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Seed Abortion in Crosses between Diploid and Tetraploid Grapes (*Vitis vinifera* and *V.* complex) and Recovery of Triploid Plants through Embryo Culture

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Interploid crosses between two diploid and two tetraploid grape cultivars were carried out to produce abortive seeds with underdeveloped triploid embryos. Growth regulators influencing the recovery of triploid plants from the underdeveloped embryos were individually examined *in vitro* using MS medium. Of six growth regulators added to the medium, malt extract (25–1600 mg/l), casein hydrolysate (20–1500 mg/l), BA (0.01–1.25 mg/l) and GA_3 (0.1–1 mg/l) did not show obvious effect on the recovery, whereas IAA and NAA showed small promotive effect at the concentration of 0.25 and 1.25 mg/l, and 0.01 mg/l respectively. Secondly, 23 interploid crosses with four diploid and four tetraploid grape cultivars were carried out reciprocally to produce triploid plants through embryo culture using MS medium supplemented with 1 mg/l GA_3 and 100 mg/l casein hydrolysate. In the 2x×4x crosses, 50 triploid seedlings were successfully recovered from 7.3% of underdeveloped embryos cultured, while 33 seedlings were recovered from 2.8% of those in the reciprocals. Of a total of 88 seedlings recovered, 38 were obtained through secondary embryo formation *in vitro*. From these results, it was suggested that the production rates of triploid plants from the interploid crosses increase with the aid of embryo culture, and that in this case *in vitro* formation of secondary embryos is an important key factor to increase the rates.

INTRODUCTION

Seedlessness in grape is one of the desirable characters for breeding table grape and raisin grape cultivars. In addition to the breeding with stenospermocarpic grape cultivars (Winkler *et al.*, 1962; Einset and Pratt, 1975; Mullins *et al.*, 1992), breeding of hypo- and hypertetraploid using tetraploid cultivars (Park *et al.*, 1999) and, especially, triploid breeding with diploid and tetraploid cultivars (Yamashita *et al.*, 1993, 1995; Wakana *et al.*, 2002) have been suggested to be the hopeful way for establishing new seedless grape cultivars. Some triploid seedless cultivars were bred in Japan, but so far, no triploid cultivars

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producing large-sized and/or high quality berries have been established.

In interploid crosses between diploid and tetraploid plants, seed abortion due to the endosperm degeneration during early embryogenesis is a severe barrier for the production of triploid plants (Esen and Soost, 1973; Sanford, 1983). In the previous study of interploid crosses with seven diploid and four tetraploid grape cultivars (Wakana, *et al.*, 2002), it was demonstrated that the 2x×4x crosses produced 0.42 seeds per flower and 1.9% of the seeds germinated and that the 4x×2x crosses produced 0.51 seeds per flower and 2.7% of the seeds germinated. Thus, the seed sowing was not an efficient method for breeding triploid grapes.

To heighten the breeding efficiency in the production of triploid seedlings from the interploid crosses, application of the embryo-rescue methods was studied.

MATERIALS AND METHODS

Plant materials

For interploid crosses between diploid and tetraploid grapes, four diploid cultivars and four tetraploid cultivars were used. The diploid cultivars were 'Muscat Bailey A', 'Delaware', 'Rizamat' and 'Sekirei', while the tetraploid cultivars were 'Red Pearl', 'Yufu', 'Cannon Hall Muscat' and 'Kyoho'. 'Muscat Bailey A', 'Delaware', 'Red Pearl', 'Yufu' and 'Kyoho' are intercontinental hybrid cultivars (*Vitis* complex) with *Vitis vinifera* and North American *Vitis* species in their pedigrees, while 'Rizamat' and 'Cannon Hall Muscat' are *V. vinifera* cultivars. 'Sekirei' is classified as *V. vinifera* cultivars, although it has 'Koshu' (*V. vinifera* ?; Ohmi *et al.*, 1993) in the pedigree. Relation between some of these diploid and tetraploid cultivars was described previously (Ohmi *et al.*, 1993; Wakana *et al.*, 2002). All of these cultivars were 10- to 15-year-old trees grown in a greenhouse located at the Sasaguri orchard of University Farm, Kyushu University, Fukuoka.

