Ovules Culture and Plant Formation of Hybrid Progeny of Seedless Grape

Embryokulturen und Regeneration bei Kreuzungen samenloser Reben

Qi Guimei1 and Ding Haiteng2

Abstract

Ovules of seedless grapes (Vitis spp.) limited by controlled pollination increased in size during berry development. Hybrid bunches picked at 30, 35, 40, 45, 50 and 55 days after pollination. Ovules were dissected and then cultured in media for 60 to 70 days. Developing ovules were placed in germination medium. After embryos germinated for 10 days, they were transferred in plant medium to promote them turned normal plants. The result indicated that the most appropriate medium was Nitsch with GA 30.2 mg/L, IAA 1.5 mg/L, ZT 7.0 mg/L. The suitable sampling period was after pollination 35 to 50 days. The germinate and the plant rate of 'perlette' X 'Flame seedless' were the highest in the five crosses, which were 70.2% and 24.8%.

Chemical name used: Gibberellin (GA3), 3-Iodoactetisic acid (IAA), 6-Benzylaminopurine (6BA), Trans-zeatin (7Z).

Keywords:

ovule culture, embryo germination, grape.

Introduction

In recent years, seedless grapes are welcome by more and more people, breeding of high quality seedless grapes is one of the important aims. Before 1990s, using standard breeding techniques, seedless cultivars can only be used as pollen parents (Perini 1995). However, with ovule culture it is now possible to use seedless vines as allthers (Cain et al., 1983; Evershed and Ramming, 1984; Gray et al., 1987; Spiegel-Roy et al., 1985; Gray DJ et al., 1990). This approach dramatically increases potential allthesis combinations and allows heretofore impossible crosses between seedless cultivars. Recently, there were more researches on vitr ovule culture and embryo germination in our country (Dong Xiaolin, 1990; Zhang Hongming, Zhang Li, Meng Xinzle 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).

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 55 days after pollination, and surface-disinfected for 10 min in 0.1 % HoCl containing a drop of surfactant and rinsed three times in sterile deionized water.

Ovules were so dissected as not to damage ovule tissue and then culture. 4-6 ovules per 50ml trygoid 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 ft) 60-70 days. Well-developmental ovules were transferred on embryo germination medium for 10-20 days, and then well-germinative embryos were placed on plant medium to induce them to turn normal plant under the airflow-mentioned 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-litre 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

<table>
<thead>
<tr>
<th>Cultural Method</th>
<th>Composition</th>
<th>Base medium</th>
<th>Growth regulators (mg/L)</th>
<th>Additional substances (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovule culture</td>
<td>No.1 B5</td>
<td>GA2,0-4,IAA1,7</td>
<td>Stock 20</td>
<td>Age powder 5</td>
</tr>
<tr>
<td>No.2 B5</td>
<td>GA2,0-4,IAA1-5,7,7T1-0</td>
<td></td>
<td></td>
<td>PH 5.8</td>
</tr>
<tr>
<td>No.3 Nitsch</td>
<td>GA2,0-4,IAA1,7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.4 Nitsch</td>
<td>GA2,0-4,IAA1,7,7T1-0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryo germination</td>
<td>No.5 I2W5</td>
<td>IAA2,0-4,BA2,0,6,6AA2,0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant culture</td>
<td>No.6 I2W5</td>
<td>IAA4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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-
Mean separation within columns by analysis of contrasts from categorical model, P<0.05

Ovular development rate of live crosses were listed in Table 3. There was clear choice among 30 to 55 days after pollination. For Himlod x Guelfi 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 desiccation of hybrid ovule is directly proportional to the mature period of parental plant.

Embryo germination and plant development

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

In 10 to 20 days when embryo were 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 acclimatization 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 Perleite x Flame seedling was 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 develop development of ovules. For instance, for Himlod x Guelfi rose, Thompson x Ruby, more developed ovules were recovered, but their plant rate was lower, only 2.0% and 2.3%. The highest plant rate was 28.4% from Perleite x Flame in our study.
the events with different responses stimuli during ovule culture. Observed re-
sponses could be due to many factors, including differences in generic compati-
ble between parental germplasm. For lack of better understanding, we cur-
rently optimize ovule culture conditions for maximum embryo development only.
As in vitro cultures become increasingly integrated into our seedling grape breed-
ing programs, Ongoing research seeks to increase ovule culture efficiency by shortening the period from pollination to plant recovery and increasing plant re-
covery rates.

Literature Cited