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Poor development of transmitting tissue in tetraploid grape pistils causing inhibition of pollen tube growth

G. Okamoto, H. Tada, A. Suyama and K. Hirano

Faculty of Agriculture, Okayama University, Tsushima-Naka, Okayama, Japan

Summary

Anatomical investigations were carried out to study the cause of poor berry set in tetraploid grape cultivars. Using 6 diploid and 6 tetraploid grape cultivars, the development of the transmitting tissue (TT) and pollen tube growth in their pistils were examined. The rates of berry set and seed number per berry were also investigated. TT was found to be cylindrical in the style and elliptic-conical in the ovaries. In the middle part of the ovary, the TT had developed along the inner surface of each septum projecting from both sides of the ovary wall. In the middle style, the TT diameter of tetraploid cultivars was larger than in diploid cultivars. However, TT development in the septum was markedly poorer in most of the tetraploid cultivars examined except for cv. Fujiminori where a sufficient number of seeded berries developed on the clusters. Most pollen tubes penetrating into the ovary tissue were inhibited to grow further in tetraploid cultivars except for cv. Fujiminori. These findings suggest that the poor set of normally seeded berries in most tetraploid grapes may be due to poor development of TT in the septum, which severely inhibits pollen tube penetration into the micropyle.

K e y w o r d s: transmitting tissue, pollen tube growth, tetraploid grapes.

Introduction

In clusters of vines of tetraploid grape cultivars such as Kyoho and Pione (hybrids of *Vitis vinifera* L. and *V. labruscana* Bailey), poor set of normally seeded berries is usually observed (Okamoto *et al*. 1984, Okamoto *et al*. 1989 a). As a main cause of the poor berry set, inhibition of pollen tube growth in tetraploid grape pistils has been reported by several researchers (Naito *et al*. 1980, 1983, Okamoto *et al*. 1984, 1989 a, Komatsu 1987). Okamoto *et al*. (1995) have discovered the presence of pollen tube growth inhibitors (PGI) in grape pistils which were mainly composed of water-soluble substances, such as quercetin glycosides. The PGI activity was commonly higher in the pistils of tetraploid grapes than in those of diploid grapes (Okamoto *et al*. 1989 b). On the other hand, morphological malformations of stigmas and ovules may be a cause of poor berry set in grapes (Carraro *et al*. 1979, Okamoto *et al*. 1984). It is generally known that a transmitting tissue (TT) and intercellular matrix in TT play critical roles for pollen tube elongation in the pistil of grapes (Ciampolini *et al*. 1996) and in other plants (Kronestedt *et al*. 1986, Herrero and Arbeloa 1989; O'brien 1994, Weber 1994, Gonzalez *et al*. 1996, Howpage *et al*. 1998). However, varietal differences of TT development, which may largely affect yield, have not yet been reported. In this study we compared the TT development of di- and tetraploid grape cultivars and its effect on pollen tube growth and set of seeded berries.

Material and Methods

P l a n t m a t e r i a l: Four to 8-year-old diploid grape cvs Campbell Early and Muscat of Alexandria, 4n-Muscat (usually called Cannon Hall Muscat) and Pione were used for this study in 1997. They were grown under a plastic foil cover in an experimental vineyard of the Okayama University. In 1998, 10-14-year-old vines of 6 diploid and 6 tetraploid grapes were used (see Tab. 1). They were grown under the plastic foil cover in the vineyard of the Okayama Prefectural Agricultural Experimental Station (San-yo Cho, Okayama Pref.). Each vine had bilateral 4 cordons (2-4 m long) trained to H-shape on a horizontal trellis 1.8 m high above the ground. All vines had been spur-pruned regularly.

P i s t i l s a m p l i n g a n d o b s e r v a t i o n o f T T d e v e l o p m e n t a n d p o l l e n t u b e g r o w t h: At full bloom, 50 flowers of 5 clusters which had a similar size in each cultivar were self-pollinated with a fine brush and marked with water paint in the morning. The pistils were sampled 3 d after pollination and fixed with 50 % ethanol, formalin and glacial acetic acid (FAA, 18:1:1 v/v) solution. They were dehydrated by EtOH-BuOH series, embedded into paraffin blocks, and sectioned into 14 µm thick cross sections using a microtome. Half of the pistils were stained with alcian blue and Shiff's reagent to observe TT development. Five sections per pistil at each part of a pistil shown in Fig. 1 were microphotographed. The size of TT, TT cell number per pistil, and the total area of the intercellular spaces per TT were measured on the photographs using an area-line meter (TAMAYA PLANIX 5000). The other pistils were stained with aniline blue and availed for counting the number of pollen tubes at various parts of a pistil under a fluorescent microscope.