Interploid cross

Twenty-three crosses between 2x and 4x cultivars were carried out using the four diploid and four tetraploid cultivars. Number of flower buds of a cluster was adjusted before emasculation so that each cluster has about 100 flower buds. The flower buds were emasculated one or two days before anthesis, sprayed with water to prevent self-pollination and bagged. When the pollination was made, pistils with wet stigma and normal morphology were chosen. Hand pollination was carried out at the full bloom stage of the clusters using fresh pollen from just opened flowers of the pollen parents. Immediately after the pollination, the pollinated flower clusters were bagged again to prevent further crosses. The pollinated fruits were collected at the mature stage in each cross.

Embryo culture

The mature fruits were surface-sterilized with 70% ethanol and seeds were extracted out of the fruits under aseptic conditions, or seeds extracted from the fruits under room conditions were sterilized in a solution of 1% sodium hypochlorite (NaClO) supplemented with 0.1% Tween-20 for ten minutes and rinsed with sterilized distilled water. Embryos were dissected out of the seeds with a forceps and a surgical knife under a stereoscopic microscope. Then, length of the embryos was measured with the microscope equipped

with a micrometer. After the measurement, embryos developing beyond a globular stage were placed on MS medium (Murashige and Skoog, 1962) supplemented with 1% agar, 2% sucrose and growth regulators, but those lacking viability were eliminated.

To determine the effect of growth regulators on embryo growth and development, the following growth regulators were individually added to MS medium: 0.01, 0.05, 0.25 and 1.25 mg/l naphthalene acetic acid (NAA); 0.01, 0.05, 0.25 and 1.25 mg/l benzyl adenine (BA); 0.01, 0.1, 1, 10 m/l gibberellin (GA_3); 25, 100, 400 and 1600 mg/l malt extract (ME); 20, 100, 500 and 2500 mg/l casein hydrolysate (CH). The medium was adjusted to pH 5.7 with 0.1 N NaOH and 0.1 N HCl, gelled with 1% agar, dispensed in a 100 ml conical flask at a rate of 20 ml per vessel, and autoclaved at 120 °C for 14 minutes. GA_3 and IAA were added to the medium through filtration with a sterilized cellulose acetate just after the medium was autoclaved. One embryo was planted per vessel.

The medium supplemented with 1% agar, 2% sucrose, 1 mg/l GA_3 and 100 mg/l CH was used for rescuing underdeveloped embryos in 23 crosses including 11 pair of reciprocal crosses between the diploid and tetraploid cultivars. After germination of the embryos, those growing to plantlets were transplanted to a proliferation medium consisting of MS medium supplemented with 1% sucrose, 1% agar and 0.01 mg/l NAA. The medium was dispensed in a 200 ml conical flask at a rate of 40 ml per vessel and autoclaved as mentioned above. When the plantlets were too large to transplant to the conical flask, they were cut into several sections with at least one leaf, and then the sections were planted in the medium to proliferate. The proliferated plantlets growing within the flasks were transplanted to pots filled with mold–soil mixture (1:1) and habituated at spring of the next year or after about six months of culture.

Chromosome observation

Chromosome observation in root tip cells from the habituated plantlets was carried out during growing season according to the procedure described by Wakana *et al.* (2002) or Park *et al.* (1999a).

RESULTS

Effect of growth regulators on embryo rescue

Because of the lack of sufficient abortive triploid seeds from same cross and because of the genetic resemblance of embryos, the seeds from reciprocal crosses between 'Muscat Bailey A' and 'Red Pearl' and those between 'Yufu' and 'Delaware' were used to study the effect of growth regulators on the growth, development and germination of underdeveloped 3x embryos cultured *in vitro*. The effect of growth regulators was examined in underdeveloped 3x embryos from 'Muscat Bailey A'×'Red Pearl' for GA_3, 'Delaware'×'Yufu' for IAA, 'Yufu'×'Delaware' for NAA (Table 1) and 'Red Pearl'×'Muscat Bailey A' for malt extract, casein hydrolysate and BA (Table 2). In these interploid crosses, sizes of the underdeveloped 3x embryos varied from very small to large and their morphology varied from highly abnormal to almost normal (Fig. 1–3). Thus, the embryos were randomly chosen to culture in each treatment.

None of these growth regulators showed prominent effect on rescuing the underdeveloped 3x embryos in any concentrations examined (Table 1 and 2). Addition of

Table 1. Effect of growth regulators on *in vitro* growth of underdeveloped embryos from crosses between 2x and 4x grape cultivars.

