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［Review］ **Physiology of Berry Set in Grapevines (2)**

Poor Berry Set in Tetraploid Grapes – Causes and Improvement of Vineyard Practices

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1. Development of Kyoho varieties and their berry setting habits

1) Origin of cv. Kyoho

The tetraploid grape cultivar, Kyoho, was established by Yasushi Oinoe, who crossed cv. Ishihara-wase and cv. Centennial in 1937 (Kamoshita, 1983). He selected one seedling that produced large berries having high sugar content and named it 'Kyoho,' which means "great mountaintop." Ishihara-wase grape is a tetraploid bud mutant of cv. Campbell Early (*Vitis labruscana* Bailey) and is grown in several commercial vineyards in Okayama Prefecture (Osumi, 1937). Ishihara-wase is cultivated as 'Large berry Campbell' with black purple skin. On the other hand, cv. Centennial, considered to be the pollen parent of Kyoho, was developed as a large berry mutant of cv. Rozaki (*V. vinifera* L.) in Australia in the 1900s. The cultivar was named 'Centennial' to commemorate the 100th anniversary of the founding of Australia. Its berry skin is yellowish green at the ripe stage.

The berry setting habits of Ishihara-wase and Centennial grapes are quite different (Fig. 1). Ishihara-wase vines usually set a sufficient number of berries to produce filled clusters, similar to the original diploid cultivar, Campbell Early. Each berry usually contains two to three seeds. In contrast, Centennial vines set poorly. In addition to the insufficient number of berries per cluster, most of the set berries are seedless and remain small in size.

The poor berry setting habit of cv. Kyoho seems to be predominantly inherited from cv. Centennial. On the other hand, the black purple skin is inherited from Ishihara-wase.

2) Progenies of Kyoho

A great number of tetraploid progenies have been developed by crossing Kyoho with other tetraploid cultivars. Most of them, including cv. Pione (Kyoho × 4n-Muscat) produced by Hideo Ikawa in 1973 and cv. Olympia (Kyoho × Kyogei) produced by Haruo Sawanobori in 1953, show poor setting habits. However, some of them, such as cv. Beni-Zuiho, Beni-Fuji, Ryuho, Honey Red, and Beni-Izu, produced by Hideo Ikawa in the 1960s (Kamoshita, 1983), set sufficient numbers of seeded berries to produce filled clusters (Fig. 2). These good-setting tetraploid grapes were obtained from hybrids of 4n-Golden Muscat and Kuroshio (Kyoho × 4n-Muscat) grapes. Cv. Fuji-Minori, also a good-setting tetraploid grape, was obtained from progenies of (4n-Golden Muscat × Kuroshio) × Pione by Kazunao Aoki in 1985 (Yamane, 1996). These suggest that 4n-Golden Muscat grape possesses genetic factors that confer good berry setting habit, although such factors could not be identified because the vine died.

2. Causes of poor berry set in tetraploid cultivar

Kyoho and Pione grapes, the major tetraploid cultivars in Japan, often set insufficient numbers of normal seeded berries per cluster and/or many seedless berries. Severe flower shatter usually occurs in shoots that grow vigorously and/or in clusters that bloom during rainy days (Fig. 3, left). Parthenocarpic set of seedless berries is stimulated when vigorous shoots are pinched before bloom (Fig. 3, middle). In both cases of severe flower shatter and parthenocarpic set of seedless berries, growers cannot harvest marketable clusters with good appearance (Fig. 3, right).

The author and his co-workers have conducted intensive

Fig. 1. Berry set in Ishihara-wase (left) and Centennial (right) grapes.

Fig. 2. Berry set in Beni-Zuiho (left), Beni-Fuji (middle), and Ryuho (right) grapes.

Fig. 3. Berry set in Kyoho grape. Severe flower shatter in a vigorous shoot (left) and parthenocarpic seedless berries after pre-bloom shoot pinching (middle) are observed, resulting in a very loose cluster with irregularly sized berries (right).

investigations to determine the causes of poor berry set in tetraploid grapes using anatomical and *in vitro* techniques.

1) Abnormal and poor development of ovules

(1) Anatomical analysis of diploid and tetraploid grape pistils

Table 1 shows the developmental conditions of ovaries and ovules at bloom as well as the percentage berry set in various tetraploid grape cultivars (Okamoto et al. 1984). A diploid cultivar, Muscat of Alexandria, was also investigated as a control of good-setting cultivar. Higher percentages of malformed or undeveloped ovules were found in tetraploid cultivars than in the diploid cultivar. More than 80% of total ovules showed normal development and seemed to be functional in Muscat of Alexandria, while 40-50% of total ovules showed normal development in such good-setting tetraploid cultivars as Beni-Zuiho and Beni-Fuji. On the other hand, Kyoho and Pione showed normal development in only 20-30% of total ovules and had much higher percentages of abnormal ovaries and ovules than the other tetraploid cultivars. Furthermore, spur-pruned Pione had a higher percentage of abnormal ovules than cane-pruned Pione, indicating that vigorous shoots tend to produce much more abnormal and impotent ovules than weak shoots.

