Induced Polyploid Grapes via *in vitro* Chromosome Doubling

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Summary

In vitro chromosome doubling of the 29 diploid-, 3 triploid-, and 1 tetraploid-grape accession, *Vitis* spp. were performed using the axillary buds of growing shoots. Treatment with 0.05% colchicine for one or two days is suitable to induce tetraploids (4X). Shoots, arising from treated-buds, often exhibited abnormal leaves, and part of them turned out to be cytochimeras (2X+4X). Additional tetraploids were isolated from parts of cytochimeras by three-divided stem cuttings. A different response to colchicine was induced in diploid cultivars, and the percentage acquisition of induced tetraploids was relatively higher in *V. vinifera* than in the American hybrid. Induced hexaploids (6X) were found to have a less satisfactory root system, but cytochimera, having only 3X cells in root tips, rooted well. No induced octoploids (8X) were obtained, and the growth of cytochimera, partly having 8X cells, were slow and dwarfed. Induced tetraploids were vigorous, rooted easily, and stable over many years, whereas cytochimera of 3X+6X and 4X+8X were likely to be lost during *ex vitro* cultivation, reverting to the source triploid and tetraploid, respectively. A comparison of induced tetraploids and the source diploids revealed no significant difference in time of full maturity, cluster and berry shape, skin color, Brix, and acidity. However, depending on the variety, the increase in berry size in the 4X to 2X ranged from 1.1 to 1.5 fold with an average of 1.3.

Key Words: chromosome doubling, colchicine, grape, *in vitro*, polyploid.

Introduction

Polyploid forms of grapevine, *Vitis* spp. are usually detected by their large berries; they have been found to arise spontaneously (Reisch and Pratt, 1996). The autotetraploids generally exhibit poor cultural characteristics, compared with source diploid grapes (Allewelt and Possingham, 1988). In contrast, the allotetraploids are more promising because the undesirable features of autotetraploids are not so evident, and selection can proceed in a wider genetic base (Jelenkovic and Olmo, 1969).

Efforts to breed tetraploid hybrids by crossing between known autotetraploids have resulted in the development of a few cultivars such as 'Niabell' and 'Early Niabell' (Olmo and Koyama, 1962). Since 1970, polyploidy breeding has had great successes in Japan where many tetraploid hybrids have been introduced by crossbreeding and are being cultivated commercially (Bessho et al., 1998). Some prominent examples of tetraploid cultivars are 'Kyoho', 'Pione', and 'Aki Queen' which produce its berry, ranging from 12 to 15

g. However, the tetraploid cultivars or lines are much fewer than the diploid ones, and closer mating between the related tetraploids result in less genetic heterogeneity, i.e. 'Kyoho' and its allied cultivars. In addition, the tetraploid sports are found to occur at a low frequency so that polyploidizing source diploid grapes with acceptable horticultural traits may lead to new cultivars.

Polyploidization of grapes has been explored by *ex vitro* colchicine treatment on growing buds of *V. vinifera* cultivars (Dermen, 1954; Gargiulo, 1960; Das and Mukherjee, 1967; Yamane and Kurihara, 1980). However, this method generally takes a long-term investigation period: it is less effective for American hybrids derived from *V. labrusca* (Yamane, 1997). However, *in vitro* approaches hold promise for inducing and screening for polyploid mutations in other crops (Murashige and Nakano, 1966; Heinz and Mee, 1970; Hermesen et al., 1981; Lyrene and Perry, 1982). Since 1984, systematic studies on induced polyploid grapes via *in vitro* chromosome doubling have been performed in our breeding station to broaden genetic variability within *Vitis* germplasm (Notsuka, 1994). Our objective is to produce table grapes with desirable qualities, notably early maturation, seedlessness, and large berry size. Numerous cultivars known for their fruit quality and desirable traits have been tetraploidized to be used as parental stocks. Thus, by hybridization among these tetraploid grapes, progenies with increased berry size

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will be assured in the future.

In this article, the effects of *in vitro* colchicine treatment on the rate of tetraploid-, hexaploid-, and octoploid-induction for table grapes are reported and the cytological results of the ploidy level in colchicine-treated explants are presented. Furthermore, fruit quality traits of induced tetraploid grapes are examined and compared with those of source diploid cultivars.

