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## **Degree of Abortion and Germination Rates in Triploid Seeds from Crosses between Diploid and Tetraploid Grapes (*Vitis vinifera* L. and *V. complex*)**

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Interploid crosses were carried out using seven diploid and four tetraploid grape cultivars to examine the degree of triploid seed development. The average number of seeds per pollinated flower was 0.42 for  $2x \times 4x$ , 0.51 for  $4x \times 2x$ , 0.78 for  $4x \times 4x$  and 1.21 for  $2x \times 2x$  crosses. About 47% of seeds from the  $2x \times 4x$  crosses and 44% of seeds from the  $4x \times 2x$  crosses were sinkers. Nineteen of 1012 seeds from the  $2x \times 4x$  crosses and 32 of 1170 seeds from the  $4x \times 2x$  crosses germinated. Among these crosses, 'Yufu'  $\times$  'Sekirei' and its reciprocal cross exhibited the highest (4.4%) and next highest (3.6%) rates of seedling production per flower. All of the seedlings examined were triploid ( $2n=3x=57$ ). In comparison with diploid and tetraploid seeds from the intraploid crosses, reduction of seed size was prominent in seeds from the  $4x \times 2x$  crosses but not in seeds from the  $2x \times 4x$  crosses. About 10% to 30% of seeds from the interploid crosses had fully developed endosperm and 80% of seeds with the fully developed endosperm contained embryos at globular to torpedo stages. The rate of seeds with embryo was higher in the  $4x \times 2x$  crosses than the  $2x \times 4x$  crosses. However, difference in embryo and endosperm development was not detected between the  $2x \times 4x$  and  $4x \times 2x$  crosses. These results suggested that in the interploid crosses endosperm block is partial or flexible and that germination rates of the triploid seeds are very low but different in different crosses.

## INTRODUCTION

Seedlessness is one of the desirable characters for breeding table grapes and raisin grapes. Seedless grapes have gained in use as table grapes in Japan as well as in the world. Winkler *et al.* (1962) divided setting of berries into four types, i.e., stimulative parthenocarpy, stenospermocarpy, empty seededness and normal set. Stimulative parthenocarpy is attributed to defective embryo sac formation and high germinability of pollen in cultivars such as 'Black Corinth'. However, stimulus of pollination and nutritional stimulus resulting from girdling or spraying open flowers with auxin or gibberellin (Coombe, 1950; Weaver and Williams, 1950; Weaver, 1952) were also necessary to satis-

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factory set of fruits. Stenospermocarpy occurs in cultivars such as 'Thompson Seedless' in which pollination and fertilization followed by embryo abortion in the very early stage of embryogenesis are prerequisite for subsequent fruit growth. The stimulative parthenocarpy and stenospermocarpy are important genetic characters for breeding seedless grapes.

Although modern breeding of seedless grape cultivars in the world was mainly dependent upon the usage of stenospermocarpic cultivars, triploid cultivars such as 'Kingdela' and 'Honey Seedless' and aneuploid ( $4x-1$ ) cultivar 'Takao' (Ashikawa, 1972; Yamane *et al.*, 1978) were also bred in Japan. Unbalanced chromosome numbers in these cultivars result in the formation of unfunctional pollen and embryo sac and, consequently, in the formation of seedless fruit. In these cultivars, gibberellin treatment just after anthesis is necessary to satisfactory set and growth of fruits.

As compared with the breeding of seedless grapes using stenospermocarpic cultivars, breeding of triploid seedless grapes with diploid and tetraploid cultivars is considered to be superior in the following points. (1) Since the seedlessness in triploid grapes results from their unbalance chromosome numbers, any diploid and tetraploid grapes would be expected to be used as seed and/or pollen parents. (2) Tetraploid sports are relatively easily induced from given diploid cultivars by colchicine treatment. (3) Very high male and female sterility is expected in the triploid grapes because of their relatively large chromosome number ( $2n=3x=57$ ), since the rates of their functional euploid ( $1x$  or  $2x$ ) gametes are calculated to be only about  $1.9 \times 10^{-6}\%$ , and since almost all of aneuploid gametes are considered to be unfunctional (Park *et al.*, 1999).

Seed abortion following crosses between diploid and tetraploid plants has been reported in *Vitis* (Einset and Lamb, 1951; Einset and Pratt, 1954; Alley, 1957; Yamashita *et al.*, 1993 and 1995), *Citrus* (Esen and Soost, 1973) and the other fruit trees (see Sanford, 1983) as well as in many genera of angiosperms (eg. Brink and Cooper, 1947). For establishing triploid grapes efficiently, it is important to know the extent of seed abortion and the rates of seed germination in various reciprocal crosses between diploid and tetraploid cultivars, and to rescue underdeveloped triploid embryos in the abortive seeds.

This study was designated to demonstrate the degree of seed abortion in various reciprocal crosses between diploid and tetraploid cultivars as the basis for triploid breeding and subsequent aneuploid breeding in grape.

## MATERIALS AND METHODS

### Plant materials

Seven diploid cultivars and four tetraploid cultivars were chosen as seed and/or pollen parents to examine the extent of triploid seed development and to produce triploid hybrids. Of the seven diploid cultivars, five cultivars 'Muscat of Alexandria', 'Rosario Bianco', 'Rizamat', 'Sekirei' and 'Neo Muscat' and one tetraploid cultivar 'Cannon Hall Muscat' are *Vitis vinifera* cultivars. However, it is uncertain whether 'Sekirei' and 'Neo Muscat' are *V. vinifera* or not, since these cultivars have 'Koshu' (*V. vinifera* hybrid?: Ohmi *et al.*, 1993) in their pedigrees. The remaining two diploid cultivars 'Muscat Bailey A' and 'Delaware' and three tetraploid cultivars 'Kyoho', 'Red Pearl' and 'Yufu' are intercontinental hybrid cultivars (*V. complex*) with *V. vinifera* and North American *Vitis*

species in their pedigrees. Of the four tetraploid cultivars, 'Red Pearl' and 'Yufu' were selected from natural giant sports of 'Delaware' and 'Muscat Bailey A', respectively. All these cultivars were 10- to 15-year-old trees grown in the Sasagri Orchard of the University Farm, Kyushu University, Fukuoka.

