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Male and female fertility in triploid grapes (*Vitis* complex) with special reference to the production of aneuploid plants

S. M. Park[1)], A. Wakana[2)], J. H. Kim[1)] and C. S. Jeong[1)]

[1)] Division of Applied Plant Sciences, College of Agriculture and Life Sciences, Kangwon National University, Chunchon, Kangwondo, Korea
[2)] Faculty of Agriculture, Kyushu University, Kasuya-gun, Japan

Summary

To produce aneuploid plants, the extent of male and female fertility in triploid grapes was studied using 187 triploid hybrid plants obtained from 2x x 4x and 4x x 2x crosses. In the triploid plants, pollen germination rates examined on agar medium ranged from 0 to 5.88 % (average: 0.24 %). In 86 out of the 187 triploid plants none of the pollen grains germinated. In the 3x x 2x and 3x x 4x crosses, 10 triploid plants showing more than 1 % pollen germination rates produced 191 seeds from 7,692 pollinations and 109 seeds from 3,862 pollinations, respectively, whereas 10 triploid plants showing no pollen germination produced 98 seeds from 5,282 pollinations and 141 seeds from 5,293 pollinations, respectively. In the 3x x 2x and 3x x 4x crosses, the percentage of ovules developing into seeds varied in different triploid hybrid plants and ranged from 0.1 to 2.3 %. Of 8 aneuploid plants derived from the 3x x 2x and 3x x 4x crosses, one grew normally, three showed slow growth rates and 5 plants died after germination. These results suggest that in triploid grapes (1) there is no relationship between the degree of male fertility and that of female fertility, but the degree of the fertility is a triploid-plant-specific character, (2) female fertility is slightly higher than male fertility, and (3) male and female fertility is very low but aneuploid plants can be produced if triploid grapes with more than 0.3 % female fertility are used as seed parents.

K e y w o r d s : aneuploid, female fertility, male fertility, pollen germination, triploid, *Vitis*.

Introduction

Aneuploids are a useful tool for cytogenetic studies with eukaryotes. At present such aneuploids do not exist in grape (*Vitis*) species (2n=2x=38) including *V. rotundifolia* (2n=2x=40). Moreover, information on the genetics of *Vitis* has been limited to several characters, since it is not a convenient plant for genetic analysis due to its long life cycle, large number of small chromosomes, partial sterility of ovules, and low seed germination (Einset and Pratt 1975).

Autotetraploid forms of grape cultivars have been found or induced by grape breeders to obtain large-berried forms of some cultivars, and studied for morphological differences from diploids (Einset and Lamb 1951; Olmo 1952; Einset and Pratt 1954; Ourecky *et al*. 1967), cytological properties (Olmo 1952; Alley 1957; Hilpert 1958) and breeding and fertility behavior (Olmo 1952; Alley 1957; Rives and Pouget 1959). However, neither spontaneous nor induced tetraploids have become economically important for several reasons such as their irregular bearing (Olmo 1942). Some of these tetraploids were subsequently used to develop triploid and tetraploid cultivars of commercial importance.

Triploid cultivars such as Osuzu and King Dela were bred in Japan by crossings between diploid and tetraploid cultivars. However, almost all work has focussed upon the morphological characteristics and seedlessness of these triploids. Thus, no detailed studies have been conducted to demonstrate triploid breeding behavior.

Found or produced autotriploids have been almost completely studied cytologically and morphologically in many annuals such as *Datura* (Satina and Blakeslee 1937 a and 1937 b; Satina *et al*. 1938), *Zea* (Punyasingh 1947), *Triticum* (Kuspira *et al*. 1986), *Lycopersicon* (Rick and Notani 1961) and *Solanum* (Vogt and Rowe 1968). In perennial fruit trees, these investigations were incomplete, although autotriploids were also found or produced and some became important cultivars in *Malus domestica*, *Pyrus communis* and *Citrus* because of their seedlessness and/or large fruit size. In *Malus domestica*, autotriploids are more or less useless as parents for further breeding since if they are selfed or crossed they produce few seeds, practically all of which produce aneuploid seedlings which were weak and seldom developed into trees (Brown 1975). Recently, Park *et al*. (1999) produced aneuploids from reciprocal crosses between 3x and 2x and between 3x and 4x grape cultivars through immature seed culture and subsequent embryo culture. This study indicated that grape is highly intolerant of aneuploidy and that the use of triploid parents with relatively high male and female fertility is the key to produce aneuploid plants. The major objectives of this study were (1) to demonstrate the degree of male and female fertility in autotriploid hybrid plants derived from various crosses between diploid and

Correspondence to: Dr. S. M. Park, Division of Applied Plant Sciences, College of Agriculture and Life Sciences, Kangwon National University, Chunchon, Kangwondo 200-701, Korea. Fax: +82-33-250-6424. E-mail: 64psm@hanmail.net

tetraploid grape cultivars, (2) to show the relation between male and female fertility in autotriploid hybrids and (3) to produce aneuploid seedlings from 3x x 2x and 3x x 4x crosses without *in vitro* culture of embryos.