Table 1 Ovule development in tetraploid and diploid grape pistils at anthesis

Cultivar	Abnormal ovule (%)		Immature embryo sac (%)			Normal ovule (%)	Berry set %	Seedless berry %
	Malfor-mation	Without embryo sac	2 or 4 nucleate	Unfused polar nuclei	Abnormal egg app.			
Tetraploid								
Cane-pruned								
Kyoho	15.7	22.9	4.3	1.4	30.0	25.7	27.4	44.1
Pione	7.4	32.0	14.8	1.2	21.0	23.6	29.1	45.4
Beni Zuiho	0.0	12.9	4.8	9.7	21.3	51.3	35.8	9.1
Beni Izu	0.0	14.1	9.4	14.1	22.8	39.6	31.8	6.6
Red Queen	4.6	17.2	4.6	10.3	21.8	41.5	21.7	1.9
Honey Red	4.6	31.3	3.8	2.5	22.5	36.1	27.0	3.8
Spur-pruned								
Pione	7.4	32.0	14.8	1.2	21.0	23.6	45.5	66.1
Diploid								
Spur-pruned								
Muscat of Alexandria	7.1	0.0	1.4	0.0	7.1	84.4	24.4	0.6

(2) *In vitro* culture test of diploid and tetraploid grape pistils

The effects of cluster nutrition on ovary and ovule development in diploid and tetraploid cultivars were examined using an *in vitro* culture test that involved modifying nutrient concentrations of the culture medium (Okamoto et al. 1989a; Okamoto and Omori, 1991). Pre-bloom pistils of Muscat of Alexandria (diploid) and Pione (tetraploid) grapes, estimated to be one week before anthesis, were embedded onto Nitsch (1951) medium of various strengths and cultured at 25°C. The ovary size measured at bloom was increased as medium strength was increased. A similar increase in ovary size was observed when the concentration of only KNO_3 was increased (Fig. 4). However, ovule development was inhibited when pistils were cultured in medium with high N (Fig. 5). In another culture test that used MS medium, the percentage of normal ovules was decreased significantly as the strength of the basal medium was increased. Such inhibition of ovule development was more obvious in Pione pistils than in Muscat of Alexandria pistils.

The results of *in vitro* culture tests of pre-bloom grape pistils suggest that in tetraploid grape pistils, an abundant supply of nitrogenous nutrients inhibits ovule development, resulting in poor setting of normal seeded berries. This finding corresponds to the previous result that spur-pruned

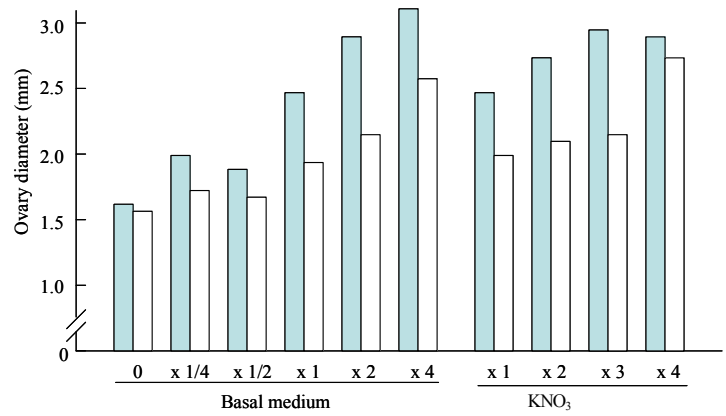

Fig. 4 Effect of strength of basal medium and KNO_3 on *in vitro* ovary growtn in Pione and Muscat of Alexandria grapes. Pre-bloom florets were cultured in Nitsch (1951) medium. Ovary diameters were measured at anthesis.

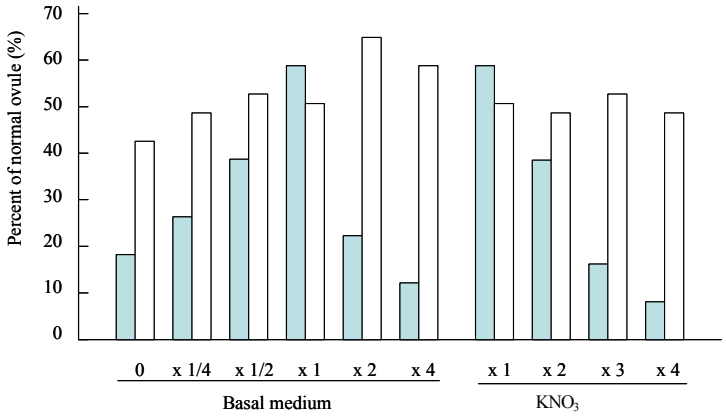

Fig. 5 Effect of strength of basal medium and KNO_3 on *in vitro* ovule development in Pione and Muscat of Alexandria grapes. Pre-bloom florets were cultured in Nitsch (1951) medium. Ovule development were analyzed at anthesis.

Pione vines that usually develop vigorous shoots produce a higher percentage of abnormal ovules than cane-pruned vines.

2) Poor development of transmitting tissue

Pollen tubes usually grow into pistils through the intercellular space in the transmitting tissue (TT) formed in the central part of the style and along the inner surfaces of both septa in the ovary (Fig. 6). TT development in six diploid and six tetraploid cultivars was investigated anatomically and TT diameters in cross sections of various parts of pistils were compared (Okamoto et al. 2001a). In the middle part of the style and the upper ovary, TT diameter of tetraploid cultivars was larger than that of most diploid cultivars. However, in the upper part of the locule where TT became elliptical, TT width was larger in most diploid cultivars than in tetraploid ones except cv. Fuji-Minori, where sufficient numbers of normal seeded berries usually set. This tendency was notable in the upper and middle parts of ovule where TT width was larger in cv. Fuji-Minori than those in other tetraploid cultivars.

TT area, total TT cell number, and total area of intercellular spaces per TT were measured at the cross sections of pistils of two diploid and two tetraploid cultivars. No obvious difference in those parameters was found in the upper part of the ovary between diploid and tetraploid cultivars. However, in the middle part of the ovule, total TT cell number and total area of intercellular spaces were considerably smaller in tetraploid cultivars than in diploid cultivars. The negative effects of such poor TT development on pollen tube growth in tetraploid cultivars were demonstrated by the distribution of pollen tubes in cross sections of the middle part of ovary (Okamoto et al. 2001a). Pollen tubes penetrating ovary tissue in Muscat of Alexandria pistils were found mostly in TT zones that developed at the inner surfaces of both septa (Fig. 7A). In contrast, pollen tubes were found outside septa and not in septum TT in Kyoho pistils (Fig. 7B).

