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Characteristics of Seedless Berries of Triploid Hybrid Grapes (*Vitis vinifera* Complex) Derived from Eighteen Crosses

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To evaluate fruit quality of 92 triploid hybrid grapes obtained from 18 interploid crosses between six diploid and five tetraploid cultivars, the flower clusters trimmed and adjusted at about 200 were immersed in a solution of GA_3 (100 mg/l) at full bloom and examined at the mature stage. Fruit set in non–treated clusters was observed in 78.3% of the hybrids, whereas GA_3 treatment of the clusters resulted in fruit set in all the hybrids. In almost all hybrids that set fruits without GA_3 treatment, GA_3 treatment to the flower clusters increased not only berry weight but also the berry set rates, especially in those setting small number of berries without GA_3 treatment. The mean weight of non–treated berries of hybrids in each cross ranged from 0.8 to 2.7 g, except for the hybrid from a 'Muscat Bailey A'×'Rizamat 4x' cross that produced 6.5 g berry. In the GA_3–treated clusters, the mean berry weight ranged from 2.1 to 16.5 g. Hybrids producing large–sized berries were derived from crosses with 'Rizamat', while those producing small–sized berries were derived from crosses with 'Delaware' and its tetraploid 'Red Pearl'. In the non–treated clusters, fertilized seeds were rarely detected in a few berries with the range of 0–6% per berry, whereas in the GA_3–treated clusters the rates of berries with the seeds decreased and ranged from 0 to 1%. The rind color of berries was roughly classified into black, red or white in the 92 hybrids.

INTRODUCTION

Seedlessness is one of the desirable characters in table grape. Although breeding of seedless grapes is mainly due to stenospermocarpy, triploid breeding has several superior points to that of stenospermocarpic grapes (Wakana *et al.*, 2005). Although triploid hybrid plants were rarely obtained *in vivo* from interploid crosses between diploid and tetraploid cultivars (Yamashita *et al.*, 1993; Wakana *et al.*, 2002), they have been produced with high frequencies through *in vitro* culture of abortive seeds and embryos (Yamashita *et al.*, 1995, 1998; Wakana *et al.*, 2003; Hiramatsu *et al.*, 2003).

In Japan, seedless berries have been commercially produced with the aid of twice treatments of gibberellin (GA_3) with a concentration of 100 ppm for seeded diploid cultivars and 30ppm for seeded tetraploid cultivars with 'Kyoho' in their pedigrees. In the previous study (Wakana *et al.*, 2005), we produced triploid hybrid plants from reciprocal crosses between diploid cultivar 'Muscat Bailey A' and tetraploid cultivar 'Red Pearl' and evaluated the characteristics of the GA_3–treated and non–treated fruits. The result suggested that only one time of GA_3 treatment at full bloom is necessary for the improvement of fruit set rates and the enlargement of seedless berries in the triploid grapes because of their greatly high male and female sterility (Park *et al.*, 2002) but low or no parthenocarpic ability. Except information for this result, however, we have no knowledge about the characteristics of triploid grapes derived from the other various crosses with various cultivars.

The aim of this study is broaden our knowledge in terms of fruit set rate, berry size, seedlessness and quality in non– and GA_3–treated berries of 92 triploid hybrids from 18 interploid crosses with diploid and tetraploid cultivars.

MATERIALS AND METHODS

Plant materials

Six diploid cultivars, 'Muscat Bailey A', 'Muscat of Alexandria', 'Delaware', 'Rizamat', 'Rosario Bianco' and 'Sekirei', and five tetraploid cultivars 'Red Pearl', 'Yufu', 'Kyoho', 'Cannon Hall Muscat' and 'Rizamat–4x', were used to produce triploid hybrid plants. 'Muscat of Alexandria', 'Delaware', 'Rizamat', 'Rosario Bianco', 'Sekirei', 'Cannon Hall Muscat' and 'Rizamat 4x' belong to *Vitis vinifera* and the other cultivars are complex intercontinental (European–American) hybrids with *Vitis vinifera* in their pedigrees. 'Red Pearl' and 'Yufu' are tetraploid forms of diploid cultivar 'Delaware' and 'Muscat Bailey A', respectively. 'Rizamat 4x' is a colchiploid induced from diploid 'Rizamat' in our laboratory. Although it has been known that 'Cannon Hall Muscat' is a tetraploid form of 'Muscat of Alexandria', Ohmi *et al.* (1999) indicated the possibility that it is not

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the tetraploid form of 'Muscat of Alexandria'.