Growth regulator (Parentage of embryos)	Concentration (mg/l)	No. of embryos cultured	No. of embryos germinating (%)	No. of plantlets established (%)
GA_3	0	22	4 (18)	4 (18)
(Muscat Bailey A	0.01	22	5 (23)	5 (23)
× Red Pearl)	0.1	22	1 (5)	1 (5)
	1	22	5 (23)	5 (23)
	10	22	0 (0)	0 (0)
IAA	0	10	0 (0)	0 (0)
(Delaware×Yufu)	0.01	10	1 (10)	1 (10)
	0.05	10	1 (10)	0 (0)
	0.25	10	2 (20)	2 (20)
	1.25	10	2 (20)	2 (20)
NAA	0	19	0 (0)	0 (0)
(Yufu×Delaware)	0.01	19	2 (11)	2 (11)
	0.05	19	2 (11)	1 (5)
	0.25	19	1 (5)	1 (5)
	1.25	19	0 (0)	0 (0)

Table 2. Effect of growth regulators on *in vitro* growth of underdeveloped embryos from 'Red Pearl' (4x)×'Muscat Bailey A' (2x).

Growth regulator	Concentration (mg/l)	No. of embryos cultured	No. of embryos germinating (%)	No. of plantlets established (%)
Malt extract	0	35	4 (11)	2 (6)
	25	25	4 (16)	3 (12)
	100	25	2 (8)	2 (8)
	400	25	1 (4)	0 (0)
	1600	25	3 (12)	2 (8)
Casein hydrolysate	20	25	3 (12)	2 (8)
	100	25	3 (12)	3 (12)
	500	25	2 (8)	1 (4)
	1500	25	3 (12)	3 (12)
BA	0.01	10	1 (10)	1 (10)
	0.05	10	1 (10)	1 (10)
	0.25	10	1 (10)	1 (10)
	1.25	10	1 (10)	0 (0)

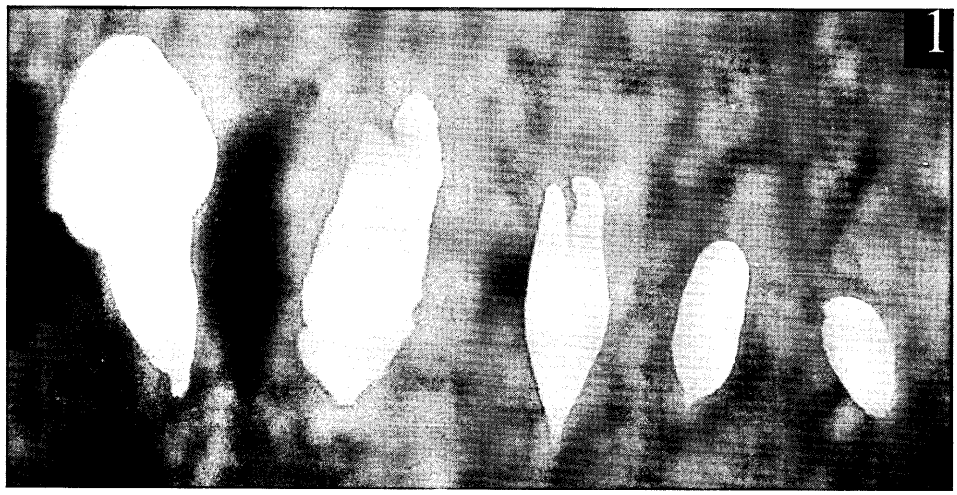

Fig. 1. Morphology of embryos used for embryo culture. The embryos were derived from 'Red Pearl'×'Muscat Bailey A' about three months after pollination.

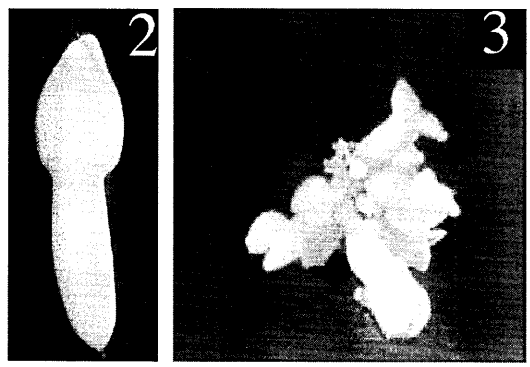

Fig. 2, 3. Embryo morphology *in vivo* and *in vitro*.
Fig. 2. An embryo with normal morphology. The embryo was derived from self-pollinated 'Muscat Bailey A' about three months after pollination.
Fig. 3. Multiple embryo formation in a cultured embryo derived from 'Muscat Bailey A'×'Red Pearl' about three months after pollination. Note the various sizes of embryos at globular to torpedo stage of embryo development. Note also some embryos show almost normal morphology.