The developmental process of TT in grape pistils was investigated (Okamoto et al. 2002a). TT initiation in ovary of grape pistils was first observed two weeks before anthesis in both diploid (Muscat of Alexandria and Campbell Early) and tetraploid (Kyoho and Suiho) cultivars. However, TT development proceeded quickly in diploid cultivars up to six days before anthesis, while TT development was slow in tetraploid cultivars and found in only 20% to 50% of ovaries six days before anthesis. Such retardation of TT development in tetraploid grape pistils may be caused by the exceedingly vigorous shoot growth, because TT development can be improved by root-zone restriction where shoot growth is retarded (Okamoto et al, 2001b).

3) Pollen tube growth inhibition in pistils

(1) Pollen tube growth in diploid and tetraploid grape pistles

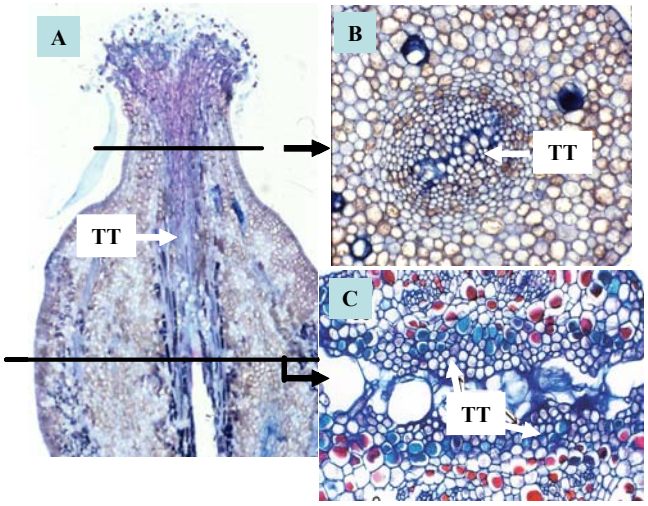

Fig. 6 Pollen tube transmitting tissue (TT) in grape pistils. A, Longitudinal section; B, cross section of style; C, cross section of middle ovary.

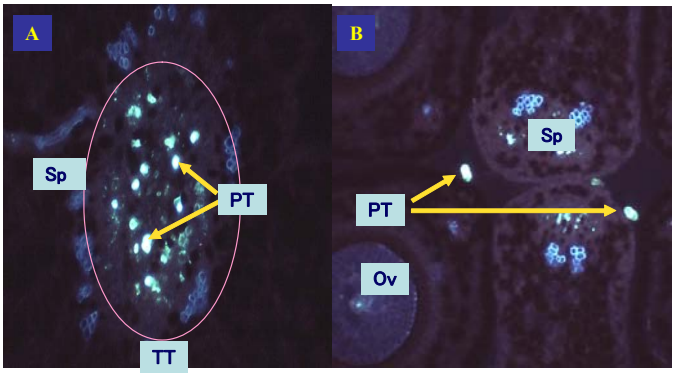

Fig. 7. Pollen tubes grow inside TT in Muscat of Alexandria pistils (A), and outside septa, not in TT, in Kyoho pistils (B). PT, pollen tube; TT, transmitting tissue; Ov, ovule; Sp, septum

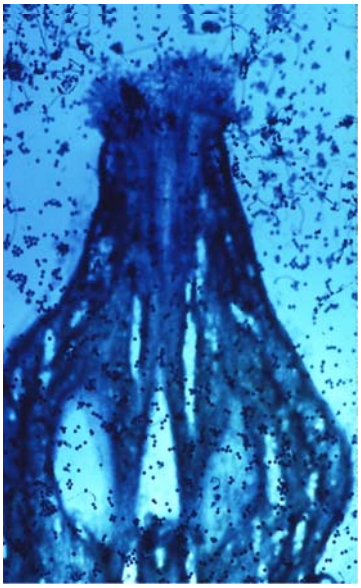

Fig. 8 Effect of pistil diffusates on *in vitro* pollen tube growth on agar medium. A slice of Pione pistil was covered with a thin film of liquid agar. Then, pollen grains of Muscat of Alexandria were scattered on the agar surface and incubation was carried out at 25℃ for 8 hr.

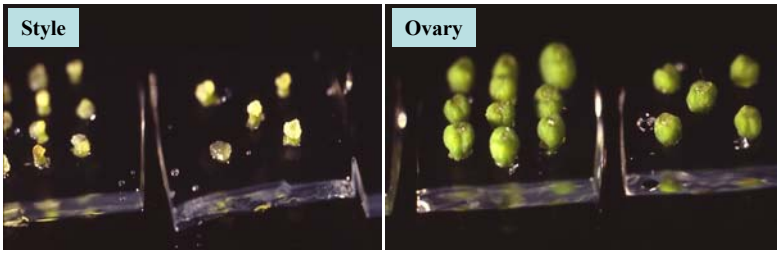

Fig. 9. Tissue standing to collect diffusates from style and ovary. Tissues were removed from agar blocks after standing for 15 min.

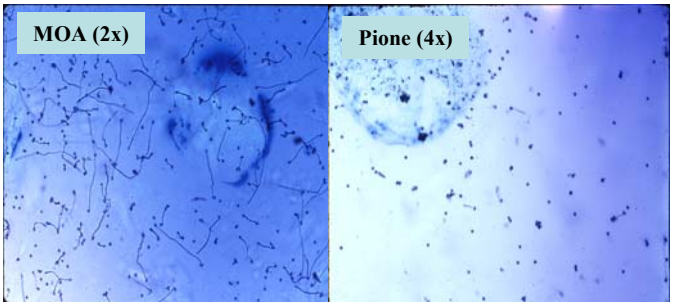

Fig. 10. Effect of standing ovary tissues on *in vitro* pollen germination. After standing ovary tissues on agar blocks, Muscat of Alexandria pollen grains were scattered and incubation was carried out at 25℃ for 8 hr.