B e r r y s e t a n d s e e d f o r m a t i o n: At full bloom 8-10 clusters of each cultivar were bagged with a poly-ethyl-

Correspondence to: Dr. G. Okamoto, Okayama University, Faculty of Agriculture, Tsushima-Naka, Okayama 700-8530, Japan. Fax: +81-86-251-8388. E-mail: gokamoto@cc.okayama-u.ac.jp

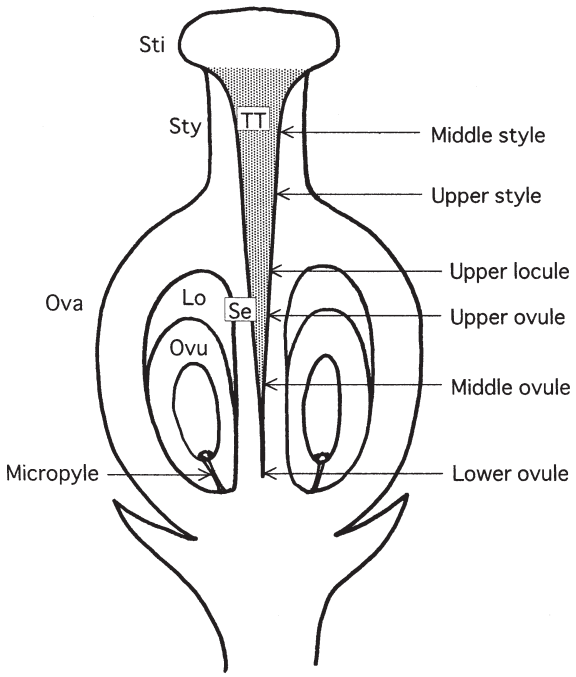

Fig. 1: Pollen tubes and the transmitting tissue (TT) developed in a grape pistil. The number of pollen tubes and TT development were examined in each section of the pistil as shown by arrows. Sti, stigma; Sty, style; Ova, ovary; Lo, locule; Ovu, ovule; Se, septum.

ene net. The percentage of berry set was determined 3 weeks after anthesis by dividing the number of berries by the total number of set and abscised pistils. Seed number per berry was counted for 50 berries per cultivar at the stage of seed hardening.

Results

TT development in various parts of pistils: In the cross section of styles, the TT was easily distinguished from the surrounding cortical tissue because of the small-sized cells with a thicker cell wall and wider intercellular spaces (Fig. 2 A). Such anatomical characteristics of TT cells were also observed in the upper part of the ovaries (Fig. 2 B). In the upper part of ovules, TT was found along the surface of the inner side of each septum projecting from both sides of the ovary wall (Fig. 2 C). The intercellular matrix in the TT was stained deeply blue-purple at each section of the pistil.

The TT sizes measured for the sections of the middle part of the style and various parts of the ovary are shown in Tab. 1. In the middle part of the style and the upper ovary, the TT diameter of tetraploid grapes was larger than in most diploid grapes. However, in the upper part of the locule, where the TT became elliptical, TT was thicker in most diploid cultivars than in tetraploid ones, except for cv. Fujiminori. This trend was more obvious in the upper and middle ovule sections where the larger size of the TT was recorded in cv. Fujiminori again.

The number of TT cell layers per septum in the diploid and tetraploid grape pistils are shown in Tab. 2. In the upper