Materials and Methods

Plant materials

Twenty-nine diploid table cultivars, 3 triploid breeding lines, and 1 tetraploid table cultivar were used. These vines were grown at the experimental vineyards

of Fukuoka Agricultural Research Center.

In vitro chromosome doubling

A schematic illustration for *in vitro* chromosome doubling, having nine stages (Fig. 1), show that throughout the culture, room temperature was maintained at 25 °C and under 16-hr photoperiod with a light intensity of ca. 3000 lx utilizing cool-white fluorescent tubes. Shoot tips 3–5 cm in length were surface-sterilized in 0.5% sodium hypochlorite for 15 min., then rinsed three times in sterilized water. Shoot tips which were dissected to 0.5 to 2.0 mm, were imbedded on MS liquid medium (Murashige and Skoog, 1962) supplemented with benzyladenine (BA) at 5 μ M and 3% sucrose (stage 1). After two weeks, the explants were subcultured onto

Fig. 1. Schematic illustration for *in vitro* chromosome doubling in grapevine.

A youngest axillary bud was used for colchicine treatment.

MS: Murashige and Skoog medium BA: Benzyladenine.

fresh MS medium gelled with 0.7% agar to induce shoot multiplication (stage 2). Subsequent to the shoot division, shoot elongation was forced by reducing BA to 2 μ M (stage 3). Stems with the youngest axillary bud were excised and subcultured on the same medium net containing 0.05% sterilized colchicine for one or two days while avoiding contact of a leaf with medium (stage 4). The colchicine-treated cuttings were rinsed in sterilized water, then grown on agar MS medium without colchicine for about one month (stage 5). After removing the brown tissue (generally under 4th expanded leaf), the explants were placed on a solid 3 μ M BA MS medium for additional shoot elongation (stage 6). The stem piece between the 6th to 8th expanded leaf was divided into three cuttings (stage 7). Each cutting was subcultured on the same MS medium continued shoot elongation (stage 8). For chromosome counting (stage 9), shoot tips of explants were collected and transferred to water at 2 °C for 24 hr. After the tips were fixed in a Carnoy solution (glacial acetic acid: ethanol, 1:3, v/v) for at least 2 hr; they were stained in 2% acetic orcein: 1N HCl (10:1) at 60 °C for 30 seconds. The squashed preparations were examined and photographed under a light microscope at a magnification of 400 to 1000.

Acclimatization

Elongated shoots (2–3 cm in length) of induced polyploid explants were rooted *in vitro* on 1/4 strength MS medium containing 0.3% gellan gum, 0.01 μ M α -

naphthaleneacetic acid (NAA), and 3% sucrose. The culture conditions were the same as above for *in vitro* chromosome doubling. Rooted explants (plantlets) were washed in sterilized water, then transferred to a polyethylene container (12 × 17 × 6 cm) containing vermiculite. The container was covered with a glass plate to maintain 80 to 90% relative humidity (RH) and applied liquid fertilizer (N-P-K, 100–200–100ppm) at bi-weekly. RH was gradually decreased to 50%. After about eight weeks, the acclimated plantlets were replanted in a 0.5-liter pot and kept in a glasshouse before being field-tested.

Results

Induction of tetraploids

The colchicine concentration and its duration on a rate of tetraploid (4X, 2n=76) induction were tried on three diploid (2X, 2n=38) cultivars (Table 1). At concentration of 0.2%, at two- and five-day exposures led to high mortality of the cultivars with the exception of 'Rizamat', *Vitis vinifera*. This cultivar was very susceptible, yielding 25% tetraploids with the 2 day exposure. At 0.05% colchicine, the number of explants which survived increased, especially after 2-day treatment. The 5-day exposure decreased the survival rate. A similar trend appeared for the rate of tetraploid induction. The 2-day treatment yielded 9.5% tetraploid in 'Rizamat'; 4.2% in 'Buffalo'; 13.6% in 'Schuyler'. When explants were treated with 0.05% colchicine for

Table 1. Effects of colchicine treatment on the rate of tetraploid induction in diploid cultivars.