Pollen tube penetration into an ovule via the micropyle is essential to ovule fertilization. This enables seed development, resulting in the setting of normal seeded berries. The number of pollen tubes per pistil in various diploid and tetraploid cultivars was examined three days after pollination (Okamoto et al, 1989b). At both middle and basal parts of styles, no obvious difference in pollen tube number was found between diploid and tetraploid cultivars. However, at both middle and basal parts of ovary tissues, a significantly large number of pollen tubes were noted in diploid cultivars compared with tetraploid ones. At the micropyle, 1.3 to 3.5 pollen tubes penetrated the ovule in diploid cultivars, while only 0.4 to 1.0 pollen tubes were found in tetraploid ones. Usually, four or five ovules develop in each pistil, which means that one to four ovules, out of the four or five ovules, can be fertilized in each diploid grape

pistil, while less than one ovule is fertilized in each tetraploid grape pistil.

From these data, it is noted that most pollen tubes penetrating through the style stopped growing at the upper and middle parts of the ovary.

(2) Pollen tube growth inhibitor in tetraploid grape pistils

As a simple *in vitro* test, a thin longitudinal slice of blooming Kyoho pistil was prepared using a freezing microtome and covered with heated liquid agar (1% agar and 20% sucrose). After cooling, sound pollen grains of Muscat of Alexandria grapes were scattered on the agar surface and incubation was carried out at 25°C for 8 hours. Significant inhibition of pollen germination and tube growth was observed above the upper and middle parts of the ovary slice, although no such inhibition was found above the style tissue (Fig. 8). This finding was further supported by a tissue standing test using agar blocks, where various numbers of styles and ovaries were vertically set on agar blocks as shown in Fig. 9 (Okamoto et al, 1989c). After removing plant materials, pollen grains were scattered on the agar surface and incubation was commenced at 25°C for 8 hours. Figure 10 shows that strong pollen tube growth inhibitors (PGIs) originating from Pione and ovaries (tetraploid grapes) diffused into the agar blocks, although no such inhibitory effect was detected from Muscat of Alexandria ovaries. These results indicate the possibility of incompatibility and/or the presence of PGIs in tetraploid grape ovaries.

To examine the possibility of incompatibility in tetraploid cultivars, self- and cross-pollination tests and several treatments to overcome self incompatibility were conducted (Okamoto et al, 1989a). However, cross-pollination, repeated pollination, bud or delayed pollination, and heat treatment of pistils at bloom did not improve pollen tube growth in Pione grape pistils.

On the other hand, the involvement of PGIs could be easily demonstrated in tetraploid grape pistils. The effects of H_2O and MeOH extracts of diploid and tetraploid grape pistils on pollen tube growth were examined by adding the extracts to a liquid culture medium for *in vitro* pollen culture (Okamoto et al. 1989c; Okamoto et al. 1989d). Both pollen germinability and tube growth were significantly inhibited when H_2O extracts of tetraploid grape pistils were added. The inhibitory activity was constant during the pre-bloom stage and resistant to heat. From these findings, it was concluded that the inhibition of pollen tube growth in tetraploid grape pistils may be caused by mechanisms different from those operating in self-incompatibility reported in many incompatible plants.

Separation of the extracts by successive partitioning with hexane, ethyl acetate, MeOH, and H_2O revealed that the components exhibiting inhibitory activity existed in the MeOH and H_2O fractions. After further separation of the MeOH extract by Sephadex LH-20 and ODS column chromatography, two major components, PGI-1 and PGI-3, were purified (Okamoto et al, 1995). They were identified as quercetin glycosides and finally, PGI-1 was determined to be quercetin glucuronide (Fig. 11) (Arisawa, 1996).

These results indicate that ovary tissues of tetraploid cultivars have higher contents of PGIs than those of diploid ones, and this may be one of the reasons for the suppressed pollen tube growth in tetraploid grape pistils.

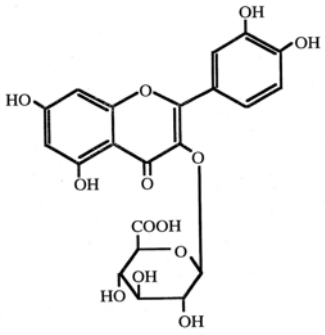

Fig. 11 PGI-1 from Pione pistils identified as quercetin glucuronide

(3) Pollen tube growth promoter extracted from TT in tetraploid grape pistils

Pollen tubes penetrate the style and ovary of grape pistils through TT extracellular spaces, as discussed above. The extracellular spaces are usually filled with matrix that is stained with PAS and alcian blue. TT extracellular matrix (TT-ECM) is thought to control pollen tube growth directly because TT-ECM supplies nutrients to pollen tubes. However, it is difficult to extract only TT-ECM from thriving grape pistils because the diameter of TT is less than

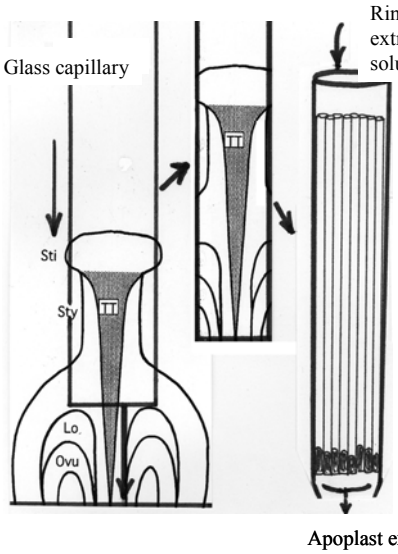

Fig. 12 Apoplast extraction and separation of TT-ECM. TT was excised with a micro capillary and subjected to apoplast extraction with H_2O→NaCl→$MgCl_2$, in that order.