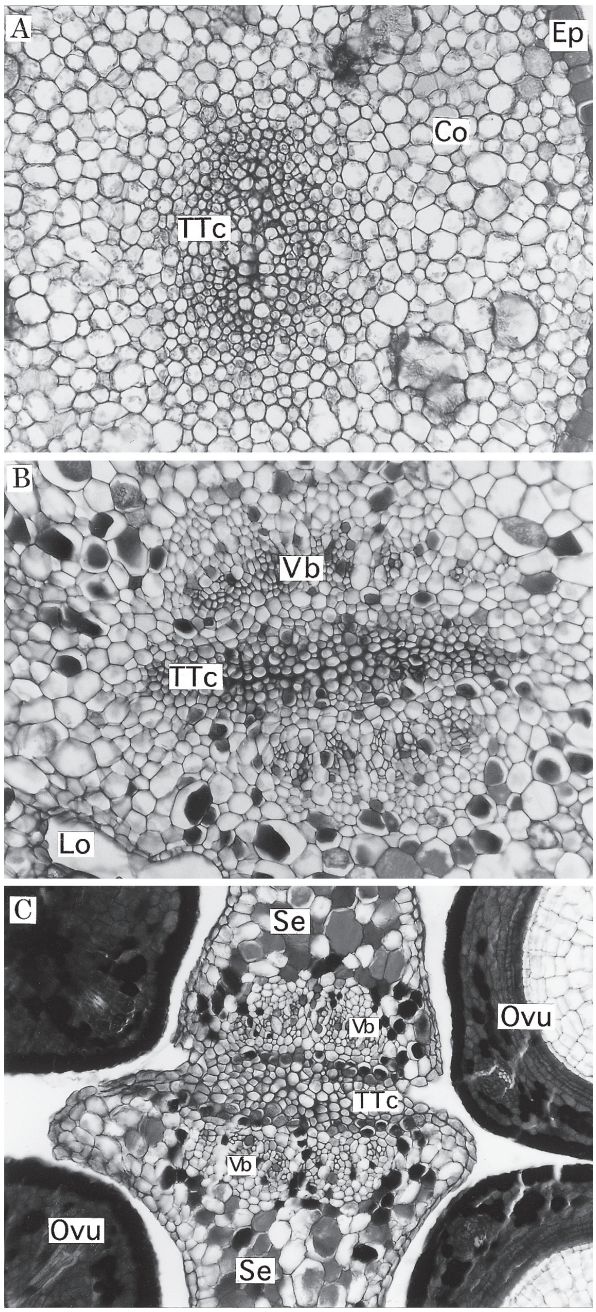

Fig. 2: Transmitting tissue (TT) development in the cross sections of middle style (**A**), upper locule (**B**), and middle ovule (**C**) in a Campbell Early pistil (x200). Ep, epidermis; Co, cortex; TTc, TT cell; Lo, locule; Se, septum; Vb, vascular bundle; Ovu, ovule.

part of the ovules, 3-6 layers of TT cells were found in the diploid grape pistils, while less than 3 layers were found in the tetraploid ones. The number of TT cell layers was lower in the middle part of the ovules; only one or two layers of the TT cells were formed in the tetraploid grape pistils except for cv. Fujiminori. However, 2-4 layers of TT cells were found in most diploid grapes and cv. Fujiminori.

Tab. 3 shows the TT area, total TT cell number, and the total area of the intercellular spaces per TT counted at the cross sections of pistils of two diploid and two tetraploid grape cultivars. There was no obvious difference between the diploid and the tetraploid cultivars in the upper part of the ovary. However, in the upper and middle parts of the

Table 1

Transmitting tissue (TT) size (10^{-3} mm) in cross sections of various parts of the pistils of 6 diploid and 6 tetraploid grape cultivars[1)]

Ploidy and cultivar	Style	Ovary			
	Middle	Upper ovary	Upper locule	Upper ovule	Middle ovule
Diploid					
Campbell Early	144.0 c[2)]	145.6 a b	118.6 a	34.8 a b	18.0 b
Glos Colman	150.3 b c	129.8 b	87.1 b	18.4 b c	13.3 b c
Kaiji	165.0 b c	156.7 a b	60.6 b c	20.3 b	13.0 c
Muscat Bailey A	142.4 c	117.1 b c	79.1 b	25.6 b	14.2 b c
Muscat of Alexandria	210.5 a	185.2 a	123.4 a	57.0 a	25.6 a
Neo Muscat	117.1 d	99.7 c	79.1 b	16.8 b c	12.8 c
Tetraploid					
Aki Queen	194.6 a b	186.7 a	69.2 b c	13.1 c	9.5 d
Fujiminori	221.5 a	177.3 a b	99.1 a b	25.0 b	14.9 b c
Kyoho	178.3 b	144.4 a b	53.8 c	11.3 c d	8.2 d e
4n-Muscat	196.2 a b	172.5 a b	31.6 d	9.5 d	7.3 e
Pione	196.6 a b	210.5 a	50.8 c	14.3 c	7.0 e
Suiho	178.9 b	159.9 a b	42.8 c d	12.7 c	8.8 d e

[1)] TT sizes are expressed as the diameter in the middle style and the upper ovary and as the thickness in the other parts of the ovary.
[2)] DUNCAN's multiple range test, $p<0.05$, n=13-15.