Colchicine treatment		Source diploid cultivar ²	No. of explant treated	Ploidy levels		
Concentration (%)	Duration (days)			2X (2n=38)	2X+4X	4X (2n=76)
0.2	2	Rizamat(V)	20(7) ^y	1	1	5(25.0%) ^x
		Buffalo(AH)	26(0)	0	0	0(0.0%)
		Schuyler(AH)	23(0)	0	0	0(0.0%)
0.2	5	Rizamat(V)	22(1)	0	1	0(0.0%)
		Buffalo(AH)	37(0)	0	0	0(0.0%)
		Schuyler(AH)	21(0)	0	0	0(0.0%)
0.05	1	Buffalo(AH)	21(15)	4	6	5(23.8%)
0.05	2	Rizamat(V)	21(11)	2	7	2(9.5%)
		Buffalo(AH)	24(16)	6	9	1(4.2%)
		Schuyler(AH)	22(12)	5	4	3(13.6%)
0.05	3	Buffalo(AH)	20(5)	4	1	0(0.0%)
0.05	5	Rizamat(V)	20(8)	1	7	0(0.0%)
		Buffalo(AH)	23(1)	1	0	0(0.0%)
		Schuyler(AH)	22(8)	1	7	0(0.0%)

² V, *Vitis vinifera* AH, American hybrid which have *V. labrusca* in their parentage.

^y Number of explant survived after colchicine treatment.

^x (induced tetraploid/explant treated) × 100%.

Table 2. Typical cytochimeral forms observed in three-divided stem cuttings in clonal explants of 'Rizamat' after colchicine treatment with 0.05% for 2 days.

Type	Division ²	No. of Cells		Cytochimeral form
		2X	4X	
I	a	26	39	2X+4X(mixoploid)
	b	40	18	2X+4X(mixoploid)
	c	53	34	2X+4X(mixoploid)
II	a	57	0	2X(diploid)
	b	0	47	4X(tetraploid) ^y
	c	31	15	2X+4X(mixoploid)
III	a	6	51	2X+4X(mixoploid)
	b	0	76	4X(tetraploid) ^y
	c	6	72	2X+4X(mixoploid)
IV	a	55	0	2X(diploid)
	b	53	52	2X+4X(mixoploid)
	c	41	28	2X+4X(mixoploid)
V	a	0	60	4X(tetraploid) ^y
	b	30	0	2X(diploid)
	c	30	0	2X(diploid)

² See stage 7 in Fig. 1.^y An additional tetraploid was isolated from cytochimera by three-divided stem cutting.

one day, tetraploidy in 'Buffalo' increased to 23.8%. Thus, *in vitro* treatment of axillary buds with 0.05% colchicine for one or two days was suitable for tetraploid induction in grapevine.

Shoots arising from colchicine-treated axillary buds often exhibited abnormal leaves, i.e. loss of serration, and distorted, dark green mosaic patterns; parts of the leaves were cytochimeric (2X+4X). Some typical forms of cytochimera in shoot tips from three-divided stem are illustrated as a, b, c in stage 7 (Table 2, Fig. 1) in 'Rizamat' following treatment of 0.05% colchicine for two days. When a stem cutting, having only tetraploidized cells, was found in a cytochimera, such as type II, III, and V, it was also possible to isolate a complete tetraploid.

Chromosome doubling in 29 diploid table grapes was attained for breeding material by *in vitro* culture between 1984 and 1988 (Table 3). The data showed that source diploid cultivars responded differently to colchicine treatments. Success ranged from 6 ('Hakata White') to 47% ('Pusa Seedless') in *V. vinifera* and 4 ('Fuefuki') to 35% ('Prima Seedless') in the American

Table 3. Diploid grape cultivars for inducing traploids via *in vitro* chromosome doubling with 0.05% colchicine treatment on the axillary buds.