Table 2 Effect of apo-plast NaCl extracts from the TT in cv. Pione pistils on *in vitro* pollen germination[z]

Molecular weight (range)	No. of pistils extracted	Pollen germination[y](%)	
		Campbell Early (2x)	Pione (4x)
5,000 - 10,000	25	17.3 *	49.7 *
	50	13.7 **	70.9 **
	100	14.1 **	73.4 **
10,000 - 30,000	25	15.4 *	0.0 **
	50	21.9	68.5 **
	100	13.5 **	75.8 **
＞30,000	25	16.4 *	51.9 *
	50	11.2 **	59.1 **
	100	9.8 **	74.3 **
Blank		23.6	40.4

[z] Extracts were dried *in vacuo* and added to 100 μl of pollen germination media.
[y] Mean ± SE. Means were compared with blank test (t-test), * $p<0.05$; ** $p<0.01$.

40 μm. However, inserting a micro capillary (I.D. 100 μm) from the top of a pistil (stigma) to the base of the ovary could excise the central column of the pistil, including mainly TT, with minimum contamination by other ovary tissues (Okamoto et al, 2002b). The excised TTs in the micro capillary were washed successively with H_2O, 1 M NaCl, and 50 mM $MgCl_2$, and each extract was obtained by centrifugation at low speed as 3,000 rpm (Fig. 12). This extraction procedure using mineral solutions enables release of ion-bound ECM from the cell wall, and is called the apoplast extraction method. Sample pistils were collected from two diploid (Muscat of Alexandria and Campbell Early) and two tetraploid (Kyoho and Pione) grapes and the effects of the extracts on pollen tube growth were compared. Interestingly, NaCl extracts of tetraploid grape pistils possessed strong pollen tube growth promoting activity (pollen tube growth promoter; PGP), while extracts of diploid ones were inactive (Table 2). After separating the NaCl extracts on a TSK gel column and biochemical analyses, a PGP having a molecular mass of approximately 40 kDa was purified, which was assumed to be a polysaccharide composed of D-glucose units (Murakami and Okamoto, 2005). The PGP may facilitate passage of pollen tubes through the central part of the ovary of tetraploid cultivars where TT develops poorly. In diploid cultivars, pollen tubes can easily penetrate ovary tissue because of the well-developed TT.

Together, the results indicate that pollen tube growth in grape pistils is controlled by a balance of several factors, including the different developmental patterns of TT and the existence of PGI and PGP in ovary tissues. Further experiments should be performed to clarify the mechanisms of TT development and PGI and PGP production, as well as the effects of cultivation conditions on those mechanisms.

3. Methods to overcome poor berry set in tetraploid cultivars

After the development of Kyoho and other tetraploid cultivars in the 1960s, growers have made great efforts to overcome poor berry set. Several cultivation conditions were found to improve berry set. Some were temporary solutions while others are practiced to this day.

1) "Protected" cultivation

Grape cultivation in a film-covered plastic house began in the 1960s. In general, berry set of Kyoho and other tetraploid cultivars is significantly improved when the vines are planted in plastic houses. Covering clusters can improve

berry set if the blooming period overlapped with the rainy days. Such "protected" cultivation conditions present two major advantages for berry set: one is that it protects the cluster from rainwater, ensuring sufficient self-pollination, and the other is that it keeps higher temperatures than outside, resulting in improvement of both pollen tube growth into pistils and ovary development after ovule fertilization. Together with the other advantages for cluster production, such as less infection by diseases and good appearance of harvested clusters, tetraploid cultivars are usually grown under protected cultivation conditions.

2) Foliar and cluster application of SADH

The effectiveness of pre-bloom application of N-dimethylamino succinamic acid (SADH), a plant growth retardant, in improving Kyoho berry set was found in the 1970s. Foliar spray of SADH two to three weeks before full bloom was widely practiced in many Kyoho vineyards, producing filled clusters. The spray retarded significantly shoot growth in Kyoho vines where vigorous shoots often caused severe flower shatter. It is generally accepted that the improvement of berry set after SADH spray is caused by decreasing nutritional competition between shoots and clusters. However, Naito (1974, 1976, 1980) demonstrated that SADH application to only clusters improved berry set. He analyzed changes in endogenous plant hormones after SADH application and concluded that SADH increases cytokinin activity in florets at bloom, ultimately promoting Kyoho berry set. Okamoto et al, (1985) found that the SADH spray for Kyoho clusters increased significantly normally developed florets at the blooming stage, which results in seeded berry setting.

In 1985, SADH was found to induce growth of cancer cells in humans, and its production was stopped worldwide. Thereafter, other growth retardants, such as CCC (2-chloroethyl trimethylammonium chloride), were found to be effective. However, most growers had already improved the cultivation conditions of Kyoho by using pruning systems and fertilizer application, realizing good berry set without having to apply such growth retardants.

3) Canopy enlargement with weak pruning

The greatest improvement in Kyoho cultivation was the development of the cane pruning system with an enlarged canopy (Tsuchiya, 1980). The system was predominantly established by grape growers in Yamanashi Prefecture in the 1970s. The canopy size ranged from 100 to 300 m^2 for each vine. Kyoho vines growing on such a large canopy develop a lot of short shoots and no vigorous ones. In such shoots, Kyoho can set a sufficient number of normal seeded berries to produce marketable clusters. This fact agrees with the finding of Okamoto et al. that the development of ovules and TT at anthesis, as well as pollen tube growth after pollination, was better in short shoots than in long and vigorous ones.