Material and Methods

Plant material: Muscat Bailey A, Yufu and 187 triploid hybrid plants were used for crossing to produce aneuploid seedlings. Yufu is a tetraploid form of the diploid Muscat Bailey A. All these cultivars and triploid plants are so-called American-European hybrids with *V. vinifera* and North American species (*Vitis* spp.) in their pedigrees. The triploid hybrid plants were 5- to 10-year-old seedlings obtained from 15 crosses between diploid and tetraploid cultivars (Tab. 1). Triploid hybrid names were given as follows: abbreviation of cross combination followed by year and plant number. For example, RiY9006 indicates a sixth triploid plant obtained from Rizamat x Yufu in 1990. All plants were grown in greenhouses located at the Kyushu University Farm, Kasuya-gun, Japan. In addition, 6 diploid and 4 tetraploid cultivars (Tab. 2), that are the parental cultivars of the triploid hybrids, were used for pollen germination test.

Table 1

Parentage and number of triploid hybrid plants used

Parentage of triploid plants[a] (abbreviation)	No. of triploid hybrid plants used
Muscat of Alexandria x Kyoho (AK)	3
Muscat of Alexandria x Red Pearl (AR)	1
Muscat Bailey A x Cannon Hall Muscat (BC)	5
Muscat Bailey A x Kyoho (BK)	24
Muscat Bailey A x Red Pearl (BR)	25
Delaware x Cannon Hall Muscat (DC)	13
Delaware x Kyoho (DK)	5
Delaware x Yufu (DY)	14
Red Pearl x Muscat Bailey A (RB)	79
Rizamat x Yufu (RiY)	1
Red Pearl x Rizamat (RRi)	3
Sekirei x Red Pearl (SR)	4
Yufu x Delaware (YD)	4
Yufu x Rizamat (YRi)	3
Yufu x Sekirei (YS)	3

[a] Muscat of Alexandria, Cannon Hall Muscat and Rizamat are *V. vinifera* cultivars, and the other cultivars are American-European hybrid cultivars with *V. vinifera* and North American *Vitis* species in their pedigrees. Red Pearl, Kyoho, Cannon Hall Muscat and Yufu are tetraploid and the other cultivars are diploid. Red Pearl and Yufu originated from tetraploid sports of diploid Delaware and Muscat Bailey A, respectively.

Pollen germination test: Pollen samples were collected from freshly opened flowers of the diploid cultivars, tetraploid cultivars and 187 triploid hybrid plants at the full bloom stage. Immediately after collection, pollen grains were cultured on a pollen germination medium in 50 mm x 15 mm sterile plastic disposable Petri dishes so that they were dispersed. The medium consisted of 8 g l^{-1} agar, 20 g l^{-1} sucrose and 10 mg l^{-1} boric acid. Cultures were maintained at 25 °C. After 4 h of incubation, pollen germination was examined under a microscope at 200x magnification. At least 1000 pollen grains were examined in each genotype. Pollen germination score was determined as the ratio of germinated pollen grains to the total pollen grains examined. Pollen samples were examined in two or three replicates for three years, but the pollen germination rate in the year that crossing was carried out was used for the evaluation of success in the crosses between diploid and triploid and between tetraploid and triploid grapes.

Table 2

Pollen germination rates of diploid and tetraploid cultivars used as parents to produce triploids

Cultivar	Ploidy	No. of pollen observed	Pollen germination, %
Delaware	2x	1018	10.5
Neo Muscat	2x	1118	36.2
Muscat Bailey A	2x	1162	34.0
Rizamat	2x	1042	40.3
Muscat of Alexandria	2x	1123	34.9
Sekirei	2x	1021	29.1
Kyoho	4x	1346	56.9
Red Pearl	4x	1206	26.4
Cannon Hall Muscat	4x	1242	19.8
Yufu	4x	1102	24.1