Intended use	Source diploid cultivar ²	Duration (day)	No. of explant treated	Ploidy level			No. of induced tetraploid
				2X	2X+4X	4X	
Early maturation	Buffalo (AH)	1	21	4	6(2) ^y	5	7(33%) ^x
	Redport (AH)	1	30	3	13(0)	3	3(10%)
	Schuyler (AH)	2	22	5	4(1)	3	4(18%)
Seedlessness	Aki Seedless (AH)	2	28	2	21(6)	1	7(25%)
	Bronx Seedless (AH)	2	33	1	6(3)	2	5(15%)
	Himrod (AH)	1	22	3	14(1)	2	3(14%)
	Monukka (V)	1	22	2	11(4)	3	7(32%)
	Prima Seedless (AH)	1	26	2	17(6)	3	9(35%)
	Pusa Seedless (V)	2	19	0	11(5)	4	9(47%)
	Ruby Seedless (V)	2	21	0	1(1)	2	3(14%)
	Suffolk Red (AH)	2	27	5	17(6)	3	9(33%)
Large - berry	Baladi (V)	2	25	4	14(7)	2	9(36%)
	Fuefuki (AH)	2	25	4	8(1)	0	1(4%)
	Hakata White (V)	2	16	0	1(1)	0	1(6%)
	Halili Sev (V)	2	26	0	4(3)	3	6(23%)
	Houman (AH)	2	10	1	3(1)	0	1(10%)
	Italia (V)	2	27	5	16(3)	3	6(22%)
	Kaiji (V)	1	30	2	28(3)	0	3(10%)
	Khusaine (V)	1	16	2	6(3)	2	5(31%)
	Morgen schon (V)	2	12	1	4(0)	1	1(8%)
	Neo Muscat (V)	2	15	2	13(2)	0	2(13%)
	Rizamat (V)	2	21	2	7(4)	2	6(29%)
	Ruby Okuyama (V)	2	17	1	7(4)	4	8(47%)
	Sabalkanski (V)	2	26	0	17(6)	1	7(27%)
Others	Benisanjaku (V)	1	24	2	18(6)	4	10(42%)
	Hakunan (V)	2	13	1	11(5)	1	6(46%)
	Koshusanjaku (V)	2	19	4	1(1)	2	3(16%)
	Pizzutello Bianco (V)	2	23	0	8(5)	5	10(43%)
	Super Hamburg (AH)	1	28	9	17(3)	2	5(18%)

² V, *Vitis vinifera*; AH, American hybrid which have *V. labrusca* in their parentage.^y Isolation of tetraploid from cytochimeras.^x Percentage acquisition of tetraploid (induced tetraploid / explant treated) × 100%.

Fig. 2. *In vitro* colchicine-treated triploid grapevine, F4347 ('Kyoho' x 'Bronx Seedless').

- A. Triploid plants (3X)
- B. Triploid cell at metaphase stage (2n=57, x 2,000)
- C. Mixoploid cell at metaphase stage (3X+6X, x 800)
- D. Hexaploid plants (6X)
- E. Hexaploid cell at metaphase stage (2n=114, x 2,000)

hybrids. This percentage was relatively higher in *V. vinifera* (avg. 27%) than in the American hybrid (avg. 20%). It was difficult to determine whether 1- or 2-day treatment was more suitable for exposure to colchicine. When the source diploid cultivars appeared vigorous during *in vitro* culture (stage 2 to 3, Fig. 1), the two-day duration was more effective.

Induction of hexaploids and octoploids

With respect to hexaploid induction, when three triploid breeding lines (F4346, F4347, and F4349: progenies of 'Kyoho' x 'Bronx Seedless') were treated with 0.05% colchicine for three days because these triploids (Fig. 2A) are more vigorous than the diploids; a mixture of ploidy levels was detected in the shoots arising from the treated buds: 3X (2n=57: Fig. 2B), cytochimera (3X+6X: Fig. 2C) and 6X (2n=114: Fig. 2E). Of these cytochimeras, additional hexaploids were

Fig. 3. *In vitro* colchicine-treated tetraploid grapevine, 'Kyoho'.

- A. Octoploid cell at metaphase stage (2n=152, x 1,500)
- B. Mixoploid cell at metaphase stage (4X+8X, x 800)
- C. A mixoploid (left) and a tetraploid (right) after acclimatization

isolated from the three-divided cuttings. The percentages of hexaploid, derived are: 8% (2/25: F4347), 38% (3/8: F4349), and 58% (7/12: F4346). These hexaploids had a poor root system, being thick and short (Fig. 2D). However, the cytochimera, having only 3x cells in root tips, rooted well so that subsequent cultivation was possible.