Interploid crosses between these diploid and tetraploid cultivars were made in 1999 for a 'Yufu' × 'Muscat of Alexandria' cross, 1997 for a 'Yufu' × 'Rosario Bianco' cross and reciprocal crosses between 'Sekirei' and 'Yufu', 1985 for a 'Muscat' of 'Alexandria' × 'Kyoho' cross, 1989 and 1990 for the other 13 crosses (Wakana *et al*., 2002). The triploid hybrid seedlings were derived either by seed sowing, embryo culture (Wakana *et al*., 2003) or immature seed culture and subsequent embryo culture (Hiramatsu *et al*., 2003). A total of 92 triploid hybrid seedlings derived from the 18 interploid crosses were used in this study. In addition, a total of 81 hybrids derived from reciprocal crosses between 'Muscat Bailey A' and 'Red Pearl', whose berry characteristics had been reported previously (Wakana *et al*., 2005), were used to examine only for the rind color. They were grown in a greenhouse located at Sasaguri orchard of the University Farm, Kyushu University.

Cluster thinning and GA_3 treatment

The methods of flower cluster thinning and GA_3 treatment were the same as those described previously (Wakana *et al*., 2005).

Analysis of berry characters

In each triploid hybrid, ten clusters with ripened berries were randomly harvested about 100 days post–anthesis or in September 1996 and 1997 for most triploid hybrids, and in 2005 for the hybrids derived from a 'Yufu' × 'Muscat of Alexandria' cross, a 'Yufu' × 'Rosario Bianco' cross and reciprocal crosses between 'Sekirei' and 'Yufu'. To compare the difference of berry characteristics between GA_3–treated and non–treated clusters and among triploid hybrids, the cluster weight, berry number per cluster, berry weight, berry cracking and rind color were examined for the ten clusters and number of seeds and sugar content (Brix) were examined for five of the ten clusters. The seed number was examined only for large seeds that contained endosperm and/or embryo or the traces of them. The data for unfertilized small seeds, which were less than one–half in length (Wakana *et al*., 2005; Sarikhani, 2007), were excluded from this examination and will be published in elsewhere. The segregation of plants with different rind color was examined for the triploid hybrids. The rind color was roughly classified into three categories, i.e., black (black to purple), red (pink to dark red) and green (light yellow to green). The linear regression analysis was performed in terms of cluster weight, berry weight and fruit ser between non–treated and GA_3–treated clusters.

RESULTS

Characteristics of non–treated cluster

The flower clusters set small seedless berries without GA_3 treatment in 83 of the 92 triploid hybrid plants derived from the 18 crosses, but the remainder, 9 hybrids, did not set fruit and the clusters with undeveloped ovaries withered and dropped within two or three weeks after anthesis (Table 1). It was also found that some of the clusters dropped in several hybrids. Three crosses with 'Kyoho' used as a pollen parent and six crosses with 'Delaware' or 'Red Pearl' frequently produced this type of hybrids that did not set fruit without GA_3 treatment. The triploid hybrids derived from a 'Muscat Bailey A' × 'Kyoho' cross showed the lowest fruit set rates with small variation (Fig. 1). The mean number of berries per cluster was very different in different hybrids with the range from about 10 to 100 (Fig. 1) and different for different crosses with the range from 19.2 to 54.7.

The mean berry weight was the highest (6.5 g) in BRi4x9201, a hybrid derived from a 'Muscat Bailey A' × 'Rizamat 4x' cross (Table 1). Except this hybrid, however, the variation of the mean berry weight was relatively small among hybrids (Fig. 2), i.e., the means varied from 0.5 g to 3.2 g. The larger the berry size in these parental cultivars, the larger the berry size in their progenies was.

The cluster weight of hybrids derived from each cross highly varied (Fig. 3) and the means ranged from 12 g to 195 g (Table 1). YS9101, a hybrid from 'Yufu' × 'Sekirei', produced the largest cluster of 370 g with 100 berries, while BRi4x9201, a hybrid from 'Muscat Bailey A' × 'Rizamat 4x', produced the next heaviest cluster of 175 g with 26 berries. However, most of the hybrids produced clusters less than 100 g in weight (Fig. 3).

Fertilized seeds were detected in large berries that were rarely formed in a half of the 92 hybrids from the 18 crosses (Table 1). The mean rate of large berries with fertilized seeds ranged from 0 to 6.0%.