Embryo sac fertility: Ten clusters of the 187 triploid hybrids each were bagged several days before anthesis to prevent outcrossing, and the flowers were self-pollinated by tapping the bags at the full bloom stage. Large seeds were extracted from mature berries about 4 months after self-pollination. To determine the degree of embryo sac fertility in triploids, 10 triploids with a pollen germination rate of 0 % and 10 triploids with more than 1 % germination rates were chosen as pistillate parents. Controlled crosses were carried out between the triploids and the diploid cv. Muscat Bailey A, and between the triploids and the tetraploid cv. Yufu. Clusters of the two cultivars were bagged before anthesis. Pollen was collected from bagged flower clusters and either immediately used for crossing or stored at -14 °C until use. Flowers of the triploid hybrid plants were emasculated a few days before anthesis, washed with running water and bagged. Pollination was carried out by placing the fresh or stored pollen directly onto the wet stigma on which a sugar solution was secreted. The pollinated flower clusters were bagged to exclude random pollination and allowed to develop. Large seeds were extracted from mature berries as mentioned afore. All seeds obtained were divided into two categories, floaters and sinkers. Only sinkers were sown in 300 mm x 400 mm seedling culture

boxes filled with moist sand in September 1998 and stored under natural conditions. They were carried in a greenhouse in February of the next year to facilitate seed germination.

Chromosome observation: Root tips of hybrid seedlings obtained from the 3x x 2x and 3x x 4x crosses were collected for chromosome observation. The procedure for chromosome observation was described by PARK *et al.* (1999).

Results

Pollen fertility of triploid hybrid plants: Results of the pollen germination test from 6 diploid cultivars and 4 tetraploid cultivars (the parents of the 187 triploid hybrid plants) are listed in Tab. 2. Pollen germination rates ranged from 10.5 to 40.3 % for the 6 diploid cultivars among which Delaware showed the lowest rate of pollen germination. Pollen germination rates in 4 tetraploid cultivars ranged from 19.8 to 56.9 %. Pollen germination rate in somatically doubled tetraploid cvs. Yufu was lower than that in the original diploid cvs. Muscat Bailey A, respectively. However, in tetraploid Red Pearl, which originated from somatic doubling of Delaware, the rate of pollen germination was much higher than that in the original cultivar. It is concluded that all these cultivars are male fertile and that Muscat Bailey A and Yufu have high fertility as pollen parents in this study.

Distribution frequencies of triploid hybrids with different rates of pollen germination on agar medium were almost the same in those from Muscat Bailey A x Red Pearl and those from the reciprocal cross (Fig. 1). Furthermore, the distribution frequencies were also almost the same in the other interploid cross combinations between diploid and tetraploid cultivars. Hence, all data of the 187 triploids were pooled and listed in Fig. 2. The rates of pollen germination in the 187 triploids ranged from 0 to 5.88 % with an average rate of 0.24 %. About 46 % of the 187 triploids showed no germination, while only 1 % showed germination percentages >1 %. There was no large variation for the rates of pollen germination in the triploids during three years of evaluation. In all triploids, various sizes of empty pollen

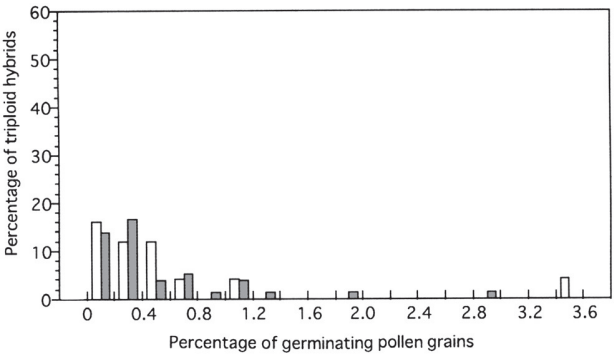

Fig. 1: Frequency for rate of pollen germination of 104 triploid hybrid grapes. White bar: 25 BR triploid hybrids from Muscat Bailey A x Red Pearl; solid bar: 79 RB triploid hybrids from Red Pearl x Muscat Bailey A. The percentage of triploid hybrids without pollen germination was 48 % (Muscat Bailey A x Red Pearl) and 52 % (Red Pearl x Muscat Bailey A).

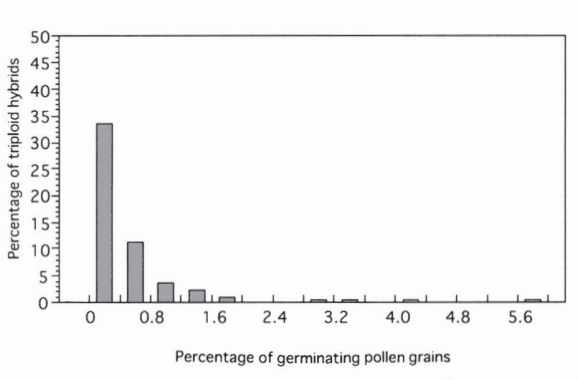

Fig. 2: Frequency for rate of pollen germination of triploid hybrid grapes. A total of 187 triploid hybrid grapes from 15 crosses was pooled. The percentage of triploid hybrids without pollen germination was 46 %.

grains and well-filled pollen grains were observed. Germination was observed in a few well-filled, large- and medium-sized pollen grains. In contrast to the pollen tubes observed in diploid and tetraploid cultivars, those in triploid hybrids often showed abnormal behavior such as slow growth rates, branching, twisting and swelling.