### Seed germination test

Sixteen reciprocal interpoll cross and seven intrapoll cross were carried out using five diploid and three tetraploid cultivars. Additional three interpoll cross between 'Yufu' and three diploid cultivars were also made.

Number of flower buds per cluster was adjusted before pollination so that each cluster had about 50–100 flowers. The flower buds were emasculated one or two days before anthesis, washed with running water or sprayed with water to prevent self-pollination and bagged. Hand pollination was carried out at the full bloom stage of the clusters using fresh pollen collected from just opened flowers of the pollen parents. Pistils with wet stigma were used for pollination, but those with dry stigma and/or abnormal morphology were removed from the clusters. Immediately after the pollination, the pollinated flower clusters were bagged again to prevent further pollination.

Berries were harvested at the fully mature stage. Seeds extracted from the berries were separated using water into two categories, floaters and sinkers. Only sinkers were set in 30×40 cm nursery boxes filled with wet sand and stored under natural conditions. In May of the next year, the nursery boxes were carried to a green house to facilitate seed germination, and number of seeds germinating was scored in each cross. The seedlings were planted in 24×30 cm pots filled with mold and soil (1:1) for about two to three months and were transplanted in the green house in spring of the next year.

### Chromosome observation

Chromosome observation was carried out as follows. The root tips from the seedlings were pretreated with 0.08M hydroxyquinoline for 2 hours, fixed with alcohol propionic acid (2.5:1), hydrolyzed with 1N HCl at 60°C for 5 minutes, stained with 1% lacto propion orcein (Dyer, 1963) for 12 hours in room temperature, squashed on microslides and observed under a microscope.

### Anatomy of seeds

For anatomical study of seeds, reciprocal cross between diploid and tetraploid cultivars and self-pollination treatments of the cultivars were carried out using two tetraploid cultivars 'Yufu' and 'Red Pearl' and two diploid cultivars 'Muscat Bailey A' and 'Delaware'. In addition, two 2x×4x cross and four 4x×2x were also made using four diploid cultivars and four tetraploid cultivars. Crosses were carried out according to the procedure as mentioned above. Berries were harvested at the mature stage in September. Seeds extracted from the berries were dissected with a pair of forceps and a surgical knife and the extent of endosperm development was examined under a dissecting microscope equipped with a micrometer. The extent of endosperm development was classified into three categories, i.e., (1) fully developed, (2) partially developed, and (3) almost or perfectly degenerated endosperm. Then, embryos were extracted from the dissected seeds and the morphology and length of them were examined under the dissecting microscope.

## RRESULTS

**Seed set**

In reciprocal interpollid crosses between five diploid and three tetraploid grape cultivars, 0.42 and 0.51 seeds per flower were obtained from  $2x \times 4x$  and  $4x \times 2x$  crosses respectively (Table 1). In self-pollination and reciprocal crosses in the diploid cultivars, number of seeds per flower ranged from 0.80 to 1.55 with the average of 1.21, while in self-pollination and reciprocal crosses in the tetraploid cultivars it ranged from 0.61 to 0.95 with the average of 0.78 (Table 1). Thus, the average rate of seed set was the highest in the  $2x \times 2x$  crosses, followed by the  $4x \times 4x$ ,  $4x \times 2x$  and  $2x \times 4x$  crosses. Significant difference in the rate was not detected between the  $4x \times 2x$  and  $2x \times 4x$  crosses at 5% level.

In reciprocal interpollid crosses between three diploid cultivars and tetraploid 'Kyoho', the rate of seed set per flower was high when 'Kyoho' was used as a pollen parent, whereas in three of four reciprocal crosses between four diploid cultivars and tetraploid 'Red Pearl' it was high when 'Red Pearl' was used as a pistillate parent (Table 1). In reciprocal crosses between 'Yufu' and 'Sekirei', the rate was higher in 'Yufu'  $\times$  'Sekirei' than that in the reciprocal. Additional interpollid crosses between 'Yufu' and three diploid vinifera cultivars showed very high rate of seed set per flower (0.72–1.35) among the interpollid crosses carried out in this study (Table 2).

**Table 1.** Germination rates in seeds derived from reciprocal crosses between  $2x$  and  $4x$  grape cultivars.

Cross <sup>a</sup>	No. of flowers pollinated (No. <sup>b</sup> )	No. of seeds obtained			No. of seeds germinating (%)	No. of 3x plants obtained
		Floaters	Sinkers	Total		
<b>2x×4x</b>						
Muscat A.×Kyoho	351( 6)	182	66	24	86 ( 2.4)	2
Muscat B.A×Kyoho	277( 4)	46	112	158	2 ( 1.3)	2
Neo Muscat×Kyoho	169( 3)	24	34	58	0 ( 0 )	0
Delaware×Red Pearl	158 ( 6)	28	28	56	0 ( 0 )	0
Muscat A.×Red Pearl	669 ( 8)	154	131	285	6 ( 2.1)	1
Muscat B.A×Red Pearl	549 ( 7)	91	84	175	5( 2.9)	2
Neo Muscat×Red Pearl	240 ( 3)	8	24	32	0 ( 0 )	0
Sekirei×Yufu	195 ( 3)	33	29	62	7 (11.3)	7
Total	2413 (37)	533	479	1012	19 ( 1.9)	14
<b>4x×2x</b>						
Kyoho×Muscat A.	245 ( 2)	12	10	22	0 ( 0 )	0
Kyoho×Muscat B.A	296 ( 2)	60	75	135	0 ( 0 )	0
Kyoho×Neo Muscat	203 ( 2)	18	50	68	0 ( 0 )	0
Red Pearl×Delaware	192 ( 5)	46	2	48	0 ( 0 )	0
Red Pearl×Muscat A.	344 ( 9)	208	62	270	0 ( 0 )	0
Red Pearl×Muscat B.A	142 ( 3)	15	89	104	0 ( 0 )	0
Red Pearl×Neo Muscat	193 ( 6)	60	64	124	2 ( 1.6)	2
Yufu×Sekirei	680 (10)	234	165	399	30 ( 7.5)	30
Total	2295 (39)	653	517	1170	32 ( 2.7)	32