high concentration of GA_3 (10 mg/l) and NAA (1.25 mg/l) depressed the growth and development of underdeveloped embryos cultured. IAA and NAA were slightly promoted the growth and development of embryos at the range between 0.01 and 1.25 mg/l and between 0.01 and 0.25 mg/l respectively, although the numbers of cultured embryos were small in both media added these regulators. Malt extract and casein hydrolysate slightly promoted the growth of embryos at the concentration of 25 mg/l, and 100 and 1500 mg/l respectively.

Production of seedlings through embryo culture

The 2x×4x crosses produced 687 seeds of which 326 (47.5%) seeds contained embryos with various morphology and these embryo sizes varied from very small (< 0.5 mm) to large (>2 mm) (Table 3), while the reciprocal crosses produced 1122 seeds of which 834 (74.3%) seeds contained various embryos similar to those observed in the 2x ×4x crosses (Table 4). After about three months culture, a total number of germinated embryos was 62 (19.0%) for the 2x×4x crosses and 66 (7.4%) for the 4x×2x crosses. Generally, the germination rate of embryos increased with increase of their size when planted on the culture medium (Fig. 4). In the embryos from 'Muscat Bailey A'×'Red Pearl' and those from the reciprocal cross, germination rates were more than 50% when the embryo length was more than 1mm.

In the 2x×4x crosses, rates of plants obtained through embryo culture ranged from 0 to 42.9% with the average of 7.3%, whereas in the 4x×2x crosses the rates ranged from 0 to 5.7% with the average of 2.9%.

In the 2x×4x crosses, when 'Delaware', 'Muscat Bailey A' and 'Sekirei' were used as

Table 3. Results of *in vitro* culture of underdeveloped embryos from 2x×4x crosses in grape.

Cross	No. of flowers pollinated	No. of seeds obtained (N[a])	No. of embryos cultured	No. of embryos germinating	No. of plants acclimated (%[b])
Delaware×C. H. Muscat	96	54 (38)	32	10	6 (11.1)
Delaware×Kyoho	12	10 (4)	4	1	1 (10.0)
Delaware×Yuhu	885	177 (54)	54	7	6 (3.4)
Subtotal	993	241 (96)	90	18	13 (5.4)
Muscat B. A×C. H. Muscat	7	7 (5)	5	4	3 (42.9)
Muscat B. A×Kyoho	25	51 (33)	32	16	13 (25.5)
Muscat B. A×Red Pearl	433	260 (126)	119	18	16 (6.2)
Subtotal	465	318 (164)	156	38	32 (10.1)
Rizamat×C. H. Muscat	ne[c]	51 (28)	25	0	0 (0)
Rizamat×Red Pearl	45	21 (11)	11	0	0 (0)
Rizamat×Yufu	105	21 (10)	9	1	1 (4.8)
Subtotal	–	93 (49)	45	1	1 (1.1)
Sekirei×C. H. Muscat	ne[c]	17 (9)	9	1	0 (0)
Sekirei×Red Pearl	167	20 (8)	7	4	4 (20.0)
Subtotal	–	37 (17)	16	5	4 (10.8)
Total	–	687 (326)	307	62	50 (7.3)

[a] No. of seeds with embryo. [b] No. of acclimated plants / No. of seeds. [c] Not examined.

Table 4. Results of *in vitro* culture of underdeveloped embryos from 4x×2x crosses in grape.