Characteristics of GA_3–treated clusters

The berry set rate was greatly improved by GA_3–treatment of the flower clusters in most of the progenies, but was slightly improved in three progenies from 'Yufu' × 'Delaware', 'Yufu' × 'Rosario Bianco' and 'Yufu' × 'Sekirei' crosses (Fig. 1). The mean number of berries per cluster ranged from 20 to 100 for the 92 hybrids and from 33 to 72 for the progenies derived from the 18 crosses (Table 2).

All GA_3–treated flower clusters set larger–sized berries than non–treated clusters in all of the 92 hybrids derived from the 18 crosses (Fig. 2). The mean berry weight ranged from 2.1 g to 16.5 g for the progenies derived from the 18 crosses (Table 2). Thus, the mean cluster weight ranged from about 20 g to 650 g for the 92 hybrids (Fig. 3) and from 95 g to 636 g for the progenies derived from the 18 crosses (Table 2). As seen in non–treatment of GA_3, the larger the berry size in these parental cultivars, the larger the GA_3–treated berry size in their progeny was. BRi4x9201 produced the largest seedless berry of about 17 g in weight.

In the hybrids derived from a 'Yufu' × 'Sekirei' cross, the significant positive correlation between GA_3–treated and non–treated berries was found in the number of berries, berry weight and cluster weight (Fig. 4). In almost all hybrids that set fruits without GA_3

Table 1. Characteristics of non–treated berries of triploid hybrid plants derived from 18 interploid crosses in grape

	No. of seedlings setting berries (N[a])	Mean weight of cluster (g) ±SD	Mean No. of berries per cluster ±SD	Mean weight of berry (g) ±SD	% berries with large seeds ±SD (N[b])
Muscat Bailey A × Cannon Hall Muscat	5 (5)	51.5±19.9	50.5±25.8	1.4±0.6	3.4±2.3 (5)
Muscat Bailey A × Kyoho	12 (20)	32.9±21.2	24.6±28.4	1.7±0.6	2.3±1.8 (11)
Muscat Bailey A × Rizamat (4x)	1 (1)	195.0	30.0	6.5	0 (0)
Yufu × Muscat of Alexandria	1 (2)	43.0	46.9	0.9	0 (0)
Yufu × Delaware	4 (5)	97.4±65.9	54.7±33.3	2.1±0.7	0.9±0.6 (4)
Yufu × Rizamat	4 (4)	103.4±87.4	34.6±22.3	1.9±1.3	1.8±0.5 (2)
Yufu × Rosario Bianco	7 (7)	83.5±55.5	43.4±20.4	1.6±0.6	0.3±0.3 (3)
Yufu ×Sekirei	20 (21)	61.7±31.9	41.1±14.8	1.4±0.5	0.5±0.8 (6)
Delaware × Cannon Hall Muscat	3 (5)	58.6±31.5	41.1±27.5	1.4±0.1	5.3±3.9 (2)
Delaware × Kyoho	1 (4)	11.7	23.5	0.5	2.1 (1)
Delaware × Yufu	4 (4)	43.4±28.5	36.7±10.0	1.1±0.7	1.3±1.1 (4)
Red Pearl × Rizamat	2 (3)	24.3±7.3	28.7±21.5	0.8±1.5	1.2±0.6 (2)
Muscat of Alexandria × Kyoho	1 (2)	86.9	42.8	2.1	1.4 (1)
Rizamat ×Yufu	1 (1)	63.3	38.9	1.6	1.4 (1)
Sekirei ×Red Pearl	2 (3)	19.9±3.1	22.3±1.3	0.9±0.2	6.0±2.8 (2)
Sekirei ×Yufu	2 (2)	41.7±48.3	34.7±7.8	1.2±1.0	0 (0)
Rosario Bianco × Kyoho	1 (1)	51.8	19.22	2.7	2.1 (1)
Rosario Bianco × Yufu	2 (2)	38.0±0	0.4±0	1.9±0	0.1±0.1 (1)

[a] No. of seedlings examined. [b] No. of seedlings producing large berries with large seeds.