**Table 1.** Continued.

Cross <sup>a</sup>	No. of flowers pollinated (No. <sup>b</sup> )	No. of seeds obtained			No. of seeds germinating (%)	No. of 3x plants obtained
		Floaters	Sinkers	Total		
2x×2x						
Delaware×Delaware	240 ( 4)	19	174	193	—	—
Delaware×Muscat A.	213 ( 4)	7	248	255	—	—
Muscat A.×Delaware	173 ( 3)	7	211	218	—	—
Muscat A.×Muscat A.	264 ( 4)	26	382	408	—	—
Total	890(15)	59	1015	1074	—	—
4x×4x						
Red Pearl×Yufu	241 ( 4)	24	124	148	—	—
Yufu×Yufu	191 ( 3)	67	101	168	—	—
Yufu×Red Pearl	112 ( 2)	53	53	106	—	—
Total	544 ( 9)	144	278	422	—	—

<sup>a</sup> Muscat. A.: Muscat of Alexandria; Muscat B. A: Muscat Bailey A. <sup>b</sup> Number of clusters used.

**Table 2.** Germination rates in the seeds obtained from 'Yufu' crossed with 2x cultivars.

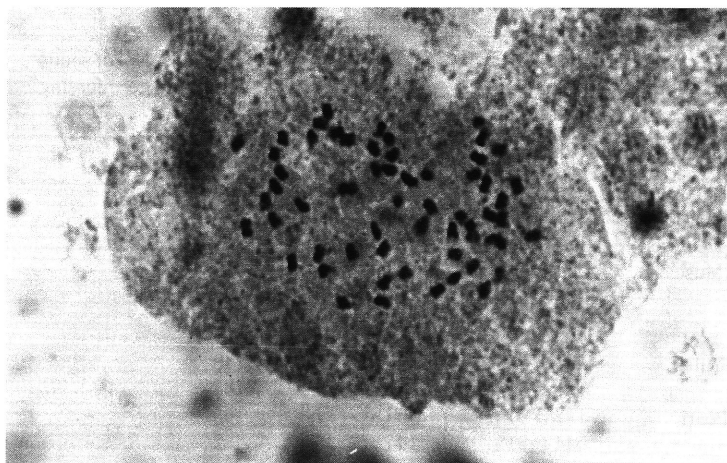
Cross <sup>a</sup>	No. of flowers pollinated (No. <sup>b</sup> )	No. of seeds obtained			No. of seeds germinating (%)	No. of 3x plants obtained
		Floaters	Sinkers	Total		
Yufu×Muscat A.	295 ( 6)	181	45	226	3 (1.3)	0
Yufu×Rozario Bianco	494 ( 4)	212	143	355	3 (0.8)	2
Yufu×Rizamat	206 ( 3)	206	73	279	1 (0.4)	1
Total	995 (13)	599	261	860	7 (0.8)	3

<sup>a</sup> Muscat A.: Muscat of Alexandria. <sup>b</sup> No. of clusters used.

### Germination of triploid seed

In 16 reciprocal crosses between five diploid and three tetraploid grape cultivars, a total of 1012 and 1170 seeds were obtained from 2x×4x and 4x×2x crosses respectively (Table 1). About 47% of the seeds from the 2x×4x and 44% of the seeds from the 4x×2x were sinkers reflecting the presence of endosperm and/or embryos, while more than half of the seeds were floaters in each of the interpollid crosses. The floaters were empty seeds without endosperm and embryos. Although the rate of sinkers was not significantly different between the 2x×4x and 4x×2x crosses, it was significantly different between the 2x×2x and 4x×4x crosses at 5% level, i.e., it was very high (95%) in the 2x×2x crosses and high (66%) in the 4x×4x crosses as compared with the 2x×4x and 4x×2x crosses. The rates of sinkers were also low (20–40%) in three additional interpollid crosses between tetraploid 'Yufu' and diploid cultivars (Table 2).

Of the 1012 seeds derived from the 2x×4x crosses, 19 seeds germinated in five of eight cross combinations. In this case, 'Sekirei' × 'Yufu' showed the highest rate (11.3%)



**Fig. 1.** Metaphase figure in the root tip cell of triploid seedling RN-8601 derived from 'Red Pearl'  $\times$  'Neo Muscat', showing 57 chromosomes.

of seed germination. In the  $4x \times 2x$  crosses, 32 of the 1170 seeds germinated. The 32 seeds were derived from two of eight cross combinations, and seeds from 'Yufu'  $\times$  'Sekirei' showed the highest germination rate (7.5%) (Table 1). In the three additional  $4x \times 2x$  crosses with 'Yufu' as a seed parent, however, seed germination rates (0.4–1.3%) were lower than that in 'Yufu'  $\times$  'Sekirei' (Table 2).