Embryo sac fertility of triploids: Based on the results of the pollen germination test for the 187 triploids (Fig. 2), 20 triploids were chosen for the study of embryo sac fertility. The 20 triploids consisted of two groups, 10 showing pollen germination rates >1 % and 10 showing no pollen germination (Tab. 3).

Embryo sac fertility in triploids with pollen germination rates >1%: In self-

Table 3

Pollen germination rates of triploid hybrid grapes chosen as pistillate parents for self-pollination, 3x x 2x and 3x x 4x crosses

Triploid hybrid	No. of pollen observed	Pollen germination, %
RiY9006	1,140	5.88
YD9056	1,130	4.34
RB9135	1,290	2.81
RB9025	1,635	1.96
DY9102	1,301	1.84
BK9101	1,052	1.52
AK8609	1,654	1.45
RB9161	1,264	1.34
BR9111	1,292	1.16
RB9008	1,254	1.12
RB9111	2,000	0.00
RB9113	2,000	0.00
DK9110	2,000	0.00
RB9002	2,000	0.00
RB9153	2,000	0.00
RB9121	2,000	0.00
RB8977	2,000	0.00
BC9101	2,000	0.00
RB9004	2,000	0.00
BR9035	2,000	0.00

pollination of the 10 triploids, a total of 16 seeds was derived from 11,670 pollinations (Tab. 4). The mean percentage of ovules developing into seeds was 0.036 %. Five of the 16 seeds were sinkers but no seed germinated. There was no relationship between the rate of pollen germination and the rate of ovules developing into seeds in the 10 triploids. In the 10 triploids crossed with diploid Muscat Bailey A, 191 seeds were obtained from 7,692 pollinations (Tab. 5), while in the 10 triploids crossed with tetraploid Yufu 109 seeds were obtained from 3,862 pollinations (Tab. 6). The mean percentage of ovules developing into seeds was 0.76 % in the crosses with Muscat Bailey A and 0.70 % in the crosses with Yufu. These mean percentages were 20 times as high as that for the self-pollination. In crosses with Muscat Bailey A, 54 out of the 191 seeds were sinkers from which 5 seedlings were derived. However, two of the 5 seedlings died soon after germination and three seedlings grew. Chromosome number of the three seedlings

Table 4

Seed set following self-pollination of triploids showing >1 % pollen germination on agar medium

Triploid hybrid plant	No. of flowers pollinated	No. of berries with seeds (%)	No. of seeds obtained			Ovules developing into seeds[a], % (Average)	No. of seeds germinating
			Floaters	Sinkers	Total		
RiY9006	1,124	3 (0.3)	2	1	3	0.07	0
YD9056	1,035	5 (0.5)	3	2	5	0.12	0
RB9135	1,118	0 (0.0)	0	0	0	0.00	0
RB9025	1,221	2 (0.2)	2	0	2	0.04	0
DY9102	1,024	0 (0.0)	0	0	0	0.00	0
BK9101	1,252	2 (0.2)	2	0	2	0.04	0
AK8609	1,231	0 (0.0)	0	0	0	0.00	0
RB9161	1,311	0 (0.0)	0	0	0	0.00	0
BR9111	1,191	0 (0.0)	0	0	0	0.00	0
RB9008	1,163	4 (0.3)	2	2	4	0.09	0
Total	11,670	16 (0.1)	11	5	16	0.03 (0.04)	0

[a] No. of seeds obtained x (No. of flowers pollinated x 4 ovules)$^{-1}$ x 100.

Table 5

Seed set in 10 triploid grapes pollinated with diploid Muscat Bailey A; pollen germination rates in the 10 triploid grapes were >1 % on agar medium

Seed parents	No. of flowers pollinated	No. of berries with seeds (%)	No. of seeds obtained			Ovules developing into seeds, % (Average)	No. of seeds germinating
			Floaters	Sinkers	Total		
RiY9006	205	9 (4.4)	5	4	9	1.10	0
YD9056	1,071	43 (4.0)	30	16	46	1.07	0
RB9135	472	2 (0.4)	1	1	2	0.11	0
RB9025	3,115	35 (1.1)	34	3	37	0.30	1[a]
DY9102	325	22 (6.8)	12	11	23	1.77	1[b]
BK9101	262	9 (3.4)	5	4	9	0.86	1[b]
AK8609	321	5 (1.2)	4	1	5	0.39	0
RB9161	453	4 (0.7)	2	2	4	0.22	0
BR9111	462	9 (1.9)	10	2	12	0.65	0
RB9008	1,006	42 (4.2)	34	10	44	1.09	2[c]
Total	7,692	180 (2.3)	135	54	191	0.62 (0.76)	5

[a] Chromosome number of seedling was 2n=2x+2=40.
[b] Seedlings died soon after germination.
[c] Chromosome number of the two seedlings was 2n=2x+4=42 and 2n=2x+6=44.