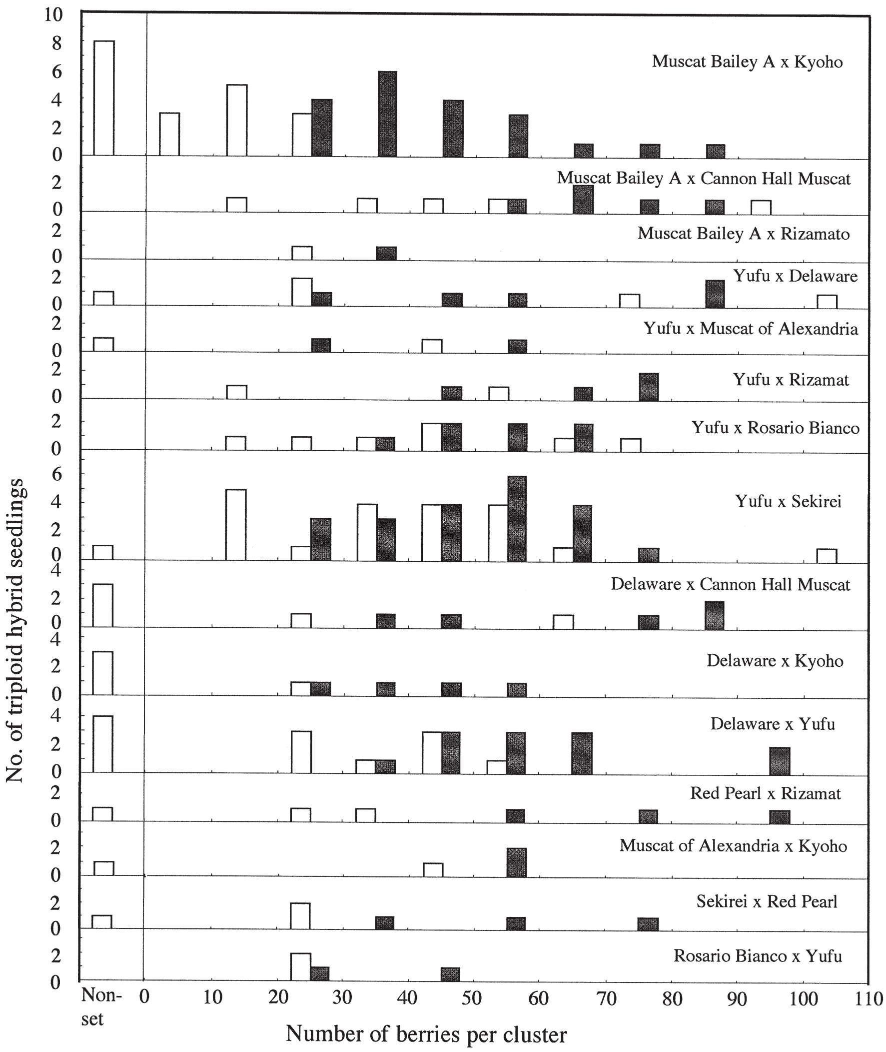

Fig. 1. Frequent distribution of triploid hybrid plants setting berries with different rates in 15 crosses. White bar: non–treatment; solid bar: GA_3 treatment.