A total of 49 seedlings were derived from the reciprocal crosses between diploid and tetraploid cultivars and from the three additional interploid crosses (Table 1 and 2). However, five seedlings from the  $2x \times 4x$  crosses died of damping-off just after germination. Thus, ploidy of the five seedlings could not be determined. Chromosome number observation in root tip cells indicated that all seedlings obtained were triploid plants with 57 chromosomes (Fig. 1). In the reciprocal crosses between diploid and tetraploid cultivars, number of triploid seedlings obtained was  $5.8 \times 10^{-3}$  per flower in the  $2x \times 4x$  crosses and  $13.9 \times 10^{-3}$  per flower in the  $4x \times 2x$  crosses, while for the three additional  $4x \times 2x$  crosses it was  $3.0 \times 10^{-3}$  per flower in average. These triploid seedlings were very vigorous and showed rapid growth as compared with diploid seedlings from self-pollinated diploid cultivars.

### Variation of seed size

When the sizes of triploid seeds from interploid crosses were compared with those of diploid and tetraploid seeds from the self-pollinated seed parents, significant reduction of seed size was detected in two of four  $2x \times 4x$  crosses and all six  $4x \times 2x$  crosses, i.e., the length of triploid seeds was about 93% for two crosses of 'Delaware' with two  $4x$  cultivars, 92% for three crosses of 'Red Pearl' with three  $2x$  cultivars and 90% for three crosses of 'Yufu' with three  $2x$  cultivars (Table 3). However, the sizes of triploid seeds from two crosses of 'Muscat Bailey A' with two  $2x$  cultivars were almost the same as those of diploid seeds from self-pollinated 'Muscat Bailey A'.

**Table 3.** Variation of seed size in 2x×2x, 2x×4x, 4x×2x and 4x×4x crosses.

Cross	No. of seeds examined	Seed length±SE (mm)
2x×4x		
Delaware×Cannon Hall Muscat	54	6.65±0.65
Delaware×Yufu	165	6.89±0.03
Muscat Bailey A×Kyoho	81	8.45±0.07
Muscat Bailey A×Red Pearl	244	8.56±0.04
4x×2x		
Red Pearl×Muscat Bailey A	138	6.08±0.05
Red Pearl×Rizamat	52	6.18±0.06
Red Pearl×Sekirei	70	6.16±0.04
Yufu×Delaware	144	7.97±0.05
Yufu×Rizamat	113	7.90±0.06
Yufu×Sekirei	64	7.88±0.09
2x×2x (self-pollination)		
Delaware	88	7.27±0.07
Muscat Bailey	119	8.47±0.04
4x×4x (self-pollination)		
Red Pearl	85	6.65±0.17
Yufu	89	8.81±0.07

### Extent of endosperm development

In reciprocal interploid crosses between 'Muscat Bailey A' and 'Red Pearl' and between 'Delaware' and 'Yufu', partially developed endosperm was observed in 60–80% of seeds, while perfectly or mostly degenerated endosperm was observed in 10–30% of seeds (Table 4). These percentages were very high as compared with those in self-pollinations of the four parental cultivars. In the four self-pollinations, about 90% of seeds had fully developed endosperm and about 90% of the seeds with fully developed endosperm had embryos, whereas in 2x×4x crosses about 10% of seeds had fully developed endosperm and 83% of the seeds with fully developed endosperm had embryos. In 4x×2x crosses, however, some different results from those in the 2x×4x crosses were observed, i.e., about 30% of seeds from 'Yufu'×'Delaware' had fully developed endosperm, and in 'Red Pearl'×'Muscat Bailey A' only 30% of seeds with fully developed endosperm had embryos.

In all of the self-pollinations and reciprocal crosses except for 'Red Pearl'×'Muscat Bailey A', the more the endosperm developed, the higher the rate of seeds with embryo was observed (Table 4). The rates of seeds with embryo were very low (about 40%) in the 2x×4x crosses as compared with those in the 4x×2x (about 75%). This result was mainly due to the difference in the rates of embryo formation in seeds with imperfectly or partially developed endosperm between the 2x×4x and 4x×2x crosses. Generally, the partially developed endosperm was watery in the 2x×4x crosses and brittle in the 4x×2x crosses.

**Table 4.** The extent of endosperm and embryo development in triploid seeds from reciprocal crosses between 2x and 4x cultivars.