Table 6

Seed set in triploid grapes pollinated with tetraploid Yufu; pollen germination rates in triploid grapes were >1 % on agar medium

Seed parents	No. of flowers pollinated	No. of berries with seeds (%)	No. of seeds obtained			Ovules developing into seeds, % (Average)	No. of seeds germinating
			Floaters	Sinkers	Total		
RiY9006	198	8 (4.0)	4	4	8	1.01	0
YD9056	625	10 (1.6)	6	5	11	0.44	1[a]
RB9135	447	3 (0.7)	1	2	3	0.17	0
RB9025	462	6 (1.3)	3	3	6	0.32	1[a]
DY9102	322	24 (7.5)	14	10	24	1.86	0
BK9101	566	21 (3.7)	15	6	21	0.93	0
AK8609	153	2 (1.3)	1	1	2	0.33	0
RB9161	159	1 (0.6)	1	0	1	0.16	0
BR9111	448	7 (1.6)	4	3	7	0.39	1[a]
RB9008	482	23 (4.8)	12	14	26	1.36	1[b]
Total	3,862	105 (2.7)	61	48	109	0.71 (0.70)	4

[a] Seedlings died soon after germination.
[b] Chromosome number of seedling was 2n=3x+3=60.

was 40 (2x+2), 42 (2x+4) and 44 (2x+6). In the crosses with Yufu, 48 of the 109 seeds were sinkers from which 4 seeds germinated. However, three of the 4 seedlings died and only one survived. Chromosome observation of the root tip cells indicated that the surviving seedling was an aneuploid plant with 60 (3x+3) chromosomes. In the 10 triploid hybrids crossed with 2x and 4x cultivars, no correlation was found between the rate of pollen germination and the rate of ovules developing into seeds. The rate of ovules developing into seeds was different in different triploid hybrid plants, but individual triploid hybrid plants showed almost the same percentage in both crosses with 2x and 4x (Tabs 5 and 6). Hence, it was considered that the average of the percentages of ovules developing into seeds in both crosses could be used to determine the embryo sac fertility in the triploid plant. The average percentages of fertile embryo sacs in the 10 triploid hybrid plants ranged from 0.14 to 1.82 %.

Embyo sac fertility in triploids with no pollen germination: In self-pollination of 10 triploids, most flower clusters dropped within 4 weeks after pollination and a small number of seedless berries set in a few clusters. Thus, no seeds were obtained from 12,151 pollinations in the 10 self-pollinated triploids (Tab. 7). When the 10 triploids were crossed with diploid Muscat Bailey A, 98 seeds were derived from 5,282 pollinations (Tab. 8). On the other hand, when the 10 triploids were crossed with tetraploid Yufu, 141 seeds were derived from 5,293 pollinations (Tab. 9). The mean percentage of ovules developing into seeds was 0.49 % in the crosses with Muscat Bailey A and 0.59 % in the crosses with Yufu. In the crosses with Muscat Bailey A, 37 out of 98 seeds were sinkers from which two seeds germinated and grew into seedlings. Observation of chromosomes in the root tip cells of the two seedlings indicated that they were aneuploid plants with 40 (2x+2) and 42 (2x+4) chromosomes (Fig. 3). In the crosses with Yufu, 50 of the 141 seeds were sinkers from which three seeds germinated. However, one of the three seedlings died soon after germination and was not available for chromosome observation. The two surviving seedlings were aneuploid with 69 and 65 chromosomes (Fig. 3).

Table 7

Seed set in self-pollination of triploids showing no pollen germination on agar medium

Triploid hybrid	No. of flowers pollinated	No. of berries with seeds
RB9111	1,148	0
RB9113	1,246	0
DK9110	1,389	0
RB9002	945	0
RB9153	1,254	0
RB9121	1,189	0
RB8977	1,005	0
BC9101	1,327	0
RB9004	1,236	0
BR9035	1,412	0
Total	12,151	0

In the crosses with Muscat Bailey A and Yufu, percentage of ovules developing into seeds was different in various triploid hybrid plants. However, each hybrid plant, except BC9101 and BR9035, showed almost the same percentage in both crosses (Tabs 8 and 9). In the two exceptional cases, the percentages in BC9101 and BR9035 crossed with