4) Root-zone restriction

To control shoot vigor in tetraploid cultivars, a root-zone restriction planting system was developed by the Hiroshima Prefectural Institute for Fruit Production (Imai, et al. 1987; Imai, 1991). This new system improved berry set of tetraploid grape vines and enabled production of filled clusters. Anatomical analyses of florets at anthesis revealed that ovule and TT development, as well as pollen tube growth after anthesis, were significantly improved compared to vines cultivated with the conventional method (Okamoto et al, 2001b). Imai et al. (1991) planted Kyoho vines at 0.5 m intervals in a raised bed (0.6 m wide and 0.3 m high) in a film-covered house. Such a high-density planting system can produced fruit clusters at a normal level in mature vineyards (15 to 20 tons per ha) even in the second year after nursery planting. Recently, developed a 'Hiroshima bilateral cordon system' for tetraploid grape cultivation where vines were planted at 4 to 8 m intervals in raised beds furnished with an automatic irrigation system (Kato and Imai, 2000). This new root-zone restriction system with "enlarged canopy" vines has been adopted widely in Hiroshima Prefecture, because of the promising marketable cluster production a few years after nursery planting.

5) GA_3 treatment to induce parthenocarpy

Seedless berry production of cv. Delaware and Muscat Bailey A was established in the 1960s by treating pre-bloom

Table 3 Effects of GA_3 treatment on berry set and seed development in Aki-Queen and Pione grape clusters.

Cultivar	Time of GA application [z]	Berry set %	Seedless berry %	No. of seeds per berry
Aki-Queen	At blooming	74.8	92.1	0.07
	Untreated	42.0	23.2	1.11
		**	**	**
Pione	3 days after full bloom	96.5	100.0	0.00
	Untreated	73.9	98.6	0.01
		**	**	*

[z] Clusters were dipped into 25 ppm GA_3 solution. Means in each column and each cultivar were separated by the t-test (**, $p<0.01$, *, $p<0.05$, n = 8).

clusters with 100 ppm GA_3. However, GA treatment of tetraploid clusters was unsuccessful because of excessive elongation and severe hardening of the rachis, resulting in insufficient berry development. After several trials, successful GA treatment at concentrations as low as 15 to25 ppm was achieved in cv. Pione in the 1980s, where GA_3 was applied to post- and not pre-bloom clusters. A high percentage (50 to 70%) of florets in the treated clusters usually set seedless berries. To produce compact clusters weighing 400 to 500 g, flower clusters should be trimmed, leaving only a 3 to 3.5 cm portion at the tip during blooming time. The second GA treatment must be carried out using 25 ppm GA_3 10 to 14 days after the first treatment.

As noted previously, tetraploid cultivars, such as Kyoho and Pione, spontaneously set a lot of small seedless berries (Okamoto et al, 1984). This means that the cultivars tend to exhibit parthenocarpy, which may be due to high levels of plant hormones such as gibberellin. It is considered that the exogenous application of GA_3 at low concentrations even at post-bloom can stimulate seedless berry set.

Miura and Okamoto (2004) studied berry set of Aki-Queen (selected from Kyoho seedlings) and Pione grapes in both GA-treated and untreated clusters. As shown in Table 3, the number of seedless berries per cluster of Aki-Queen was increased significantly by applying GA_3 at full bloom. On the other hand, in Pione clusters, 98.6% of set berries were seedless even in untreated clusters. The percentage berry set, however, was increased significantly by applying GA three days after full bloom. These data indicate that Pione clusters tend to exhibit parthenocarpy and that GA_3 stimulates seedless berry set. Measurements revealed that pollen tube growth in pistils was inhibited by GA treatment in Aki-Queen but not in Pione because tube growth was already completed by the time GA_3 was applied in Pione. The most important effect of GA treatment on Pione pistils was on the growth of the ovary wall. GA treatment was found to significantly increase ovary wall width in Pione pistils when measured five and ten days after bloom, although cell number was not increased. From these results, it is concluded that GA treatment of tetraploid grape clusters stimulates ovary wall tissue growth, resulting in a high percentage of seedless berry set in the clusters.

Literature cited

Arisawa, M. 1996. Purification and characterization of pollen tube growth inhibitors present in 'Pione' grapes. Master Thesis of Okayama Univ.

Imai, S., G. Okamoto and M. Endo. 1987. Effects of dense planting and root-zone control on attaining greater early production and fruit set stability of tetraploid grapes. Bull. Hiroshima Fruit Tree Exp. Sta. 12: 1-9. (In Japanese with English summary)

今井俊治．1991．密植・根域制限栽培による 4 倍体ブドウの早期成園化の実証．岡山大学博士論文.

Imai, S., T. Fujiwara, S. Tanaka and G. Okamoto. 1991. Effect of soil moisture on vine growth and fruit production of Kyoho grapes growing on restricted rooting volume. Environ. Control in Biol. 29: 133-140. (In Japanese with English summary)

鴨下繁利・井川秀夫・土屋長男．1983. 井川系ブドウの系譜．pp. 117-160.

Kato, J. and S. Imai. 2000. Facilitating grapevine cultivation operations by improved trellis training — the "Hiroshima T-training system"—. J. ASEV Jpn. 11: 15-21. (In Japanese with English summary)

Miura, K. and G. Okamoto. 2004. Effect of gibberellin A_3 on pollen tube growth and berry set in diploid and tetraploid grape clusters. J. ASEV Jpn.: 2-16. (In

Japanese with English summary)

Murakami, M. and G. Okamoto. 2005. Chemical properties of pollen tube growth promoters extracted from transmitting tissue in Pione grape (*Vitis vinifera x V. labrusca*) pistils. Vitis 44: 153-156.

Naito, R., H. Ueda and T. Hayashi. 1974. Promotion of berry set in grapes by growth retardants. 2. Effect of SADH and CCC applied directly to clusters on berry set and shoot growth in Kyoho and Muscat of Alexandria grapes. J. Japan. Soc. Hort. Sci. 43: 109-114.

Naito, R. and T. Hayashi. 1976. Promotion of berry set in grapes by growth retardants. 3. Effect of prebloom application of SADH and CCC on gibberellin and cytokinin activity in florets of grape varieties, Kyoho and Muscat of Alexandria grapes. J. Japan. Soc. Hort. Sci. 45: 135-142.