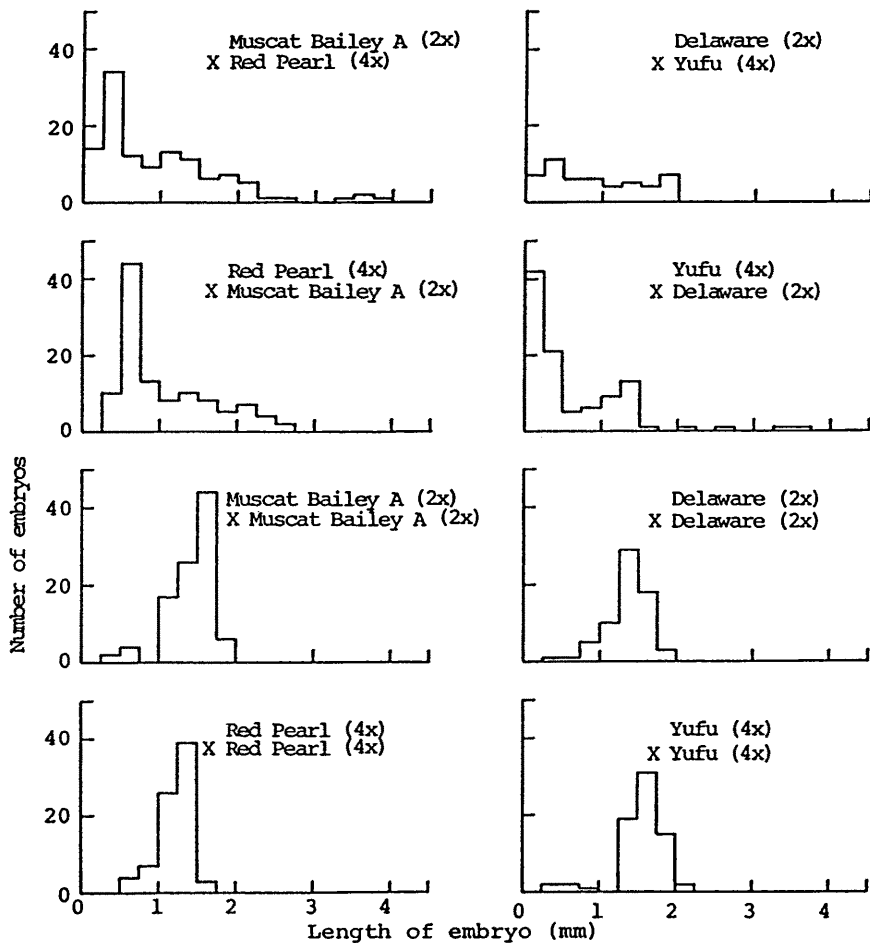
Cross	Percentage of seeds with embryos in indicated degree of endosperm development (N <sup>a</sup> )			
	Fully developed	Partially developed	Perfectly or mostly degenerated	Total
<b>2x×4x</b>				
Muscat B.A×Red Pearl	80 ( 26)	55 (145)	23 ( 73)	48 (244)
Delaware ×Yufu	87 ( 15)	25 (126)	21 ( 24)	30 (165)
Total	83 ( 41)	41 (271)	23 ( 97)	41 (409)
<b>4x×2x</b>				
Red Pearl×Muscat B.A	30 ( 10)	94 (108)	35 ( 20)	80 (138)
Yufu ×Delaware	80 ( 45)	70 ( 86)	39 ( 13)	70 (144)
Total	71 ( 55)	84 (194)	36 ( 33)	75 (282)
<b>2x×2x (self-pollination)</b>				
Muscat B.A	90 (110)	33 ( 3)	0 ( 6)	84 (119)
Delaware	90 ( 71)	14 ( 7)	30 ( 10)	77 ( 88)
Total	90 (181)	20 ( 10)	19 ( 16)	82 (207)
<b>4x×4x (self-pollination)</b>				
Yufu	88 ( 81)	0 ( 2)	17 ( 6)	81 ( 89)
Red Pearl	92 ( 78)	33 ( 6)	0 ( 1)	87 ( 85)
Total	90 (159)	25 ( 8)	14 ( 7)	84 (174)

<sup>a</sup> Number of seeds examined.

### Extent of triploid embryo development

With regard to the variation of embryo size and morphology, no clear differences were found in reciprocal interploid crosses between 'Yufu' (4x) and 'Delaware' (2x) and between 'Red Pearl' (4x) and 'Muscat Bailey A' (2x) (Fig. 2). Sizes of most embryos from self-pollinations of the diploid and tetraploid cultivars ranged from 1 to 2 mm in length, whereas in the crosses between diploid and tetraploid cultivars various sizes of embryos were observed (Fig. 2 and 3). Most of these embryos were smaller than those in the self-pollinations. However, it was noticeable that in the interploid crosses some embryos were larger than embryos in the self-pollinations, probably because of hormonal unbalance in the endosperm. No relationships were found between seed and embryo sizes not only in the self-pollinations of diploid and tetraploid cultivars but also in the 2x×4x and 4x×2x crosses (Fig. 4).

Most embryos derived from self-pollination had a normal hypocotyl and cotyledons, whereas in the interploid crosses most of embryos, except undifferentiated small globular-shaped embryos, showed abnormal morphology, i.e., malformation of hypocotyl and/or cotyledons, blimp-shaped embryos without distinct hypocotyl and cotyledons, and proliferation of embryos by budding (polyembryo formation).

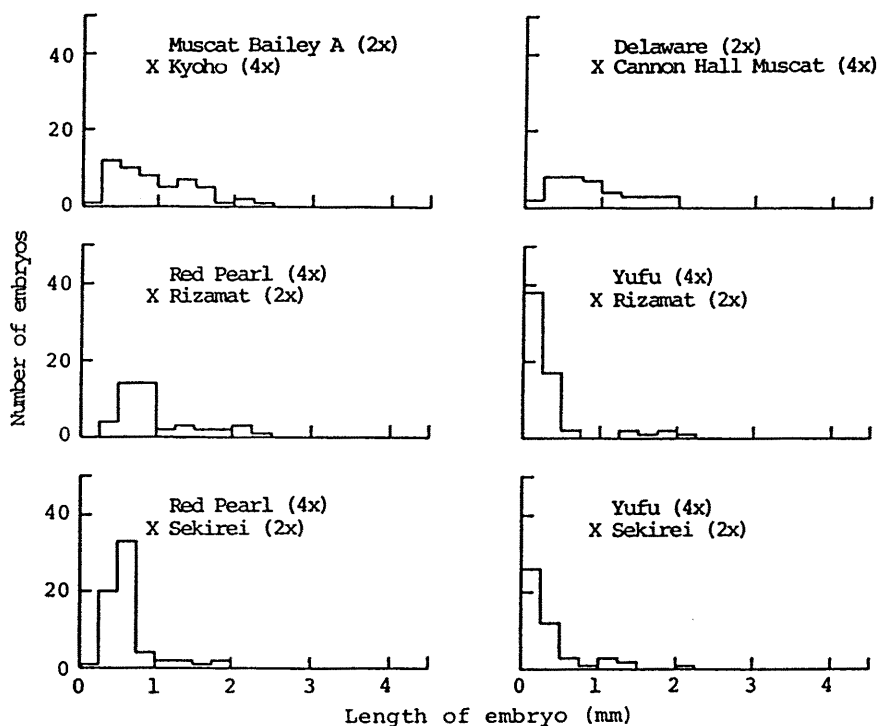


**Fig. 2.** Distribution frequencies of embryo with different lengths in  $2x \times 4x$ ,  $4x \times 2x$ ,  $2x \times 2x$  and  $4x \times 4x$  crosses in *Vitis*.