Table 2

The number of transmitting tissue (TT) cell layers in the cross section of the upper and middle ovules in 6 diploid and 6 tetraploid grapes

Ploidy and cultivar	Upper ovule	Middle ovule
Diploid		
Campbell Early	4.04 b[1)]	2.96 a b
Glos Colman	3.33 c d	2.06 d f
Kaiji	3.46 c	1.92 e
Muscat Bailey A	3.36 c d	2.41 c d
Muscat of Alexandria	5.17 a	3.33 a
Neo Muscat	3.00 c d	1.77 e f
Tetraploid		
Aki Queen	2.08 f g	1.45 f g
Fujiminori	2.86 d f	2.55 c
Kyoho	2.44 e f	1.39 f g
4n-Muscat	1.73 g	1.05 g
Pione	2.21 f g	1.45 f g
Suiho	1.71 g	1.00 g

[1)] DUNCAN's multiple range test, $p<0.05$, n=20.

ovule, the TT cell number and the area of intercellular space were considerably smaller in the tetraploid cultivars than in diploid cultivars (Tab. 3, Fig. 3).

Pollen tube growth in styles and ovaries: The number of pollen tubes penetrating the various parts of the pistils are shown in Tab. 4. Far more pollen tubes were found in each part of the pistils of Muscat of Alexandria compared to other cultivars. Except for this cultivar, there was no distinct difference in the number of pollen tubes penetrating the middle style. However, the number of pollen tubes that penetrated the ovary tissue, especially in the upper, middle, and lower parts of the ovule in the tetraploid cultivars was significantly lower than in the diploid ones. Among the tetraploid cultivars, the number of pollen tubes penetrating each section of the ovule and the micropyle was significantly higher in pistils of Fujiminori than in other cultivars.

The pollen tubes penetrating the ovary tissue in the diploid grape pistils were found mostly in their TT zones which had developed at the inner side of both septa (Fig. 4 A). However, in the tetraploid cultivars, small numbers of pollen tubes were found to be passing through outside of the septa (Fig. 4 B).

Berry set and seed formation: The rate of berry set and seed number per berry are shown in Tab. 5. There was no obvious difference in berry set between the diploid and the tetraploid cultivars. All berries of diploid cultivars were seeded, containing on the average 1.6-3.2 seeds per berry, while in tetraploid cultivars around 30-62 % of the set berries were seedless. The average seed number per berry was <0.65 except for cv. Fujiminori which contained 1.9 seeds per berry.

Discussion

It is obvious that the TT in grape pistils plays a critical role for further growth of pollen tubes into the ovary tissue. In the ovaries of tetraploid grapes, pollen tubes were found to be passing through the outside of the septa, not inside of

Table 3

Morphology of the transmitting tissue (TT) in cross sections of the ovaries of two diploid and two tetraploid grapes

Ploidy and cultivar	Upper ovary			Middle ovule		
	TT area (10^{-2} mm^2)	TT cell number	Intercellular space/TT (10^{-3} mm^2)	TT area (10^{-2} mm^2)	TT cell number	Intercellular space/TT (10^{-3} mm^2)
Diploid						
Campbell Early	2.4 b[1)]	261.2 a b	5.3 b	1.5 b	175.0 b	3.8 a b
Muscat of Alexandria	3.1 a b	304.1 a	12.2 a	2.1 a	224.8 a	6.8 a
Tetraploid						
4n-Muscat	2.6 b	300.8 a	6.9 b	0.5 c	75.8 c	1.5 c
Pione	3.7 a	230.3 b	12.3 a	1.3 b c	97.2 c	3.0 b

[1)] DUNCAN's multiple range test, $p<0.05$, n=20.