Naito, R. and T. Hayashi. 1980. Promotion of berry set in grapes by growth retardants. 4. Comparison of SADH cluster dipping, shoot pinching and flower thinning with regards to their effects on berry set in Kyoho grapes. J. Japan. Soc. Hort. Sci. 49: 297-310

Nitsch, J. P. 1951. Growth and development in vitro of excised ovaries. Am. J. Bot. 38: 566-576.

Okamoto, G. and Y. Watanabe and K. Shimamura. 1980. Effect of topping and B-9 spray on the nutrition of cluster and berry setting in vigorous shoot of Kyoho grape vines. Sci. Rep. Agri. Okayama Univ. 56: 1-10. (In Japanese with English summary)

Okamoto, G. and S. Imai. 1982. A histological study of berry setting in 'Muscat of Alexandria' grapes. J. Japan. Soc. Hort. Sci. 50: 436-444. (In Japanese with English summary)

Okamoto, G., K. Shimamura and K. Yamamoto. 1984. A study of difference in the occurrence of seedless berries among the tetraploid grapes related to Kyoho vine. J. Japan. Soc. Hort. Sci. 53: 251-258. (In Japanese with English summary)

Okamoto, G., Y. Nishimura and K. Shimamura. 1985. Promotion of berry setting on the clusters of Pione grapes by cane pruning and B-9 spray on prebloom clusters. Sci. Rep. Agri. Okayama Univ. 66: 1-7. (In Japanese with English summary)

Okamoto, G., M. Yamamuro and K. Shimamura. 1989a. Effects of the levels of minerals, sugar and hormones on *in vitro* development of ovules and pollen tube elongation in excised grape florets. Sci. Rep. Agri. Okayama Univ. 73: 11-16. (In Japanese with English summary)

Okamoto, G., M. Inoue and K. Shimamura. 1989b. Effect of cross-pollination and several overcoming treatments for self-incompatibility on pollen tube growth in pistils of Pione grapes. J. Japan. Soc. Hort. Sci. 58: 311-317. (In Japanese with English summary)

Okamoto, G., I. Shibuya, M. Furuichi and K. Shimamura. 1989c. Inhibition of pollen tube growth by diffusate and extract of grape pistils. J. Japan. Soc. Hort. Sci. 58: 515-521. (In Japanese with English summary)

Okamoto, G., I. Shibuya and K. Shimamura. 1989d. Properties of pollen tube growth inhibitors extracted from grape pistils. J. Japan. Soc. Hort. Sci. 58: 523-528. (In Japanese with English summary)

Okamoto, G., M. Noda, S. Imai, and T. Fujiwara. 1991a. Effect of levels of fertilizer application on vine growth and fruit development of Kyoho grape planted on a restricted rooting volume. Sci. Rep. Fac. Agri. Okayama Univ. 78: 27-33. (In Japanese with English summary)

Okamoto, G. and N. Omori. 1991b. Effect of the levels of minerals, phytohormones and pistil extracts on in vitro ovule development and pollen tube growth in excised grape pistils. J. Japan. Soc. Hort. Sci. 60: 521-529.

Okamoto, G., Y. Fujii, K. Hirano, A. Tai and A. Kobayashi. 1995. Pollen tube growth inhibitors from Pione grape pistils. Am. J. Enol. Vitic.: 17-21.

Okamoto, G., H. Tada, A. Suyama and K. Hirano. 2001a. Poor development of transmitting tissue in tetraploid grape pistils causing inhibition of pollen tube growth. Vitis 40: 49-54.

Okamoto, G., H. Tada, A. Suyama, Y. Hayashi and K. Hirano. 2001b. Effect of shoot vigor on the development of transmitting tissue and pollen tube growth in pistils of tetraploid grape, cv Pione. Vitis 40: 105-110.

Okamoto, G., Y. Hayashi and K. Hirano. 2002a. Morphological studies on the development of transmitting tissues in diploid and tetraploid grape pistils. J. Japan. Soc. Hort. Sci. 71: 8-12.

Okamoto, G., M. Murakami, M. Mimoto and K. Hirano.2002b. Chemical properties of TT matrix in grape pistils and its effect on pollen tube growth. J. Japan. Soc. Hort. Sci. 71 (Suppl. 1): 213. (In Japanese with English summary)

大隅 正. 1937. 芽条異変による葡萄の大粒変種に関する研究. 岡山県立農事試験場臨時報告 40: 1-21.

恒屋棟介. 1971. 巨峰ブドウ栽培の新技術. 博友社.

土屋長男. 1980. 第 9 章 整枝・剪定. 葡萄栽培新説. pp. 155-216. 山梨県果樹園芸会. 甲府.

山根弘康. 1996. 第 3 節 日本育成品種解説. 「中川昌一(監修). 日本ブドウ学, pp. 371-383」. 養賢堂(東京).

［総　説］ブドウの結実生理（2）

4倍体ブドウの結実不良—その原因と改善技術

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概　要

1937年に大井上　康が4倍体ブドウの‘石原早生’と‘センテニアル’との交配を行い、その実生から得られた‘巨峰’は、果粒が巨大で食味も非常に優れる、画期的な新品種であった。しかし、花振るいが激しいという欠点を持ち、実際の栽培は困難であった。両親品種の結実特性をみると、キャンベル･アーリーの 4倍体芽条変異である石原早生には結実性は特に問題はないが、ロザキの4倍体変異であるセンテニアルは非常に結実が悪い。したがって、巨峰の結実不良はセンテニアルから由来したものと思われる。1960年代から巨峰と他の4倍体品種との相互交配によって、ピオーネ、オリンピアなど、多数の4倍体品種が育成され、ゴールデン・マスカットの4倍体個体を育種親とする竜宝、紅瑞宝、紅富士など（井川秀雄が育成）や藤稔（青木和直が育成）など、結実性の比較的良いものも生まれた。しかし、1980年代以降の主要な4倍体品種は巨峰、オリンピア、ピオーネであり、これらの結実不良の原因究明とその改善策の開発は非常に重要な研究課題であった。

