Embryogenic Callus Induction and Plant Regeneration from Unfertilized Ovule of ‘Kyoho’ Grape

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Summary

Unfertilized ovules were excised 19–21 days before anthesis and cultured in a liquid medium to induce embryogenic callus from ‘Kyoho’ grape (Viitis × labruscana). The culture medium consisted of half strength MS supplemented with 1.0 μM 2,4-Dichlorophenoxyacetic acid (2,4-D) and 0.2–5.0 μM N-(1,2,3-thiadiazol-5-yl)-N’-phenyleurea (TDZ, thidiazuron), or 1.0 μM 2,4-D and 0.2 μM N-(2-chloro-4-pyridyl)-N’-phenyleurea (CPPU). After 3–4 months of culture, the embryogenic calli produced from the ovules were successfully subcultured to maintain a high embryogenic potential for over one year. When these adventitious embryos were transferred to a modified 1/2MS hormone-free medium, plantlets were regenerated 1.25 months later. This is the first embryogenic callus induction and plant regeneration from ‘Kyoho’ grape.

Key Words: embryogenic callus, ‘Kyoho’ grape, plant regeneration, unfertilized ovule.

Introduction

Some fungal diseases cause serious damage to grapes in Japan, despite repeated spraying of agrochemicals to prevent their spread. Thus, disease-resistant grapes are highly desirable. However, genetic improvement of disease-tolerance by traditional hybridization and testing of progenies require a long time. DNA transfer, which offers an alternative method for such improvement, have yielded uniform transplants using embryogenic calli or somatic embryos in several cultivars of grape (Martinelli and Mandolino 1994, Mauro et al. 1995).

Embryogenic calli and/or somatic embryos have been induced in several grape cultivars (Mullins and Srinivasan 1976, Srinivasan and Mullins 1980, Hirabayashi and Akihama 1982, Hirabayashi 1985, Matsuda and Hirabayashi 1989, Notsuka et al. 1992, Nakano et al. 1997), but only a few in V. × labruscana (Hirabayashi and Akihama 1982, Nakano et al. 1997). ‘Kyoho’ (V. × labruscana) is a very important cultivar in Japan from which embryogenic calli have not been induced. Thus we tried to induce embryogenic calli from unfertilized ovules of ‘Kyoho’.

Materials and Methods

Flower buds from a 5-yr-old ‘Kyoho’ vine were collected at two different stages of development: 1) at 19–21 days before anthesis when anthers were a clear yellow color and 2) about 6–7 days before anthesis. Flower buds were sterilized for 20 minutes with sodium hypochlorite (1% available chlorine) containing Tween 20 (a few drops) and then rinsed with sterile distilled water. Unfertilized ovules were excised from the buds under a stereoscopic microscope, and transferred aseptically into 100ml flasks containing 20ml of liquid media. Half strength MS liquid media (3% sucrose, Murashige and Skoog 1962) were supplemented with 1.0 μM 2,4-D and 0.2–5.0 μM TDZ, or 1.0 μM 2,4-D and 0.2 μM CPPU. After the media were adjusted to pH 5.7 and autoclaved, 20 ovules were transferred into each flask. Each treatment was repeated 5 times. These flasks were agitated continuously in a gyro-rotary incubator (60 rpm) at 26°C in the dark. Unfertilized ovules were cultured for 2 months and the induced calli subculturated every month.

Results and Discussion

After one month of culture, the ovules, collected at an early stage of development, produced creamy white calli. After increasing in size, the callus partly became brown and then the surface began to break down into small, ivory-white, cellular aggregates after 2 more months (Table 1, Fig. 1A). These cellular aggregates tended to be induced from large-sized calli. The aggregates were transferred to a modified 1/2MS hormone-free medium containing 3% agar and 5% maltose, instead of sucrose to check their ability for embryogenesis. About one month later, the aggregates began to produce embryos (Fig. 1B); the calli were, therefore, considered to be embryogenic. The embryogenic callus was induced in media containing either CPPU or TDZ.
Table 1. Effect of developmental stage of ovules and addition of cytokinins on the induction of embryogenic callus in 'Kyoho' grape.

<table>
<thead>
<tr>
<th>Date of collecting explants</th>
<th>Cytokinin concentration (µM)</th>
<th>Frequency of embryogenic callus formation (%)</th>
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<tr>
<td>19 - 21 days before anthesis</td>
<td>CPPU 0.2, TDZ 0.2, 1.0, 5.0</td>
<td>9 ± 2.9, 13 ± 3.4, 8 ± 2.7, 9 ± 2.9</td>
</tr>
<tr>
<td>6 - 7 days before anthesis</td>
<td>CPPU 0.2, TDZ 0.2, 1.0, 5.0</td>
<td>0, 0, 0, 0</td>
</tr>
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</table>

1/2MS liquid media containing 1.0 µM 2,4-D were used.