Table 8

Seed set in triploid grapes crossed with diploid Muscat Bailey A; pollen germination rate of 10 triploid hybrid grapes was 0 % on agar medium

Seed parents	No. of flowers pollinated	No. of berries with seeds (%)	No. of seeds obtained			Ovules developing into seeds, % (Average)	No. of seeds germinating
			Floaters	Sinkers	Total		
RB9111	446	37 (8.3)	29	10	39	2.19	0
RB9113	653	20 (3.1)	8	12	20	0.77	2[a]
DK9110	419	7 (1.7)	4	3	7	0.42	0
RB9002	450	7 (1.6)	5	2	7	0.39	0
RB9153	328	4 (1.2)	1	3	4	0.34	0
RB9121	1,022	12 (1.2)	8	4	12	0.29	0
RB8977	463	3 (0.6)	2	1	3	0.16	0
BC9101	331	2 (0.6)	1	1	2	0.15	0
RB9004	443	2 (0.5)	2	0	2	0.11	0
BR9035	727	2 (0.3)	1	1	2	0.07	0
Total	5,282	86 (1.8)	61	37	98	0.46 (0.49)	2

[a] Chromosome number of two seedlings was 2n=2x+2=40 and 2n=2x+4=42.

Table 9

Seed set in triploid grapes crossed with tetraploid Yufu; pollen germination rate in 10 triploid grapes was 0 % on agar medium

Seed parents	No. of flowers pollinated	No. of berries with seeds (%)	No. of seeds obtained			Ovules developing into seeds, % (Average)	No. of seeds germinating
			Floaters	Sinkers	Total		
RB9111	750	70 (9.3)	52	21	73	2.43	2[b]
RB9113	775	16 (2.1)	6	10	16	0.52	1[a]
DK9110	341	6 (1.8)	4	2	6	0.44	0
RB9002	470	6 (1.3)	4	2	6	0.32	0
RB9153	440	7 (1.6)	3	4	7	0.40	0
RB9121	622	14 (2.3)	10	4	14	0.56	0
RB8977	445	4 (0.9)	3	1	4	0.22	0
BC9101	439	7 (1.6)	5	2	7	0.40	0
RB9004	858	5 (0.6)	3	2	5	0.15	0
BR9035	153	3 (2.0)	1	2	3	0.49	0
Total	5,293	138 (2.6)	91	50	141	0.67 (0.59)	3

[a] Seedling died soon after germination. [b] Chromosome number of two seedlings was 2n=3x+12=69 and 2n=3x+8=65.

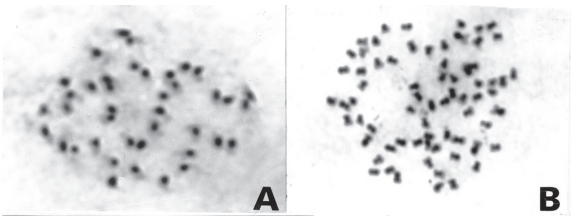

Fig. 3: Metaphase figures in root tip cells of aneuploid seedlings from 3x x 2x and 3x x 4x crosses. **A**: Seedling derived from RB9113 x Muscat Bailey A, 2n=2x+2=40. Magnification: x 3000. **B**: Seedling derived from RB9111 x Yufu, 2n=3x+12=69. Magnification: x 2500.

Yufu were about three and 7 times as high as those crossed with Muscat Bailey A, respectively. It may be considered that the difference of the percentages in the two exceptional cases were due to a small number of pollinations. Thus, it was also concluded that the average of the percentages of ovules developing into seeds in both crosses revealed the embryo sac fertility in the triploids. The average percentage of fertile embryo sacs in the 10 triploids ranged from 0.13 to 2.31 %. Among the triploids examined in this study, RB9111 showed the highest embryo sac fertility (2.31 %), although this triploid showed no pollen germination on the agar medium.

Growth of aneuploid plants: Of the 8 aneuploid seedlings obtained, one seedling with 69 chromosomes showed normal growth and morphology, but three seedlings with 44, 60 and 65 chromosomes showed slow growth rates and abnormal morphology such as small leaves and short internodes. Four seedlings with 40 or 42 chromosomes died within three months after seed germination.