著者らは、巨峰、ピオーネの結実不良の原因を研究の目標とし、まず、雌ずいの組織形態学的実験や*in vitro*実験を行って、結実不良の主な原因が胚珠の発育不完全と花粉管生長の不良にあることを認め、さらに花粉管生長をコントロールしている物質が雌ずい内に存在することを明らかにした。これらの研究の概要を示すとともに、4 倍体品種の結実不良を克服するために、各地で行われた改善策の開発とその成果について、簡単に紹介する。

1. 結実不良の原因

1）胚のうの不完全

開花期の雌ずい中の胚のうの形態を調査した結果、2倍体品種では 80%以上の胚珠が形態的に正常であったのに対し、4 倍体品種では、比較的結実の良い紅瑞宝、紅富士などでも正常胚珠率は40～50%、結実の不良な巨峰、ピオーネでは胚珠や胚のうの形態異常や未発達が多く見られ、正常胚珠はわずかに20～30%であった。また、長梢剪定樹に比べて短梢剪定樹では正常胚珠率がさらに低いことから、新梢の勢力や体内栄養が4倍体品種の胚珠の発達不良に関与していると推察された。そこで、無機塩濃度を変えた Nitsch（1951）の基本培地を用いて開花前の小花を培養したところ、ピオーネ小花では、培地の窒素濃度を高めるにつれて子房直径は増大したが、胚珠および胚のうの発達は著しく不良となった。一方、マスカット･オブ･アレキサンドリアでは、子房の直径、胚珠の発達に及ぼす窒素濃度の影響は比較的小さかった。これらのことから、4倍体品種では、開花期までの胚珠、胚のうの形態異常や未発達が結実不良の一因で、特に窒素栄養が豊富であると、その傾向が助長されることが明らかになった。

2）花粉管誘導組織の未発達

雌ずい内での花粉管の通路である誘導組織（Pollen tube transmitting tissue, TT）の発達は、花柱および子房上部にかけては2倍体品種と 4 倍体品種に明確な差はないが、子房組織内では 4 倍体品種の TT 発達は非常に不完全あるいは未形成であり、これが花粉管生長停止の一因になっていると推察される。根域制限栽培などで新梢の勢力を制御すると、TT の発達がある程度改善されることから、胚のうの発達不良と同様に、樹の栄養条件は TT 発達にも関与していると思われる。

3）雌ずい内における花粉管生長の阻害

開花期の花柱または子房をカンテンブロック（Sucrose 20%を含む）上に一定時間置床した後、そのカンテン上で花粉を培養すると、4 倍体品種の子房か

らは花粉管成長を阻害する物質（Pollen tube growth inhibitor, PGI）がカンテンに拡散することが確かめられた。雌ずいのホモジネートを各種の溶媒で連続抽出し、PGI 活性をアッセイした結果、MeOH および水抽出物中に PGI の多くが存在した。MeOH 抽出物を各種のクロマトで分離した結果、2 種類の PGI が単離され、その 1 つは quercetin 3-O-glucuronide と同定された。

4）4 倍体品種の雌ずい内に含まれる花粉管生長促進物質

花粉管の通路である TT の細胞間隙には、PAS やアルシアンブルーに強く染色される TT 細胞間隙充填物質（extracellular matrix, TT-ECM）が存在する。開花期の雌ずいから TT 部分を切り出して、NaCl および $MgCl_2$ 溶液で TT-ECM をアポプラスト抽出した。ピオーネ雌ずいの NaCl 抽出物には、予想外にも花粉管生長を促進する活性物質（Pollen tube growth promoter, PGP）が存在し、キャンベル･アーリーの雌ずいではその活性はなかった。このことは、TT が発達している 2 倍体品種では、特に花粉管の生長を助長する物質の存在が必要ではないが、その発達が不良な 4 倍体品種では花粉管生長促進物質の存在によって、辛うじて受精、種子形成が可能になっているものと考えられる。なお、ピオーネ雌ずいの TT-ECM に含まれる PTT の 1 つは D-glucose を主成分とする分子量約 40 kDa の多糖類であることが判明した。

2. 4 倍体ブドウ結実不良の改善技術

1960 年代に始まった巨峰の栽培において、各産地では結実安定のために膨大な研究が行われた。まず、1960 年代後半のビニールフィルム被覆の普及によって、巨峰の栽培は軌道に乗り始めた。その主な効果は、開花期の花房付近の保温と雨よけであった。1970 年代に入ると、矮化剤 SADH（B-ナイン）の開花前の散布が巨峰の結実安定に大きな効果をもたらすことが明らかにされ、これによって巨峰栽培は大きく進展した。同時に、山梨県の産地で開発された樹冠の拡大と長梢・弱剪定技術によって、巨峰、ピオーネの結実安定が大きく前進し、発がんの危険性による B-ナインの製造停止後は必須の栽培技術となった。一方、1980 年代に、広島県果樹試験場で「根域制限栽培」方式が開発され、巨峰などの新梢生長のコントロールを行うことによって、結実安定を確実にするとともに、根域ベッドを用いた超密植栽培によって早期成園化をも達成した。

1980 年代の後半からは、ピオーネの花穂の GA 処理によって、無核果の着粒確保が達成された。この技術開発によって、それまでは結実確保が不可能であった短梢剪定方式でも、安定した多収が可能となり、ピオーネ栽培は西日本を中心に大発展した。この技術は、藤稔、安芸クイーン、翠峰など、多くの 4 倍体品種に対しても有効で、現在はほとんどの 4 倍体品種はホルモン処理によって無核果生産されている。