## DISCUSSION

In angiosperms, embryo and endosperm are formed as the result of double fertilization. Normal fertilization within diploid species results in the formation of  $2x$  embryo associated with  $3x$  endosperm. Soon after fertilization, the endosperm grows rapidly and develops into a nutritive tissue for the embryo. In almost all species of angiosperms, therefore, the success of the embryo depends on the normal development of endosperm, and embryo abortion in interspecific and interploid crosses results from degeneration of endosperm during its growth (Brink and Cooper, 1947).

Sometimes seeds that are normal in size, have endosperm, are firm when pinches,

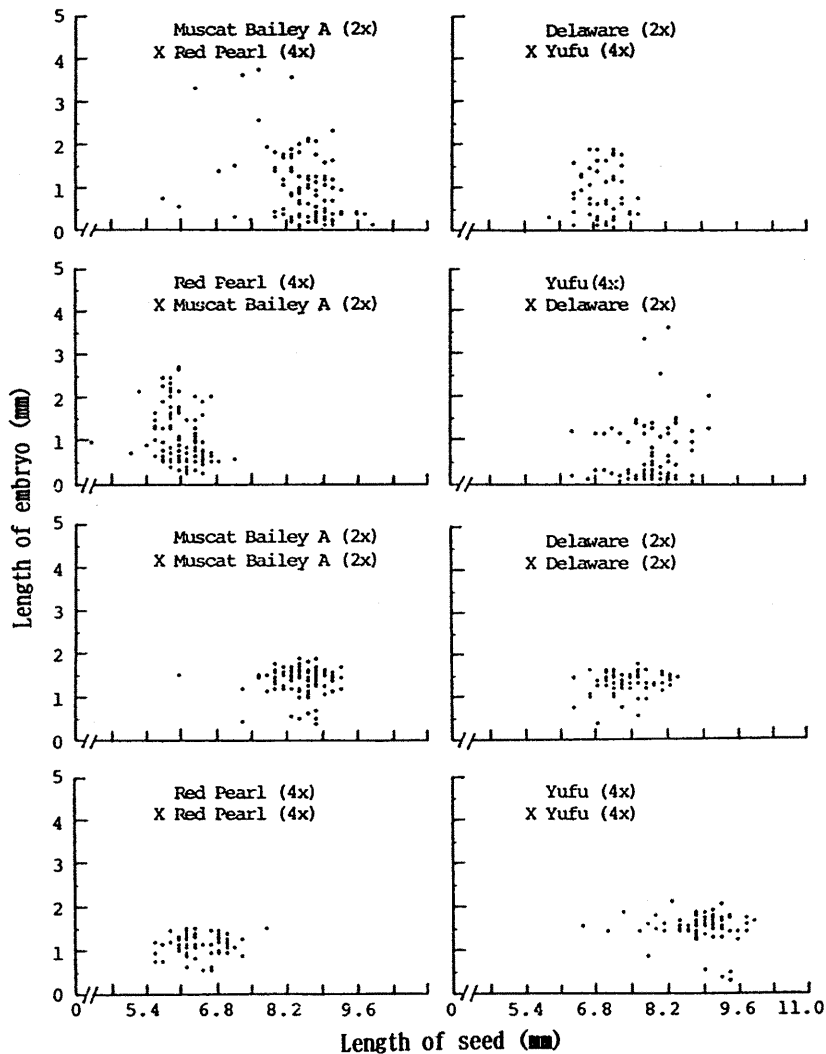


**Fig. 3.** Distribution frequencies of embryo with different lengths in  $2x \times 4x$  and  $4x \times 2x$  crosses in *Vitis*.

and are not infected with fungi do not have embryos. Embryoless seeds are quite common in Apiaceae, but they also occur in corn, wheat, rice, castor bean and so on (Baskin and Baskin, 2001). Automonous endosperm has been found in unfertilized ovules of *Viola odorata in vitro* (Wijowska *et al.*, 1999). In the ovules, parthenogenetic development of egg cells or apogamy did not accompany automonous endosperm. We also found embryoless seeds with perfectly developed endosperm in the present interploid crosses between  $2x$  and  $4x$  grape cultivars and self-pollination of the parents. These results support the hypothesis of independent pathway for embryo and endosperm development.

Johnston *et al.* (1980) proposed the endosperm balance number (EBN) to explain the basis for normal endosperm development after interspecific and interploid crosses in *Solanum*. According to them, each species has an effective ploidy (EBN) in the endosperm, and it is the effective ploidies, rather than numerical ploidies, that must be in a 2:1 maternal : paternal ratio for normal endosperm development. Studies by Ehlenfeldt and Hanneman (1988) and Johnston and Hanneman (1996 and 1999) demonstrated that a few genes and more than one chromosomes were involved in determining EBN.

According to this EBN theory, all *Vitis* species and *Vitis* complexes ( $2n=2x=38$ ),



**Fig. 4.** Relationships between embryo and seed length in  $2x \times 4x$ ,  $4x \times 2x$ ,  $2x \times 2x$  and  $4x \times 4x$  crosses in *Vitis*. Correlation coefficient ( $r$ ) is  $-0.358$  for 'Muscat Bailey A'  $\times$  'Red Pearl',  $-0.275$  for 'Red Pearl'  $\times$  'Muscat Bailey A',  $0.055$  for 'Muscat Bailey A'  $\times$  'Muscat Bailey A',  $0.093$  for 'Red Pearl'  $\times$  'Red Pearl',  $-0.34$  for 'Delaware'  $\times$  'Yufu',  $0.121$  for 'Yufu'  $\times$  'Delaware',  $0.140$  for 'Delaware'  $\times$  'Delaware', and  $-0.092$  for 'Yufu'  $\times$  'Yufu'. There were no significance at 5% level.

except *Vitis rotundifolia* ( $2n=2x=40$ ), are 2EBN and all tetraploid grapes are 4EBN, since in *Vitis* there are no interspecific crossing barriers within the same ploidy (Einset and Pratt, 1975). In  $2x \times 4x$  crosses, therefore, the resultant tetraploid endosperm with a 2:2 paternal : maternal ratio results in abortion, and in  $4x \times 2x$  crosses the resultant pen-

taploid endosperm with a 4:1 paternal : maternal ratio also results in abortion. However, this EBN theory is unable to explain the reason why the degree of endosperm breakdown after interploid crosses is different in different species and why it is different in different seeds from the same cross.