Data were collected after 4 months of culture. Values represent the mean ± SE. Induced cali were transferred to modified 1/2MS medium (3% agar, 5% maltose) to check the ability of embryogenesis.

Fig. 1. Embryogenic callus induction and plant regeneration from unfertilized ovule of 'Kyoho'.
A: Cellular aggregates (embryogenic calli) produced from unfertilized ovule callus. B: Somatic embryos differentiated from the cellular aggregates. C: Plants regenerated from the somatic embryos.

There were no significant differences among the media tested in their efficiency for embryogenic callus induction. The ovules at a later stage of development produced a small amount of calli and no cellular aggregates even though they were incubated for 5 months.

The embryogenic calli, however, turned brown and died when subcultured in the same fresh medium of callus induction. Since Matsuta and Hirabayashi (1989) induced embryogenic calli from the leaf explant of 'Koshusanjaku' and proliferated them on vitamin-, inositol- and glicine-free Nitsch and Nitsch (1969) basal medium supplemented with 1.0 µM 2,4-D, we tried to proliferate embryogenic callus on the modified 1/2 MS medium (3% agar and 5% maltose) supplemented with only 1.0 µM 2,4-D. On this medium, embryogenic calli were able to proliferate and part of them produced embryos. After about one month, calli containing somatic embryos almost doubled in size. After being subcultured for over one year, the calli still maintain high embryogenic potential.

When these calli containing somatic embryos were transferred to a modified 1/2MS hormone-free medium (0.85% agar and 5% maltose), plantlets developed from the somatic embryos after 1 - 2.5 months. The plantlets
were transferred to pots and gradually acclimatized to a lower humidity and higher light intensity in an illuminated culture room (Fig. 1C).

Notsuka et al. (1992) reported that the combination of 1.0 μM 2,4-D and 0.2 μM CPPU was most suitable for inducing callus from unfertilized ovules of ‘Ne O Muscat’. Matsuta and Hirabayashi (1989) found that CPPU and TDZ were more effective than 6-benzylaminopurine for somatic embryogenesis from leaf discs of ‘Koshusanjaku’. Harst (1995) also reported the production of embryogenic callus from leaf discs of ‘Seyval blanc’ with TDZ. In this study, we found that CPPU or TDZ combined with 2,4-D effectively induced embryogenic callus from ovules of ‘Kyoho’.

Several researchers induced embryogenic callus by using the unfertilized ovules at a late stage of development before anthesis, but in this experiment with ‘Kyoho’, embryogenic calli were obtained only when unfertilized ovules, 0.1 – 0.2 mm long and excised 19–21 days before anthesis, were used. This stage of ovules were round and were the minimum size which could be isolated under a stereoscopic microscope. In preliminary experiments, we had succeeded in producing embryogenic calli of V. riparia (male strain) by using the ovule at this stage (data not shown). In this study, we confirmed that using unfertilized ovules of this stage is critically important to induce embryogenic callus from ‘Kyoho’. Hirabayashi and Akihama (1982) and Hirabayashi (1985) found that inducing embryogenic callus was more difficult in V. × labruscana cultivars than in V. vinifera. Nakano et al. (1997) tried inducting embryogenic callus from 22 cultivars of grape by using leaves, immature ovaries and anthers; they found that the embryogenic callus could be induced from 9 out of 11 V. vinifera cultivars, but only 1 (‘Delaware’) out of 11 V. × labruscana cultivars including ‘Kyoho’. Our method for inducing embryogenic callus of ‘Kyoho’ is expected to be applicable to other grape cultivars, especially those of V. × labruscana.

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Literature Cited


ブドウ‘巨峰’の未受精胚珠からのEmbryogenic Callusの誘導と植物体再生

中島 裕子・小林光雄・中村ゆう

摘 要

ブドウ‘巨峰’について開花19–21日前の花から未受精胚珠を取り出し培養した。培地には、1.0 μMの2,4–Dと、0.2 μMのCPPUあるいは0.2, 1.0, 5.0 μMのTDZを含む1/2 MS液体培地を用いた。基質から3–4ヶ月後には、全ての培地でembryogenic callusが誘導された。これらのカルスは1.0 μM 2,4–D と5%マルトース、3%蔗糖を含む1/2 MS培地で緑化培養が可能で、1年後でも高い胚芽体再生能を有した。不定胚を含むカルスを1/2 MS ホルモンフリー培地に移したところ、1–25ヶ月後には植物体が再生された。