When axillary buds of a tetraploid cultivar, 'Kyoho' were treated with 0.05% colchicine, no complete octoploid (2n=152: Fig. 3A) was obtained. After a one day-treatment, 25 tetraploids and 1 cytochimera (4X+8X: Fig. 3B) were derived among 26 explants examined. With the three-day exposure, 16 tetraploids and 8 cytochimeras were obtained from the 26 treated explants. The cytochimeras, having 8X cells, generally grew less vigorously and was much more dwarfed compared to the stature of 'Kyoho', indicating that a complete octoploid from a 4X explant is difficult to obtain. Some of our rooted cytochimeras had only 4X cells in root tips; those with few 8X cells did not root at

all. The leaves of the acclimatized cytochimeras were distorted and/or had mosaic patterns, compared with the 4X explant (Fig. 3C). Also, the prostrate hairs on the shoot tips and on the lower surface of the mature leaves of the octoploids were much more dense than on the tetraploids.

Field trials of induced polyploids

Induced tetraploids were easily rooted, stable over many years, and vigorous, whereas the cytochimeras of 3X+6X and 4X+8X on the same plant were likely to be lost during their *ex vitro* cultivation, reverting to triploidy and tetraploidy, respectively. Some induced tetraploids grafted on the rootstock, 'Kober 5BB' have been successfully grown for at least one year.

Preliminary field trials from 1990 to 1995 revealed that fruit quality traits of the induced tetraploids were comparable with those of source diploid cultivars, except that two to three years were required depending on the tetraploids because their juvenile period differed (Table 4). The age of the vine and kind of rootstock are not always consistent between grapevines examined. However, there was no significant difference in time of full maturity, cluster and berry shape, skin color, Brix, and acidity between 2X and 4X cultivars. With regard to berry size, differences were present; doubling the chromosome number from 2X to 4X increased the berry size from 1.1 to 1.5fold with an average of 1.3.

Discussion

Tetraploidy in grapes has been induced using *ex vitro* colchicine treatments, resulting in many tetraploids of *V. vinifera*. For potted plants, a 0.25 to 0.5% colchicine containing 5 to 10% glycerin has been injected into the axillary buds several times a day for three to five consecutive days to increase ploidy (Gargiulo, 1960; Das and Mukherjee, 1967; Yamane and Kurihara, 1980). This *ex vitro* treatment is labor-intensive and time-consuming, whereas *in vitro* treatment requires: (i) a much lower concentration of colchicine; (ii) a much shorter exposure to colchicine; (iii) high penetration of colchicine to growing point because of a few or no prostrate hairs in the axillary buds within a culture flask. Greater transmission of colchicine into axillary bud through stem vessel is also expected because treated explants were imbedded on a medium containing colchicine.

When colchicine is applied to the growing point of a plant, it may penetrate into the apical histogenic cell layers, L1, L2, L3 (Satina and Blakeslee, 1941). Colchicine treatments often result in partly polyploidized cell layers leading to chimeral shoots with various abnormal leaves. A polyploid tissue can be detected visually by noting leaf characteristics, especially shape of the petiole sinus, shape of teeth, and size of stomata (Yamane and Kurihara, 1980). However, it is difficult to find the minute morphological changes reflecting

Fig. 4. Comparison of source diploid and the induced tetraploid grapevine.

- A. Early maturation: a diploid (left) and a tetraploid (right) bunch in 'Buffalo'. Bar=5cm
- B. Seedlessness: a diploid (left) and a tetraploid (right) bunch in 'Prima Seedless'. Bar=5cm
- C. Large-berry: a diploid (left) and a tetraploid (right) bunch in 'Neo Muscat'. Bar=5cm

Table 4. Comparison of fruit quality traits between source diploid – and the induced tetraploid – grapevine from 1990 to 1995.