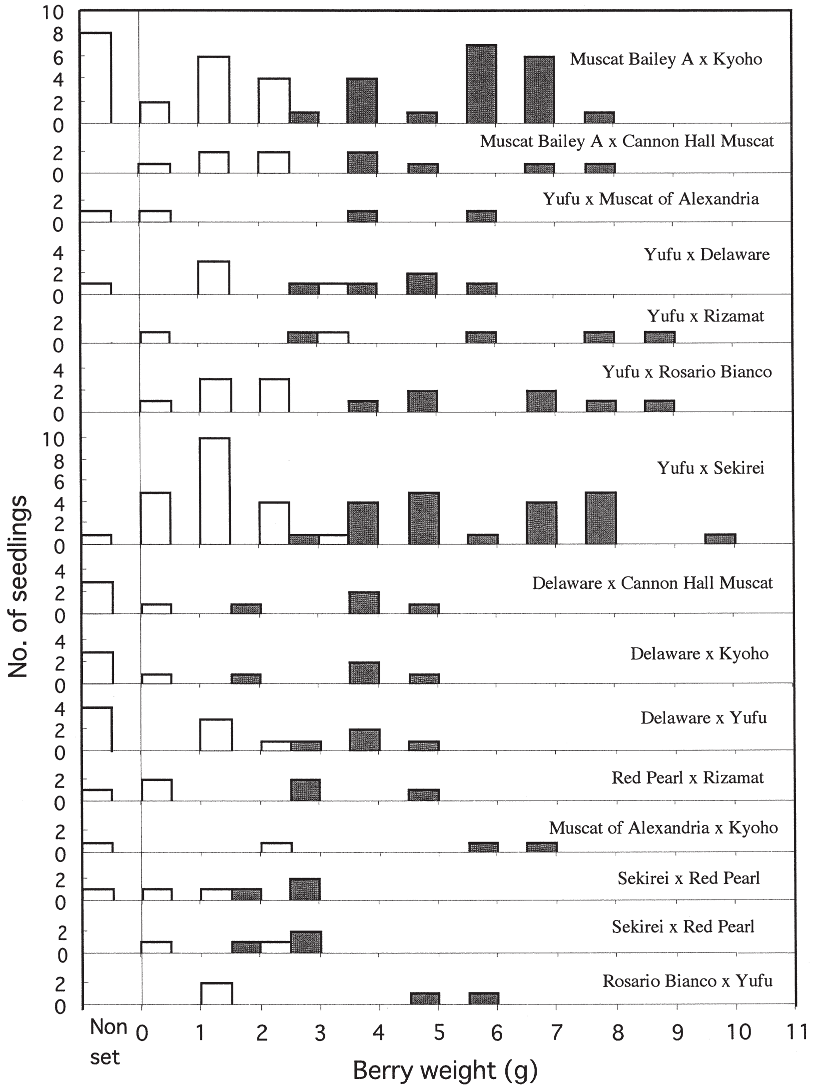

Fig. 2. Frequent distribution of triploid hybrid plants with different berry weight in 15 crosses. White bar: non–treatment; solid bar: GA_3 treatment.

Table 2. Characteristics of GA_3–treated berries of triploid hybrid plants derived from 18 interploid crosses in grape

	No. of seedlings examined ±SD	Mean weight of cluster (g) ±SD	Mean No. of berries per cluster ±SD	Mean weight of berry (g)	Brix ±SD	% berries with large seeds ±SD (N[a])
Muscat Bailey A ×Cannon Hall Muscat	5	303.6±105.0	68.0±10.8	5.0±1.	20.7±1.8	0.1±0.1 (2)
Muscat Bailey A ×Kyoho	20	214.6± 64.2	43.7±16.2	5.3±1.3	20.0±1.3	0.1±0.2 (3)
Muscat Bailey A ×Rizamat (4x)	1	635.8	38.5	16.5	17.5	0
Yufu ×Muscat of Alexandria	2	183.8±105.3	40.1±16.6	4.2±0.9	19.4±0.1	0
Yufu ×Delaware	5	284.0±157.9	61.5±19.5	5.0±1.9	19.1±2.0	0.2±0.4 (0)
Yufu ×Rizamat	4	171.0±153.9	33.5±33.6	5.9±2.2	19.5±1.3	0
Yufu ×Rosario Bianco	7	313.8±107.7	52.0±11.1	6.0±1.7	18.9±1.3	0
Yufu ×Sekirei	21	261.5±140.1	48.0±14.0	5.5±1.8	18.8±0.5	0
Delaware ×Cannon Hall Muscat	5	179.2± 85.4	65.1±18.0	2.8±0.7	20.8±0.8	0.1±0.2 (1)
Delaware ×Kyoho	4	134.1± 47.3	41.7± 9.6	3.1±0.8	20.2±0.9	0
Delaware ×Yufu	4	174.2± 73.1	59.0±17.4	3.0±0.8	20.0±1.6	0.4±0.9 (1)
Red Pearl ×Rizamat	3	188.5±105.9	71.5±16.5	2.8±1.1	19.7±1.7	0
Muscat of Alexandria ×Kyoho	2	326.9±160.9	56.6± 3.2	5.8±0.2	21.0±0.5	0
Rizamat ×Yufu	1	401.1	58.0	8.0	17.5	0.3
Sekirei ×Red Pearl	3	114.5± 39.5	52.4±14.9	2.1±0.4	23.0±2.3	1.3±0.8 (1)
Sekirei ×Yufu	2	263.1± 41.5	51.6± 1.2	5.1±0.7	18.0±0.2	0
Rosario Bianco ×Kyoho	1	163.8	33.0	5.0	20.2	0
Rosario Bianco ×Yufu	2	95.2± 67.9	42.1±14.4	5.4±0.6	19.5±0.5	0

[a] No. of seedlings producing more than large berries with large seeds.