Sanford (1983) described that in fruit crops interploid endosperm block can vary from being nearly absolute (eg. *Vassinium*), to being flexible or partial (eg. *Citrus*), to being entirely absent (eg. *Malus* × *domestica*) – all depending upon the genus. The present study shows that in *Vitis* the interploid endosperm block is flexible or partial, since the number of seeds derived from interploid crosses between 2x and 4x cultivars was about one-half of that from self-pollinations of the 2x and 4x cultivars (Table 1), and since more than 40% of seeds from the interploid crosses were sinkers (Table 1) in which more than 80% of the seeds showed various degree of endosperm development from partial to full (Table 4). Furthermore, the facts that 2.5% of seeds from 2x × 4x crosses and 1.1% of seeds from the reciprocals germinated also suggest that in grape the interploid endosperm block is flexible or partial. The reason for this flexible or partial interploid endosperm block is obscure. However, it seems that allopolyploid origin (Olmo, 1976) and large chromosome number ( $n=19$ ) in *Vitis* may have some role on the flexibility of endosperm block, since apple (*Malus* × *domestica*) exhibiting highly absence of endosperm block is also considered to originate from allopolyploid plants (intergeneric hybrid) with relatively large chromosome number ( $n=17$ ).

Yamashita *et al.* (1993) reported that triploid seedlings are more efficiently derived from 4x × 2x than 2x × 4x crosses. The present study suggests that there is no clear difference between 2x × 4x and its reciprocal crosses as to the formation of perfect 3x seeds with germination ability. In these cross combinations, the number of perfect seeds per flower was different in different cross combinations, and 'Yufu' × 'Sekirei' and its reciprocal cross showed the highest (30 seeds per 680 pollinations) and the next highest (7 seeds per 195 pollinations) production of perfect 3x seeds respectively. Interploid cross combinations exhibiting such high rates of perfect 3x seed formation as these are enough to produce triploid seedlings efficiently. However, almost all interploid crosses exhibited less than 1% of perfect seed formation per flower. In such cases, immature embryo culture and *in ovulo* embryo culture developed in crosses with stenospermocarpic grapes (Cain *et al.*, 1983; Emershad and Ramming, 1984; Spiegel-Roy *et al.*, 1985; Gray *et al.*, 1990) and interspecific crosses between *V. vinifera* and *V. rotundifolia* (Goldy *et al.*, 1988) may help us to produce triploid seedlings from these crosses. Recently, Yamashita *et al.* (1998) successfully produced triploid hybrids through *in ovulo* embryo culture in five interploid crosses with diploid and tetraploid cultivars.

Yamashita *et al.* (1993) examined the size of triploid seeds from two 2x × 4x crosses ('Muscat of Alexandria' × 'Kyoho' and 'July Muscat' × 'Kyoho') and one 4x × 2x cross ('Kyoho' × 'Muscat of Alexandria'). They found that triploid seeds from the 2x × 4x crosses are larger than seeds from the open-pollinated 2x seed parents and that triploid seeds from the 4x × 2x cross are smaller than seeds from the open-pollinated 4x seed parent. In six 4x × 2x crosses carried out in this study with 'Yufu' and 'Red Pearl' as seed parents, significant reduction of seed size was recognized in all of the six crosses. However, our results also showed that the sizes of triploid seeds from 2x × 4x crosses were almost same as those of diploid seeds from self-pollination of diploid seed parent

'Muscat Bailey A', and were smaller than those of diploid seeds from self-pollination of seed parent 'Delaware'. These results suggest that the reduction of  $3x$  seed size is common in the  $4x \times 2x$  crosses but not in the  $2x \times 4x$  crosses. Recently, Park *et al.* (2002) have found the occurrence of  $4x$  seedlings from  $4x \times 2x$  crosses in *Vitis*. Selection of the large-sized seeds in  $4x \times 2x$  crosses may be a useful method to produce highly heterozygous tetraploid hybrids without using colchicine-induced tetraploid plants as pollen parents.

In summary, the triploid hybrid seedlings derived from the interploid crosses between diploid and tetraploid grape cultivars showed higher vigor than diploid seedlings from their parents. Thus, the triploid hybrids are expected to exhibit high productivity and seedlessness. To produce such triploid hybrids more efficiently from various interploid crosses, more works such as immature embryo culture (Yamashita *et al.*, 1993 and 1995) and *in ovulo* culture will be required. However, the present results at hand indicate that some of interploid crosses between diploid and tetraploid cultivars produce relatively many triploid seeds with germination capability under natural conditions. Selection of such cross parents may also help us to produce triploid hybrids efficiently.