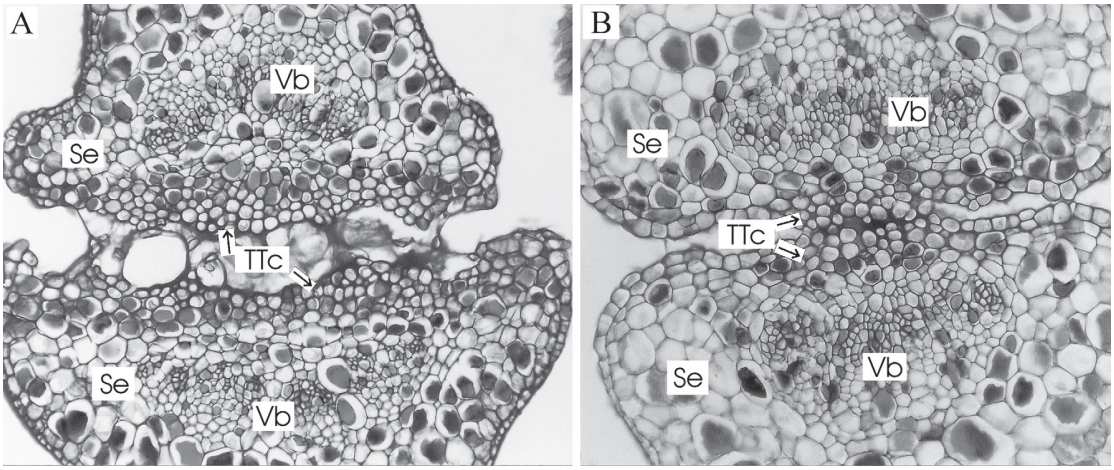

Fig. 3: Transmitting tissue (TT) development in the upper part of the ovule in Muscat of Alexandria (**A**) and Kyoho pistils (**B**) (x200). A larger number of TT cells are observed at the top of the Muscat of Alexandria septa compared to the Kyoho septa. Se, septum; Vb, vascular bundle; TTc, TT cell.

Table 4

The number of pollen tubes penetrating various parts of the pistil in 6 diploid and 6 tetraploid grapes

Ploidy and cultivar	Style		Ovary				Micro-pyte
	Upper	Middle	Upper	Locule Upper	Ovule Upper	Ovule Middle	Ovule Lower	
Diploid								
Campbell Early	25.7 c[1)]	20.7 c d	15.4 c	11.0 c d	8.70 c	6.60 c	4.20 c	1.00 a b
Glos Colman	12.0 d	9.3 d	7.6 d	6.5 d	5.50 c d	4.50 c	3.30 c	1.00 a b
Kaiji	35.3 b c	21.1 c d	16.7 c	12.5 c	8.00 c	5.50 c	3.40 c	0.97 b
Muscat Bailey A	22.0 c	12.5 c d	7.7 d	6.2 d	4.30 c d	4.70 c	3.00 c	0.24 c
Muscat of Alexandria	216.9 a	101.5 a	64.5 a	45.2 a	30.10 a	20.30 a	10.0 a	1.40 a
Neo Muscat	62.2 b	50.8 b	29.5 b	20.6 b	15.00 b	11.00 b	7.40 b	1.40 a
Tetraploid								
Aki Queen	17.2 c d	8.4 d	2.9 e	1.2 e	0.73 e	0.22 e	0.28 e	0.04 d
Fujiminori	28.9 c	25.1 c	10.1 c d	3.6 d e	2.10 d	0.94 d	0.86 d	0.41 c
Kyoho	13.7 d	9.9 d	3.1 e	1.5 e	0.73 e	0.20 e	0.14 e	0.04 d
4n-Muscat	23.8 c	14.7 c d	5.6 e f	2.2 e	0.65 e	0.29 e	0.14 e	0.11 d
Pione	26.6 c	16.3 c d	6.4 d e	2.5 e	1.70 d e	0.39 e	0.25 e	0.16 d
Suiho	32.6 b c	17.0 c d	4.2 e	2.1 e	0.71 e	0.33 e	0.10 e	0.07 d

[1)] DUNCAN's multiple range test, $p<0.05$, n=20.

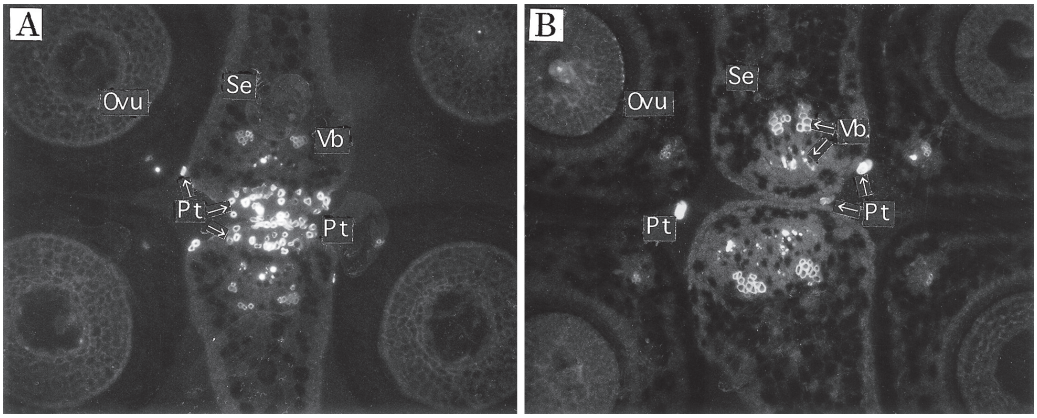

Fig. 4: Pollen tubes penetrating the middle part of the ovary tissue in Muscat of Alexandria (**A**) and Kyoho (**B**) (100). A number of pollen tubes were found inside the septa in ovaries of Muscat of Alexandria. However, only 3 pollen tubes were found outside the septa in ovaries of Kyoho. Ovu, ovule; Se, septum; Pt, pollen tube; Vb, vascular bundle.