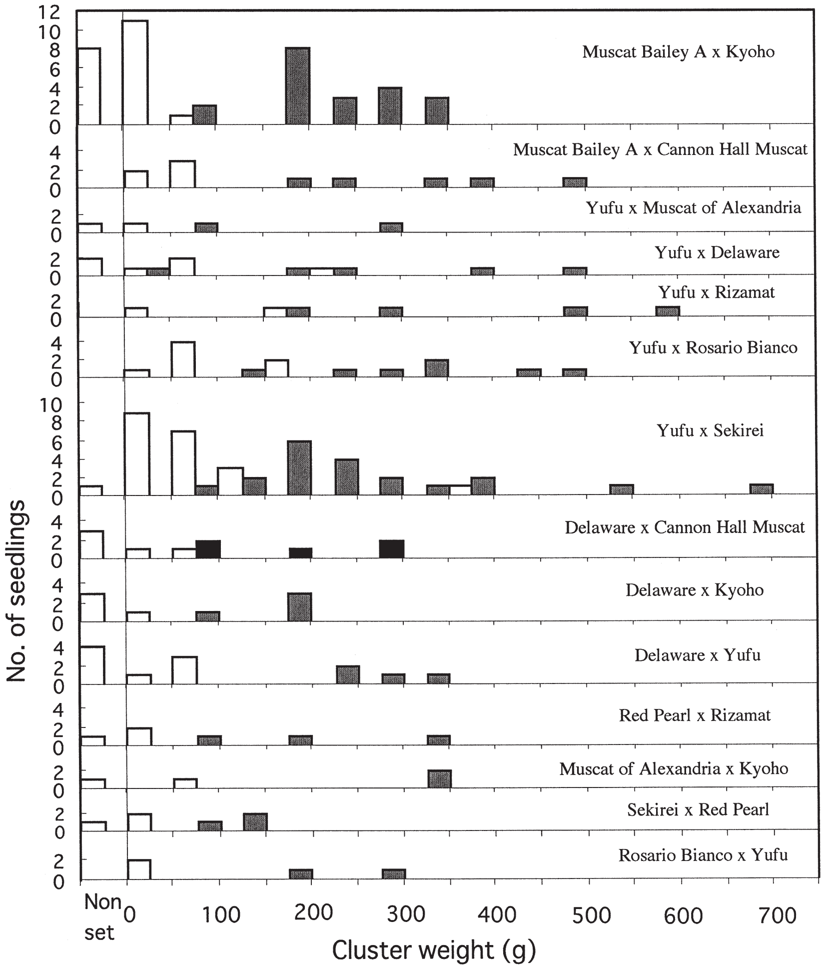

Fig. 3. Frequent distribution of triploid hybrid plants with different cluster weight in 15 crosses. White bar: non–treatment; solid bar: GA_3 treatment.

Table 3. Segregation of rind color in triploid hybrids derived from 20 interploid crosses

Fruit color Phenotype	Cross	Number of seedlings with indicated rind color (%)			
		Black	Red	Green	Total
Black×Black	Muscat Bailey A × Kyoho	14	2	2	18
Black×Red	Muscat Bailey A × Red Pearl	6	8	4	18
	Muscat Bailey A × Rizamat (4x)	1	0	0	1
	Yufu × Delaware	5	0	0	5
	Yufu × Sekirei	16	3	1	20
	Yufu × Rizamat	3	2	0	5
	Total	31	13	5	49
Red×Black	Delaware × Kyoho	2	1	2	5
	Delaware × Yufu	11	2	0	13
	Red Pearl × Muscat Bailey A	36	21	6	63
	Rizamat × Yufu	1	0	0	1
	Sekirei × Yufu	2	0	0	2
	Total	52	24	8	84
Black×Green	Yufu × Rosario Bianco	4	1	2	7
	Yufu × Muscat of Alexandria	1	1	0	2
	Muscat Bailey A × Cannon Hall Muscat	3	0	3	6
	Total	8	2	5	15
Green×Black	Muscat of Alexandria × Kyoho	2	0	1	3
	Rosario Bianco × Kyoho	0	1	0	1
	Rosario Bianco × Yufu	2	0	0	2
	Total	4	1	1	6
Red×Red	Red Pearl × Rizamat	0	2	0	2
	Sekirei × Red Pearl	0	4	0	4
	Total	0	6	0	6
Red×Green	Delaware × Cannon Hall Muscat	0	2	2	4

