
Perlette × Flame	56.0b	81.3a	32.7c	59.6b
Flame × Ruby	56.3b	67.1b	48.2b	72.6a
Thompson × Ruby	37.5d	67.6a	54.0c	60.1b
Ruby × Red globe	60.1a	55.2b	39.5c	64.4a

Percentages based on developmental ovules Mean separation within columns by analysis of contrasts from categorical modeling, P=0.05

Effect of sampling time on ovule development

Be due to difference of specific property and developmental phase of seedless cultivars. The ovule development was influenced by the weather, different year and the position of a bunch of grape. Therefore, a suit sampling time of crosses should be that ovules get the greatest degree of development, but ovular abortion doesn't begin. In this date, if ovules were dissected and cultured, ovular development rate was the highest. How to define dissecting date is very important. In our study, ovular development rate generally increased dramatically at 35 to 50 days after pollination (Table 3), and plants were eventually obtained from most crosses (Table 4)

Table 3 Comparison of ovule development rate among six sampling times all seedless × seedless crosses tested*

Crosses	Days after pollination (d)					
	30	35	40	45	50	55
Himlod × Guifei rose	52.1a	49.6a	38.1b	20.2c		
Perlette × Flame	40.5c	64.1b	78.9a	80.5a	59.1b	23.6d
Flame × Ruby	33.5d	50.8b	71.9a	74.6a	42.3c	31.1d
Thompson × Rruby	25.6e	41.8d	49.2c	65.7a	59.2b	38.9d
Ruby × Red globe	30.6e	39.6d	49.3c	58.8b	66.4a	48.5c

*The percentage of ovule development in No.4 medium

Mean separation within columns by analysis of contrasts from categorical modeling, P=0.05

Ovular development rate of five crosses were listed in Table 3. There was clear choice among 30 to 55 days after pollination. For¡Himlod × Guifei rose', the suitable sampling times were among 30 to 35 days after pollination , but for the others , the suitable sampling times were among 40 to 50 days after pollination. Therefore, the time of dissection of hybrid ovule is directly proportional to the mature period of maternal parent.

Embryo germination and plant development

After ovules were cultured for 60 to 70 days, well-developmental ovules were transferred on the embryo-germination medium for 10 to 20 days, in which the embryos began to germinate. Although a previous report documented that the embryo-germination and growth of plant could directly come from ruptured ovules (spieged-roy et al.1985) we didn't find that ovules germinated directly to become normal plants (consist of root, stem, leaf). Some ovules germinated firstly out of embryo root , the others germinated firstly out of cotyledon, then became green. But the embryo root and cotyledon were often contorted in shape abnormally.

In 10 to 20 days when embryos germinated, well-germinated embryos (i.e. small plants)were transferred to 100ml trigonal bottle to promote the formation of normal plant. Some of them developed branching root systems and shoots with numerous nodes and leaves. They became normal plants. After acclimation of 35 days, the plants were established in the pots in greenhouse , and as statistical number(Table 4). Table 4 showed that the progeny number from Perlette × Flame seedless were the most , the plant rate were the highest too.

As previously described for ovule culture, efficiency of plant development related to date of ovule culture. Culture of ovules in 40 to 50 days after pollination generally resulted in more plants compared with earlier data(Table 3). However the number of plants recovered was not always proportionate to the number of developmental ovules. For instance, for Himlod × Guifei rose, Thompson × Ruby, more developed ovules were recovered, but their plant rate was lower, only 2.0% and 2.3%, The highest plant rate was 24.8% from Perlette × Flame in our study.

Table 4 developed ovules and plants number obtained from ovule culture in 30 to 55 days after pollination for all seedless seedless crosses tested

Crosses	No. ovules cultured	No. ovules developed	No. plants obtained	Germination-rate%	Plant rate %
Himlod × Guifei rose	617	247	5	40.0	2.0
Perlette × Flame	331	234	58	70.7	24.8
Flame × Ruby	537	326	17	56.9	5.2
Thompson × Ruby	720	386	9	53.6	2.3
Ruby × Red globe	408	212	22	51.9	10.4

Differences in embryo and plant recovery between sample times and among crosses showed that the development of ovule and plant were independent on

the events with different responses stimuli during ovule culture. Observed responses could be due to many factors, including differences in genetic compatibilities between parental germplasm. For lack of better understanding, we currently optimize ovule culture conditions for maximum embryo development only.
As ovule culture becomes increasingly integrated into our seedless grape breading program, Ongoing research seeks to increase ovule culture efficiency by shortening the period from pollination to plant recovery and increasing plant recovery rates.

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