Cross	No. of flowers pollinated	No. of seeds obtained (N[a])	No. of embryos cultured	No. of embryos germinating	No. of plants acclimated (%[b])
C. H. Muscat×Delaware	24	8 (6)	5	0	0 (0)
C. H. Muscat×Muscat B. A	26	45 (35)	32	1	0 (0)
C. H. Muscat×Rizamat	ne[c]	43 (33)	32	1	0 (0)
C. H. Muscat×Sekirei	ne[c]	46 (33)	32	0	0 (0)
Subtotal	–	142 (107)	101	2	0 (0)
Kyoho×Delaware	29	10 (3)	3	0	0 (0)
Kyoho×Muscat B. A	88	30 (13)	12	0	0 (0)
Subtotal	117	40 (26)	15	0	0 (0)
Red Pearl×Muscat B. A	425	544 (434)	403	47	25 (4.6)
Red Pearl×Rizamat	55	53 (45)	43	8	3 (5.7)
Red Pearl×Sekirei	95	71 (65)	64	1	0 (0)
Subtotal	575	668 (544)	510	56	28 (4.2)
Yufu×Delaware	243	158 (111)	105	6	4 (2.5)
Yufu×Rizamat	84	114 (63)	50	0	0 (0)
Yufu×Sekirei[d]	60	76 (63)	51	2	1 (1.3)
Subtotal	387	348 (237)	206	8	5 (1.4)
Total	–	1198 (897)	832	66	33 (2.8[e])

[a] No. of seeds with embryo. [b] No. of acclimated plants/No. of seeds. [c] Not examined. [d] Reciprocal cross was not made. [e] Average value (%) includes the data of 'Yufu'×'Sekirei'. Average value (%) without that of 'Yufu'×'Sekirei' is 2.9.

seed parents, seedlings were successfully obtained from 5.4, 10.2 and 10.8% of seeds through embryo culture respectively. When 'Rizamat' was used as a seed parent, however, only one seedling was derived from 93 seeds through embryo culture. In the 4x×2x crosses, when 'Red Pearl' and 'Yufu' were used as seed parents, underdeveloped embryos from 4.2 and 1.4% of the seeds developed into seedlings *in vitro*, respectively. When 'Cannon Hall Muscat' was used as a seed parent, only two of 107 embryos germinated *in vitro*, but no plants were established. In two cross combinations where 'Kyoho' was used as a seed parent, none of cultured embryos germinated.

Production of seedlings through secondary embryo formation *in vitro*

Multiple embryo formation due to secondary embryo formation from initial embryo was observed not only in several triploid seeds but also in many embryos cultured (Fig.2). The secondary embryos often initiated in blimp-shaped embryos without hypocotyls and/or cotyledons (Fig. 3). In the cultured embryos, a total of 101 embryos showed multiple embryo formation *in vitro* (Table 5). This was 8.9% of embryos cultured. The rate of embryos showing multiple embryo formation *in vitro* was higher in the embryos from the 2x×4x crosses (23.5%) than those from the reciprocals (3.5%).

Morphology of the additional secondary embryos was different in different embryos. Among these embryos, almost normal ones with well-developed hypocotyls and cotyledons germinated and developed into plants. Thirty-eight embryos forming secondary

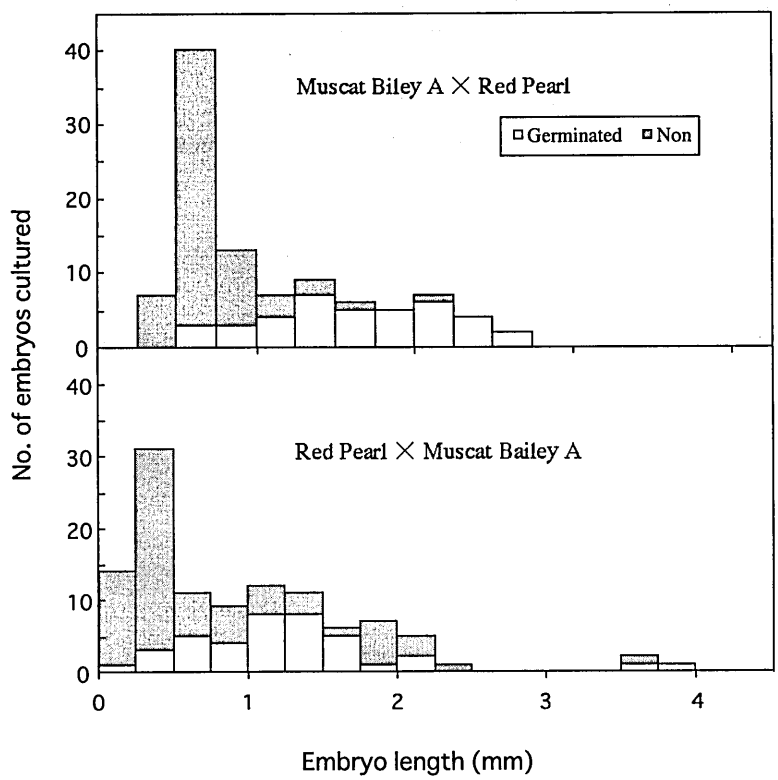

Fig. 4. Frequency distributions of embryos with different length and their germination rates after three months of culture. White bar: germinated; shaded bar: non-germinated.