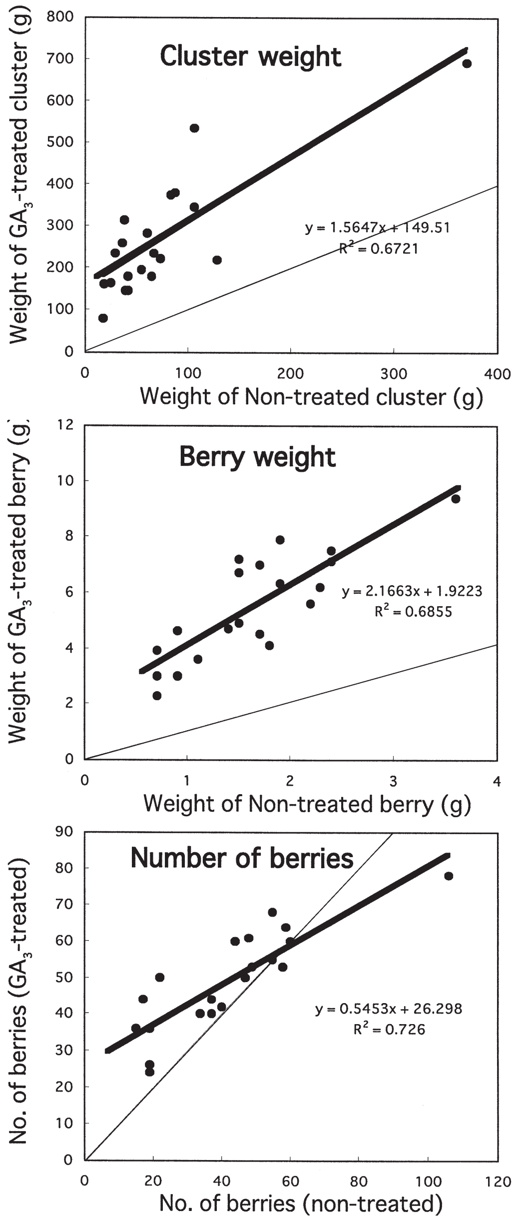

Fig. 4. Correlation of berry characters between GA_3–treated and non–treated flower clusters of triploid plants derived from a 'Yufu' × 'Sekirei' cross. The fine lines indicate y=x.

treatment, especially in those showing low fruit set rates less than about 60 berries per cluster, GA_3–treatment of the flower clusters increased the fruit set rates (Fig. 4). Berry weight and cluster weight in GA_3 treatment were 1.9 g and 150 g heavier than the non–treatment and the weight increased 2.2 g and 1.6 g respectively as the berry weight and cluster weight in non–treatment increased 1 g each.

Fertilized seeds were very rarely detected in a few berries of 5 of the 92 hybrids from the 18 crosses (Table 2). Thus, about 95% of the hybrids were perfectly seedless. The mean rate of berries with fertilized seeds ranged from 0 to 1.3% for the progenies derived from the 18 crosses.

Brix, berry cracking, lignification and rind color

There is no large difference in soluble solid content (Brix) among progenies from the 18 crosses, except for that derived from a 'Sekirei' × 'Red Pearl' cross that showed higher sugar content than the others (Table 2). The very high rates (> 90%) of berry cracking in GA_3–treated clusters were found in a 3x hybrid from a 'Yufu' × 'Muscat of Alexandria' cross and that from a 'Muscat Bailey A' × 'Rizamat 4x' cross. The high rates (10–50%) were rarely found in 3x hybrids derived from interploid crosses with 'Rizamat', 'Rosario Bianco' and 'Sekirei'. Compared with the parental cultivars without GA_3–treatment, lignification of cluster axes (pedicles, peduncles, primary branches, secondary branches and rachis) was enhanced to some extent by GA_3 treatment of the flower clusters in most triploid hybrids examined. A few hybrids showed very low extent of lignification and produced soft pedicles that are a favorable character for the commercial cultivars.

The triploid hybrids with black, red or green rind color segregated in interploid crosses when black cultivars were used as a pollen or pistillate parent (Table 3). The rates of hybrids producing black–colored berries were the highest (about 60%), followed by those producing red and green berries. The rind color segregated into red and green in the progenies derived from interploid crosses between red and green cultivars.