Grapevine (replication) ^z	Ploidy level	Full maturity	Bunch Shape	Berry				
				Shape	Size (g)	Skin color	Brix (%)	Acidity (%)
Buffalo (2)	2X	Early Oct.	Cylindric	Elliptic	3.9	Violet black	19.3	0.54
	4X	Early Oct.	Cylindric	Elliptic	5.0	Violet black	17.1	0.55
Schuyler (2)	2X	Early Oct.	Conical	Elliptic	4.1	Violet black	17.5	0.47
	4X	Early Oct.	Conical	Elliptic	5.0	Violet black	16.1	0.51
Redport (2)	2X	Early Oct.	Cylindric	Round	3.9	Violet red	14.5	0.30
	4X	Early Oct.	Conical	Round	5.9	Violet red	14.1	0.38
Halili Sev (3)	2X	Early Sep.	Many winged	Elliptic	12.0	Violet black	14.2	0.67
	4X	Late Oct.	Many winged	Elliptic	14.2	Violet black	12.9	0.77
Kaiji (2)	2X	Late Sep.	Conical	Ovate	11.2	Red	18.9	0.51
	4X	Mid Sep.	Conical	Elliptic	11.8	Red	18.4	0.42
Koshusanjaku (2)	2X	Mid Oct.	Long conical	Elliptic	5.0	Green	17.8	0.41
	4X	Late Oct.	Long conical	Elliptic	6.9	yellow	14.7	0.68
Neo Muscat (3)	2X	Early Sep.	Cylindric	Elliptic	5.4	Green	16.1	0.50
	4X	Early Sep.	Cylindric	Elliptic	7.3	yellow	16.2	0.51
Rizamat (3)	2X	Early Oct.	Many winged	Cylindric	10.5	Violet red	16.6	0.31
	4X	Early Oct.	Many winged	Elliptic	11.3	Violet red	15.0	0.45

^z Year of replication.

periclinal or sectorial chimera *in vitro*. The advantage of *in vitro* chromosome doubling is that it enables an effective screening of polyploidy on shoot tips containing L1, L2, and L3. We determined various cytochimeral forms and selected wholly polyploidized explants (L1–L2–L3=4–4–4) within four months after colchicine treatment. In particular, the L2 polyploidized sector is of primary interest because gametes arise from this layer of cells. Some polyploids can occasionally be isolated by three-divided cuttings even if one colchicine-treated stem is a cytochimera, so that the number of induced polyploids is increased. According to Kondo et al. (1998), flow cytometry is a rapid method to detect ploidy of grapevine, compared to the squash technique of counting chromosomes. This method enables one to determine the ploidy of *in vitro* colchicine-treated explants, whereas *ex vitro* colchicine treatment on axillary buds takes at least two years for the determination of ploidy level. The latter method requires cutting back of a shoot to a polyploid leaf many times and forcing the bud at its axil into growth (Dermen, 1954; Yamane and Kurihara, 1980).

The percentage success of deriving tetraploid grapes depends on the source diploid cultivar, as previously reported by Das and Mukherjee (1967) and (Yamane and Kurihara, 1980). The basic cause of the different responses to colchicine treatment in grapevine is unknown, but may be attributed to such environmental or genetic factors as temperature (Dermen, 1940; Yamane and Kurihara, 1980), growth condition of

shoots (Dermen, 1954) and species parentage (Yamane, 1997).

There is always a small chance that the ploidies of apical cell layers will change by a replacement or displacement phenomenon (Dermen, 1960). If the induced tetraploids are completely tetraploidized in L1, L2, and L3, they would be stable during *in vitro* and *ex vitro* cultures because 4X cells are not at risk of being displaced by the faster dividing 2X cells. However, no stable hexa- and octoploids were derived from their corresponding triploid (3X) and tetraploid (4X) sources, respectively. Rooted mixoploids (3X+6X or 4X+8X) in which the root tissue were not completely polyploidized were likely to be lost during field trials. Arisumi (1972) postulated that the degradation of the chimeral segment is caused by cell layer replacement of the 6X (8X) by the 3X (4X). Furthermore, available evidence suggests that the induced hexaploid and incomplete octoploid may be of little importance in grape breeding.