embryos resulted in successful establishment of plants. This was 45.8% of embryos resulting in plants. In the 2x×4x crosses, 38.9% of embryos forming secondary embryos resulted in successful establishment of plants, while in the 4x×2x crosses 34.5% of embryos forming secondary embryos resulted in successful establishment of plants. In the 2x×4x crosses, plants derived through secondary embryo formation were 28 of 50 established plants (46%), whereas in the 4x×2x crosses they were 10 of 33 (30.3%).

Characteristics of triploid seedlings established

Chromosome observation in root tip cells of the seedlings established through embryo culture indicated that all were triploid plants with 57 chromosomes, except for one tetraploid plant with 76 chromosomes. The characteristics and origin of the exceptional tetraploid seedlings derived from 'Red Pearl×'Muscat Bailey A' has been reported elsewhere (Park *et al.*, 2002a). After acclimating, the triploid seedlings grew rapidly and showed higher vigor than diploid seedlings derived from self-pollination of their parents.

Table 5. Rate of plants obtained through secondary embryo formation in cultured 3x embryos from reciprocal crosses between 2x and 4x grape cultivars.

Cross	No. of embryos cultured	No. of embryos forming secondary embryos (%)	No. of embryos developing into plants through secondary embryo formation (N[a])
2x×4x			
Delaware×C. H. Muscat	32	3 (9.4)	2 (4)
Delaware×Kyoho	4	1 (25.0)	1 (0)
Delaware×Yufu	54	18 (33.3)	4 (2)
Muscat B. A×C. H. Muscat	5	0 (0)	0 (3)
Muscat B. A×Kyoho	32	16 (50.0)	10 (3)
Muscat B. A×Red Pearl	119	26 (21.8)	8 (8)
Rizamat×C. H. Muscat	25	0 (0)	0 (0)
Rizamat×Red Pearl	11	3 (27.3)	0 (0)
Rizamat×Yufu	9	1 (11.1)	1 (0)
Sekirei×C. H. Muscat	9	2 (22.2)	0 (0)
Sekirei×Red Pearl	7	2 (28.6)	2 (2)
Total	307	72 (23.5)	28 (22)
4x×2x			
C. H. Muscat×Delaware	5	0 (0)	0 (0)
C. H. Muscat×Muscat B. A	32	1 (3.1)	0 (0)
C. H. Muscat×Rizamat	32	0 (0)	0 (0)
C. H. Muscat×Sekirei	32	0 (0)	0 (0)
Kyoho×Delaware	3	0 (0)	0 (0)
kyoho×Muscat B. A	12	0 (0)	0 (0)
Red Pearl×Muscat B. A	403	23 (5.3)	9 (16)
Red Pearl×Rizamat	43	1 (2.3)	1 (2)
Red Pearl×Sekirei	64	0 (0)	0 (0)
Yufu×Delaware	105	1 (1.0)	0 (4)
Yufu×Rizamat	50	1 (2.0)	0 (0)
Yufu×Sekirei[b]	51	2 (3.9)	0 (1)
Total[c]	832	29 (3.5)	10 (23)

[a] No. of embryos developing into plants without secondary embryo formation *in vitro*. [b] Reciprocal cross was not made. [c] Values include the data of 'Yufu' ×'Sekirei'.

They flowered by two to three years after planting and some set small seedless fruits and the other set no fruits. The characteristics of their GA_3–treated and non–treated berries will be published elsewhere.