## REFERENCES

- Ashikawa, K. 1972 On the new grape cultivar 'Takao'. *Jap. J. Breed.*, **22**: 46–51 (in Japanese)
- Baskin, C. C. and J. M. Baskin 2001 Seed. Academic Press, California
- Brink, R. A. and D. C. Cooper 1947 The endosperm in seed development. *Bot. Rev.*, **13**: 423–541
- Cain, D. W., R. L. Emershad and R. E. Tarailo 1983 *In ovulo* embryo culture and seedling development of seeded and seedless grapes (*Vitis vinifera* L.). *Vitis*, **22**: 9–14
- Coombe, B. G. 1950 Artificial parthenocarpy in grape vines. *Jour. Austral. Inst. Agr. Sci.*, **16**: 69–70
- Dyer, A. F. 1963 The use of lacto-propiono orcein in rapid squash methods for chromosome preparations. *Stain Technol.*, **38**: 85–90
- Esen, A. and R. K. Soost. 1973 Seed development in *Citrus* with special reference to  $2x \times 4x$  crosses. *Amer. J. Bot.*, **60**: 448–462
- Ehlenfeldt, M. K. and R. E. Hanneman Jr. 1988 Genetic control of endosperm balance number (EBN): three additive loci in a threshold-like system. *Theor. Appl. Genet.*, **75**: 825–832
- Einset, J. and B. Lamb 1951 Chimera sports of grapes. *J. Hered.*, **42**: 158–162.
- Einset, J. and C. Pratt 1954 "Giant" sports of grapes. *Proc. Amer. Soc. Hort. Sci.*, **63**: 251–256
- Einset, J. and C. Pratt 1975 Grape. In: Janick, J. and N. Moore (eds), *Advances in fruit breeding*. Purdue Univ. Press, West Lafayette p. 130–153
- Emershad, R. L. and D. W. Rammung 1984 *In ovulo* embryo culture of *Vitis vinifera* L. cv. Thompson Seedless. *Amer. J. Bot.*, **71**: 873–877
- Goldy, R., R. L. Emershad, D. W. Rammung and J. Chaparo 1988 Embryo culture as a means of introgressing seedlessness from *Vitis vinifera* to *Vitis rotundifolia*. *Hort. Sci.*, **23**: 886–869
- Gray, D. J., J. A. Mortensen, C. M. Benton, R. E. Durham and G. A. Moore 1990 Ovule culture to obtain progeny from hybrid seedless bunch grapes. *J. Amer. Soc. Hort. Sci.*, **115**: 1019–1024
- Johnston, S. A., and R. E. Hanneman Jr. 1996 Genetic control of endosperm balance Number (EBN) in the Solanaceae based on trisomic and mutation analysis. *Genome*, **39**: 314–321
- Johnston, S. A., and R. E. Hanneman Jr. 1999 The nature of the genetic control of endosperm balance number based on aneuploid analysis of *Datura*. *Sex. Plant Reprod.*, **12**: 71–75
- Johnston, S. A., T. P. M. den Nijs, S. J. Peloquin and R. E. Hanneman Jr. 1980 The significance of genic balance to endosperm development in interspecific crosses. *Theor. Appl. Genet.*, **57**: 5–9
- Ohmi, C., A. Wakana and S. Shiraishi 1993 Study of the parentage of grape cultivars by genetic interpretation of GPI-2 and PGM-2 isozymes. *Euphytica*, **65**: 195–202
- Olmo, H. P. 1976 Grape. In Simmonds, N. W. (ed.), *Evolution of crop plants*. Longman, London. p. 294–298

- Park, S. M., M. Hiramatsu and A. Wakana 1999 Aneuploid plants derived from crosses with triploid grapes through immature seed culture and subsequent embryo culture. *Plant Cell, Tissue and Organ Culture*, **59**: 125–133
- Park, S. M., A. Wakana, M. Hiramatsu and K. Ureshino 2002 A tetraploid hybrid plant from  $4x \times 2x$  crosses in *Vitis* and its origin. *Euphytica*, (in press)
- Sanford, J. C. 1983 Ploidy manipulations. In Moore, N. and J. Janick (eds) *Methods in fruit breeding*. Purdue Univ. Press, West Lafayette, p. 100–123
- Spiegel-Roy, P., I. Baron and U. Lavi 1985 *In vitro* culture and plant formation from grape cultivars with abortive ovules and seeds. *J. Amer. Soc. Hort. Sci.*, **110**: 109–112
- Weaver, R. J. and W. O. Williams. 1950 Response of flowers of Black Corinth and fruit of Thompson Seedless grapes to applications of plant growth-regulators. *Bot. Gaz.*, **111**: 477–485
- Weaver, R. J. 1952 Thinning and girdling of Red Malaga grapes in relation to size of berry, color, and percentage of total soluble solids of fruit. *Proc. Amer. Soc. Hort. Sci.*, **60**: 132–150
- Wijowska, M., E. Kuta and L. Przywara 1999 Autonomously endosperm induction by *in vitro* culture of unfertilized ovules of *Viola odorata* L. *Sex. Plant Reprod.*, **12**: 164–170
- Winkler, A. J., J. A. Cook, W. M. Kliewer and L. A. Lider 1962 *General viticulture*. California Univ. Press, Berkeley
- Yamane, H., A. Kurihara and R. Tanaka 1978 Studies on polyploidy breeding in grapes. 1. Chromosome numbers of large-berried grape varieties grown in Japan. *Bull. Fruit Tree Res. Stn.*, **E2**: 1–8 (in Japanese with English summary)
- Yamashita, H., S. Horiuchi and T. Taira 1993 Development of seeds and the growth of triploid seedlings obtained from reciprocal crosses between diploids and tetraploids grapes. *J. Jap. Soc. Hort. Sci.*, **63**: 249–255 (in Japanese with English summary)
- Yamashita, H., T. Haniuda and H. Shiba 1995 *In vitro* culture of embryos obtained by crossing tetraploid cultivar Kyoho with diploid cultivars. *J. Jap. Soc. Hort. Sci.*, **65**: 719–724 (in Japanese with English summary)
- Yamashita, H., I. Shigehara and H. Haniuda 1988 Production of triploid grapes by *in ovulo* embryo culture. *Vitis*, **37**: 113–117