Autopolyploidy seems to bring about principally enlargement of cells without increasing the cell number, i.e. the gigantism of a plant organ. In grape, most spontaneous autotetraploids are periclinal chimeras in which an epidermal 2x–L1 overlays the internal 4x–L2 and 4x–L3 (Einset and Pratt, 1954; Thompson and Olmo, 1963; Yamane et al., 1978). These tetraploids are less fruitful, lack vigor and have brittle shoots (Olmo, 1976; Yamane, 1997). However, the tetraploids induced *in vitro* are generally vigorous, indicating that the reversion to juvenility (vigorous growth) occurred. This

rejuvenation is explained by a phenomenon observed in micropropagated woody plants (Hackett and Murray, 1993). When *in vitro* propagated explants undergo several subcultures, juvenile characteristics, such as rooting potential, ability to flower, and tree stature are restored. In addition, there was no significant delay in the fruiting of *in vitro* derived tetraploids, which is in agreement with other fruit crops such as apple (Zimmerman, 1981), blueberry (Lyrene, 1981), and Japanese persimmon (Tao et al., 1994). Vigorous growth, along with early flowering (fruiting) found in micropropagated woody plants, has also been referred to as partial rejuvenation by Pierik (1990). Fruit quality traits of the induced tetraploids are very similar to those of the source diploids, which agrees with previous comparative studies on grapes (Reisch and Pratt, 1996). The berry sizes of our induced tetraploid are 1.1 to 1.5 times larger than those of the source diploid cultivars. Similar increases in berry size by spontaneous- or induced- tetraploid grapes have been reported as follows: 1.1 to 3.1, averaged 2.0 (Olmo, 1952); 1.5 to 1.8, averaged 1.7 (Ourecky et al., 1967); 1.1 to 1.8, averaged 1.5 (Yamane, 1997).

As breeding materials, the colchicine-induced tetraploids *in vitro* are valuable additions to the present gene pool for developing future table grapes. Progeny testings reveal that induced tetraploids are essentially tetraploid in their breeding behavior (data not shown). Of the induced tetraploids (Fig. 4), Buffalo 4X (later named as Fukuoka No. 9) is the most promising because the cultivar is vigorous, early maturing, and productive, bearing well-colored berries. Fukuoka No. 9 accumulates a large amount of sucrose in the ripe berries, i.e. a sucrose accumulator which has not yet been found within known tetraploid grapes (Shiraishi, unpublished). Since 1997, research on the adaptability of agro/morphological traits, such as the age of vine, rootstock, and training system is being conducted.

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試験管内染色体倍加による人為倍数体ブドウの作出

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摘 要

ブドウ二倍体 29 品種, 三倍体 3 系統および四倍体 1 品種について新梢腋芽の試験管内染色体倍加処理を行った。人為四倍体 (4X) の作出では, 0.05% コルヒチンの 1 日あるいは 2 日処理が適していた。コルヒチン処理芽から伸長した新梢には異常葉が多く認められ, その一部は 2X+4X の細胞キメラであった。これらの細胞キメラは, 三個体に分株することによって完全な四倍体個体が分離できる場合があった。二倍体ブドウのコルヒチン反応性には品種間差異が認められ, また, 人為四倍体の獲得率は欧州種の方が欧米雑種よりも高かった。人為六倍体 (6X) では十分な発根が認められなかったが, 3X の根端細胞を持つ細胞キメラの発根は良好であった。人為八倍体 (8X) は得られなかった。部分的に 8X 細胞をもつ細

胞キメラの生長は弱勢であり, 極端に矮化した。人為四倍体は容易に発根し, 何年も安定であり, かつ, 樹勢が強いのに対し, 3X+6X および 4X+8X の細胞キメラは圃場栽培下で消失しやすく, 元の三倍体および四倍体に戻ることが多かった。人為四倍体と二倍体間での果実品質の比較では, 成熟期, 果房形, 果粒形, 果皮色, 糖度および酸度では顕著な差異が認められなかったが, 果粒重では品種間差異が認められ, 二倍体に対する四倍体での果粒重の増加率は 1.1 から 1.5 倍まで変異し, 平均 1.3 倍であった。

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