Discussion

Pollen fertility: Pollen fertility of autotriploids has been usually determined from pollen grains stained with reagents such as aceto carmine (Kasha and McLennan 1967; Lee *et al*. 1972; Kuspira *et al*. 1986). In this case, filled or plump pollen grains containing normal cytoplasm were classified as fertile and empty ones were classified as sterile. Pollen fertility of autotriploids determined by this method has been reported to be high in *Triticum monococcum* (Kuspira *et al*. 1986), *Oryza sativa* (Rao and Reddi 1971) and many other species (Lamm 1944; Patel and Olmo 1955; Shah 1964; Gupta and Srivastava 1970). In these species, however, crosses between diploid females and triploid males produced a few seeds which produced euploid plants. These results suggest that most unbalanced pollen grains are inviable or nonfunctional. Thus, it may be concluded that pollen fertility estimated by stainability does not exactly reflect the fertility of pollen in autotriploids.

In autotriploids, pollen fertility estimated by staining with aceto carmine ranged from 46 to 73 % (data not presented). However, Park *et al*. (1999) reported that 231 seeds were derived from 3,800 pollinations or 15,200 ovules if the autotriploids were crossed with diploid and tetraploid cultivars and that only two seedlings established through culturing of immature embryos within these seeds were aneuploid plants (2n=2x+16=54 and 2n=3x+2=59). These results suggest that in autotriploid grapes a few unbalanced pollen grains are functional and result in fertilization. Hence, it is concluded that pollen fertility of autotriploid grapes is very low. This conclusion is further supported by the fact that in self-pollination no pollen grains of triploid grapes showing no pollen germination on artificial medium affected seed set (Tab. 7), whereas a few pollen grains of triploid grapes showing more than 1 % pollen germination rates resulted in seed formation (Tab. 4). Thus, to elucidate the degree of pollen fertility in autotriploid grapes pollen germination tests are essential.

Pollen germination rate in Muscat Bailey A was 1.4 times as high as that in its tetraploid form Yufu. The reduction of fertility in the tetraploid form is considered to be related to a disturbance of meiosis as was reported for *V. vinifera* (Alley 1957). However, in tetraploid Red Pearl the pollen germination rate was 2.3 times as high as that of the original diploid cultivar Delaware. The very low pollen fertility in Delaware as compared with other diploid cultivars may be related to the increase of fertility in the tetraploid form, although its precise mechanism is not known at present. Among the cultivars used as parents of triploids, tetraploid Kyoho, a hybrid between the tetraploid Campbel Early and Centennial, showed the highest rate of pollen germination. However, the degree of pollen fertility in these parental cultivars of triploids (Tab. 2) and reciprocal crosses between diploid and tetraploid cultivars (Figs 1 and 2) have no effect on the degree of pollen fertility of their triploids.

The very low pollen fertility of grape triploids is comparable to that of *Triticum monococcum* triploids in which only diploid and tetraploid plants were derived from 2x x 3x crosses (Kuspira *et al*. 1986). The appearance of euploid plants in the 2x x 3x crosses indicates that the balanced functional gametes produced by triploid males give rise to viable progenies. In *Vitis*, however, appearence of euploids is not expected because of its very large chromosome number (n=19) as compared to *Triticum* (n=7). In *Triticum*, balanced gametes (1x and 2x) constitute about 1 % of all meiotic products (Kuspira *et al*. 1986), whereas in *Vitis* they constitute 3.8×10^{-4} % of them, if random segregation is assumed in meiosis. As has been suggested for *Triticum* (Kuspira *et al*. 1986), it is considered that in *Vitis* microspores with unbalanced chromosome constitutions caused gametophytic abortion and that unbalanced gametophytes that remained viable and functional either failed to affect fertilization or resulted in fertilization and subsequent abortion of zygotes or embryos. This postulation is supported by the evidence that, in 2x x 3x crosses, embryos were derived *in vitro* from 9 of 52 seeds from 2,788 pollinations, but only two aneuploid plants were established *in vitro* (Park *et al*. 1999).

Although pollen fertility of grape triploids is very low, it is noticeable that of the 187 triploids examined 13 showed more than 1 % and 4 more than 3 % fertility. Because the degree of pollen fertility in these triploids was stable in every year pollen germination tests were carried out, it is a triploid-plant-specific character. Park *et al*. (1999) reported that the number of seeds derived from 2x x 3x and 4x x 3x crosses increased with the degree of pollen fertility of triploids and that many seeds were derived from these crosses if triploids with more than 1 % pollen fertility were used as pollen parents. Hence, if used as males, the 13 triploids with more than 1 % pollen fertility are expected to produce aneuploid plants.

Embryo sac fertility: The results of interploid crosses with 20 triploids indicate that in triploids the degree of embryo sac fertility is also a triploid-plant-specific character. Furthermore, average embryo sac fertility of the 20 grape triploids is calculated to be 0.65 % in the 3x x 4x crosses (Tabs 6 and 9) and 0.62 % in the 3x x 2x crosses (Tabs 5 and 8). This suggests that the degree of embryo sac fertility in the triploids is consistent irrespective of the crosses with tetraploid Yufu and diploid Muscat Bailey A, and that the reduction of pollen germination rate in Yufu (Tab. 2) does not result in low seed set in the 3x x 4x crosses. Hence, the mean embryo sac fertility of the triploid grapes is concluded to be 0.63 %.