DISCUSSION

The present study demonstrated that the efficiency of triploid breeding is heightened by *in vitro* culture of underdeveloped embryos from interploid crosses between diploid and tetraploid grape cultivars. In the same cross combinations, the rates of established seedlings to pollinated flowers are high in embryo culture as compared with seed sowing reported previously (Wakana *et al.*, 2002). In 'Muscat Bailey A'×'Red Pearl', for example, two seedlings were obtained from 549 flowers through seed sowing, whereas 16 seedlings

were obtained from 433 flowers through embryo culture, i.e., the rates were 0.4% for seed sowing and 3.7% for embryo culture. The similar facts that embryo culture increased the rate of seedling production were also prominent in 'Red Pearl'×'Muscat Bailey A' and 'Muscat Bailey A'×'Kyoho' where the rates were 0% and 0.7% for seed sowing and 5.9% and 52.0% for embryo culture respectively.

When reciprocal crosses between 2x and 4x cultivars are compared, the results of triploid seedling production are different from those of seed sowing reported previously (Wakana *et al.*, 2002). In the present embryo culture, the rates of established seedlings to pollinated flowers in the 2x×4x crosses were higher than those in the 4x×2x crosses, whereas in the seed sowing no difference was detected between reciprocal crosses between 2x and 4x cultivars. This may be due to the difference of plant material between the experiments, or due to delaying of embryo abortion in the 2x×4x crosses.

Yamashita *et al.* (1993, 1995) also reported that the rate of 3x seedlings established per flower increased by *in vitro* culture of embryos from crosses between diploid and tetraploid grape cultivars, although they used embryos extracted from immature berries as materials for seedling production. In the present study with underdeveloped embryos from mature berries, none of embryos from several 2x×4x cross combinations and two thirds of 4x×2x cross combinations developed into seedlings. Furthermore, the six growth regulators added at various concentrations to the MS medium did not exhibit prominent effect on the growth and germination of embryos and subsequent seedling production. These may be related to the presence of several depressive factors such as low viability and very abnormal morphogenesis in the underdeveloped embryos from mature berries.

On the other hand, our result suggests that *in vivo* and *in vitro* occurrence of multiple secondary embryos in underdeveloped embryos is important to increase the production of 3x seedlings, because *in vitro* morphogenesis in some of the multiple embryos was almost normal and they were develop into seedlings, and because the rate of underdeveloped embryos showing secondary embryo formation was considerably high in most cross combinations.

Esen and Soost (1973) have reported multiple embryo formation in the abortive triploid seeds of monoembryonic diploid citrus cultivars pollinated with tetraploid cultivars. They postulated that the abnormal polyembryo formation occurring only under abortive conditions is related to the malfunction of the endosperm such as hormonal unbalance. In this study of embryo culture, however, effect of six growth regulators on the formation of secondary embryos was not detected. So far, the method and mechanism to induce the initiation of multiple embryos are not clear.

Formation of secondary embryos or somatic embryogenesis in grape embryos has been reported in embryo culture (Yamashita *et al.*, 1993, 1995) and *in ovulo* embryo culture (Emershad and Ramming, 1984, 1994; Emershad *et al.*, 1989), while polyembryonic seed formation has been reported in the seeds from *Vitis vinifera*×*V. riparia* (Bouquet, 1982). Considering these and the present results, it seems that grape zygotic embryos have some extent of potential to initiate somatic embryos irrespective of the presence or absence of growth regulators. The fact that in many grapevine genotypes the connective of anthers is a highly regenerative tissue when cultured *in vitro* and give rise to somatic embryos with high frequencies (Mullins *et al.*, 1992) may partially support this

postulation.

The size of embryos excised from mature berries was varied from very small to very large and most of them were underdeveloped and/or very small. Another fact that the germination rate of cultured embryos decreased with decrease of their size (Fig. 4) suggests the necessity of *in vitro* culture before the embryos fall into abortive situation. Thus, to obtain 3x seedlings from these crosses with high frequencies, *in vitro* culture of embryos extracted from immature berries may be necessary before they abort or fall into abortive situation due to endosperm degeneration.

In the crosses with stenospemocarpic cultivars as seed parents, seedlings were derived through *in ovulo* embryo culture with high frequencies (Ramming and Emershad, 1982: Cain *et al.*, 1983; Emershad and Ramming, 1984; Spiegel-Roy *et al.*, 1985; Goldy *et al.*, 1988; Emershad *et al.*, 1989; Gray *et al.*, 1990). The same embryo-rescue techniques were also applied to aneuploid plant production from triploid grapes (Park *et al.*, 1999a) and triploid plant production from crosses between diploid and tetraploid grapes (Yamashita *et al.*, 1998). Application of embryo-rescue techniques such as these may further improve the efficiencies of triploid breeding in grape.

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