them. This may be due to the poor development of TT in their ovaries, especially at the upper and middle parts of the ovule. The pistils of diploid cultivars, where the pollen tubes mainly penetrated the septum tissue, have a higher number of TT cells and larger intercellular spaces compared to tetraploid pistils. It is apparent that most of tetraploid grapes cultivated in Japan have poorly developed TTs in their ovaries, which may be one of the main causes of the inhibited pollen tube growth after entering into the ovaries. However, in the pistils of cv. Fujiminori, the TT development was found to be normal, *i. e.* similar to diploid cultivars. Fujiminori had been bred from cultivars of the Kyoho group, and is known to set a sufficient number of seeded berries (YAMANE 1996). Better TT development in this tetraploid cultivar might enable pollen tubes to reach the micropyles, which can result in the set of more seeded berries.

Intercellular matrix, stained deeply by both arcian blue and Schiff's reagent, was abundantly observed in TT especially in diploid cultivars. Pistil samples for paraffin embedding were dehydrated by soaking with a series of n-BuOH-EtOH solutions, which dissolves water- and alcohol-soluble substances but not insoluble substances such as protein, insoluble pectin, and starch. The matrix observed in our microscopic examinations is supposed to be a kind of carbohydrate such as pectic polysaccharides and glycoproteins because of the stainability with both arcian blue and Shiff's reagent. CONSIDINE and KNOX (1979) and CIAMPOLINI *et al*. (1996), who investigated the intercellular material in the grape pistils histochemically, suggested that the matrix was composed of polyphenols, pectic substances, and lipids. The presence of lipids, pectin acidic polysaccharides, and proline-rich protein in the TT intercellular spaces has been reported for various species (VITHANAGE 1984; WANG and CHEUNG 1993; WEBER 1994). Comparative investigations on the intercellular material observed in the TT of tetraploid and diploid grape pistils are necessary to understand the mechanism of pollen tube growth inhibition in tetraploid grape pistils.

Table 5

Berry set and seed formation of 6 diploid and 6 tetraploid grapes

Ploidy and cultivar	Total	Berry set per 100 flowers Seed number per berry					Avg. seed number per berry
		0	1	2	3	≥4	
Diploid							
Campbell Early	57.9 a[1)]	0.0 c	1.9 d	12.6 a b	17.4 a	26.2 a	3.20 a
Glos Colman	23.3 c	0.0 c	2.7 d	6.2 b	9.3 b	3.3 c	2.30 a b
Kaiji	53.9 a	0.0 c	35.7 a	13.5 a b	6.3 b	1.8 c	1.60 b
Muscat Bailey A	54.1 a	0.0 c	8.1 c d	12.6 a b	19.9 a	18.1 a	3.10 a
Muscat of Alexandria	31.8 b c	0.0 c	0.5 d	4.8 b c	16.9 a	9.6 b	3.20 a
Neo Muscat	39.0 b	0.0 c	6.5 c	18.8 a	9.8 b	10.0 b	2.50 a b
Tetraploid							
Aki Queen	33.7 b	12.4 b	20.6 b	0.7 c d	0.0 c	0.0 d	0.65 b c
Fujiminori	41.8 a b	12.2 b	27.0 a b	2.6 d	0.0 c	0.0 d	1.09 b
Kyoho	25.5 c	12.8 b	12.3 c	0.4 d	0.0 c	0.0 d	0.52
4n-Muscat	26.0 c	16.9 a b	6.5 c	2.6 c	0.0 c	0.0 d	0.45
Pione	31.7 b c	19.3 a b	11.8 c	0.6 d	0.0 c	0.0 d	0.39
Suiho	40.2 a b	24.8 a	13.4 b c	2.0 c d	0.0 c	0.0 d	0.42

[1)] DUNCAN's multiple range test, $p<0.05$, $n=20$.

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