The very low embryo sac fertility in the triploid grapes corresponds to that of the triploids of *Lycopersicon esculentum* (Rick 1971), *Triticum monococcum* (Kuspira *et al*. 1986) and other species which are highly intolerant to aneuploidy. In these species with relatively small chromosome numbers (n=6-12), only balanced and n+1, n+2 and n+3 meiotic products form viable megagametophytes and functional

megagametes, and the viability and function rapidly decrease as the chromosome number of meiotic products increases.

The results of the 3x x 2x and 3x x 4x crosses indicate that in *Vitis* n+2, n+3, n+4, n+6, n+8 and n+12 meiotic products form functional megagametes, and the results of a previous study (Park *et al*. 1999) indicated that n+13, n+14 and n+16 meiotic products also form functional gametes in the interploid crosses with triploids used as seed parents. Except for allotriploids such as those in *Gladiolus* (Jones and Bamford 1942) and *Aster* (Avers 1954), a series of aneuploid types has been reported in the progenies of autotriploids of *Petunia hybrida* (n=7; Rick 1971), *Clarkia unguiculata* (n=9; Vasek 1956), *Zea mays* (n=10; McClintock 1929; Punyasingh 1947), *Hyacinthus* spp. (n=8; Darlington *et al*. 1951), *Collinsia heterophylla* (n=7; Dhillon and Garber 1960) and *Solanum* spp. (n=12; Vogt and Rowe 1968) crossed with diploids. Most of these species are considered to be of hybrid origin, and have more extensive genetic duplication than those which are highly intolerant of aneuploidy (Kuspira *et al*. 1986). *Vitis* is also considered to be a hybrid in its origin and to be highly diploidized genus (Patel and Olmo 1955). Therefore, one of the possible reasons for the production of a series of aneuploid types is considered to be related to the hybrid nature of *Vitis*.

Rick and Notani (1961) reported that wild or primitive types of tomato are more tolerant to chromosomal imbalance than cultivated genotypes. In addition to this finding, Kuspira *et al*. (1986) indicated that the group being highly intolerant is self-fertilizing or highly homozygous while the group being tolerant is cross-fertilizing or highly heterozygous. The two indications agree with our interpretation of the present result. Most of the parental cultivars of all triploid grapes used in this study are considered to be highly heterozygous as suggested by Ohmi *et al*. (1993) in intercontinental hybrid cultivars, because they have been bred by crossing of the complex intercontinental hybrid cultivars with *V. vinifera* and North American wild *Vitis* species (*e.g. V. labrusca*, *V. lincecumii* and *V. aestivalis*) in their pedigrees.

In addition to the production of a series of aneuploid types, number, viability and morphology of aneuploid types of progeny produced by triploid grapes are important to determine the level of tolerance. It is noticeable that very low percentages of seeds derived from the 3x x 4x and 3x x 2x crosses germinated indicating that almost all unbalanced megagametes result in the abortion of zygotes and embryos. A small number of aneuploid plants was derived and they showed low to very low viability and abnormal leaf and stem morphology, except one with 69 (3x+12) chromosomes. Considering these facts, it seems that grape is moderately tolerant to chromosomal imbalance.

Relation between female and male fertility: Until now, there has been no information available regarding the relationship between embryo sac and pollen fertility in autotriploids. In the present study, the highest embryo sac fertility (average 2.31 %) was detected in the triploid RB9111 showing no pollen germination on the artificial medium, whereas the triploid RiY9006 with the highest rate of pollen fertility (5.88 %) showed moderate embryo sac fertility (average 1.05 %). In addition, several plants with very low embryo sac fertility (0.1-0.3 %) were detected among triploid hybrid grapes with >1 % pollen fertility, while all triploid hybrid plants showing no pollen germination showed 0.1 to 2.3 % embryo sac fertility. These facts indicate that female and male fertility are indepedent traits.

One of the reasons for studying grape triploid fertility was to generate a complete series of primary single trisomics and other types of aneuploids as material for genetic analysis. The present investigation not only indicated that grape triploids were highly sterile, but also indicated that those with more than 0.3 % female fertility produce aneuploid progenies through seed sowing. The triploids that have more than 0.3 % female fertility but have no or very low male fertility are very useful seed parents for the production of aneuploid plants, because emasculation is unnecessary in the triploids. The production of aneuploids from these triploids may contribute to future cytogenetic and genetic studies in *Vitis*.

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