DISCUSSION

Fruit development is roughly divided into three phases: development of the ovary, fruit set and fruit growth. Pollination regulates a complex process of developmental events that collectively prepare the flower for fertilization and seed development in many flowering plants (O'Neill and Nadeau, 1997). The final decision to set fruit is dependent on success of pollination and fertilization which increase the amounts of plant growth hormones in the ovary (Gillaspy *et al.*, 1993). Although lack of pollination and fertilization results in senescence of flower, it is well known that exogenously applied gibberellins on emasculated flower are mimic of pollination and fertilization inducing ovary growth (Nitsch, 1970). The development of fruit without pollination and fertilization, parthenocarpy, is an important trait for seedless fruit production in horticultural crops to improve fruit quality and productivity (Spena and Rotino, 2001). Cultivated grapes with hermaphroditic flowers, which are the self–pollinated plants (Einset and Pratt, 1975; Chkhartishvili *et al.*, 2006), often show facultative parthenocarpy, that is, round small seedless berries (shot berries) may develop in a cluster depending upon the malfunction of pistils, embryo sac degeneration (Winker *et al.*, 1974) and high shoot vigor (Okamoto *et al.*, 2001), or by unfavorable environmental conditions that prevent normal pistils from pollination and fertilization. The setting rate of shot berries per cluster and maturation of shot berries vary among cultivars or individuals, and most triploid grapes produce anything but shot berries (Park *et al.*, 2002; Wakana *et al*., 2005). Recent study has suggested that some grape cultivars and hybrids have stimulative or vegetative parthenocarpic ability and the others have no or very low ability (Sarikhani, 2007). According to this report (Sarikhani, 2007), 'Yufu', 'Muscat Bailey A', 'Rosario Bianco' and 'Rizamat' used in this study belong to the grapes that have high vegetative parthenocarpy, while the other cultivars used in this study are the plants with stimulative parthenocarpic ability. This is supported by the present result that all triploid hybrids derived from the crosses between these vegetatively parthenocarpic cultivars set fruit without GA_3 treatment.

In grape with facultative parthenocarpy, there is little description of parthenocarpic mutants except for stenospermocarpy, a different type of seedlessness from parthenocarpy; relatively small–sized seedless berries with rudimental seed traces are produced after normal fertilization followed by endosperm and embryo abortion in early stage of embryogenesis (Ledbetter and Burgos, 1994). The present study indicates that most triploid hybrids derived from interploid crosses with diploid and tetraploid cultivars have vegetative or stimulative parthenocarpy and a few have no ability of parthenocarpy, since fruit set occurs in 92% (83/92) of hybrids that have very high or perfect male and female sterility as Park *et al*. (2002) have reported. In most cases, probably, dysfunctional sterile pollen of the triploid hybrids stimulates parthenocarpy in these self–pollinated plants without pollen germination.

The fruit set rates and the size of the parthenocarpic berries varied depending upon the individual hybrid and cross combination. According the report of Sarikhani (2007), these rates of fruit set and fruit growth in grapes with vegetative parthenocarpy are hybrid– or cultivar–specific characters and almost consistent in the different pollination treatments, i.e., non–pollination, pollination with sterile pollen and pollination with functional fertile pollen. Thus, it is most likely that the rates of fruit set and fruit growth are not due to the difference in hormone content in sterile pollen but due to the difference in sensitivity of or gene action in the pistil to sterile pollen in grapes with stimulative parthenocarpy. The size of the parthenocarpic berries was very smaller than the GA_3–treated ones, and positively correlated with the GA_3–treated ones. This also suggests that dysfunctional pollen stimuli may be equivalent to the normal pollination effect but not equivalent to the fertilization and subsequent fertilized seed effects. Namely, the parthenocarpic ability in grape may be due to mutation in transduction pass way for pollination stimuli. However, we could not find the types of mutation occurring in these hybrids used in this study.

Barritt and Einset (1969) have proposed two pairs of genes with epistatic action for fruit color: *B*, a dominant gene for black fruit, and *R*, a dominant gene for red fruit. White– or green–fruited grapes are considered to be recessive for both genes. The segregation of black–, red– and green–fruited triploid hybrids in crosses with black–fruited cultivars suggests that all black–fruited cultivars used in this experiment are heterozygous for the two genes.

In conclusion, the average weight of berries in non–treated clusters was less than 3.5 g in 91 triploid hybrids derived from the 17 crosses, except for BRi4x9201 producing 6.5 g parthenocarpic berries. Moreover, more than 8% of the hybrids did not set berries without GA_3 treatment. These suggest that the hybrids have low or no parthenocarpic ability and that triploid hybrids with high parthenocarpic ability will not be expected to produce from the present crosses except for those with 'Rizamat'. The berry sizes and set rates were, however, recovered by GA_3 treatment in these triploid hybrids. Thus, it is concluded that the immersion treatment of flower clusters in GA_3 solution is essential to produce clusters with high quality seedless berries.

Among the triploid plants estimated after GA_3–treatment of the clusters in this study, a triploid hybrid BK9101 derived from 'Muscat Bailey A' × 'Kyoho' and BR9001 from 'Muscat Bailey A' × 'Red Pearl' have the most superior characteristics for tree vigor, productivity, berry characters, disease resistance and management. The former produces about 10 g black–purple berries with high sugar content of 20–22 Brix, while the later produces about 3 g yellow–white berries with sugar content of about 22 Brix. Although the cluster thinning is necessary for the two triploid hybrids, berry thinning is not, i.e., after the appropriate cluster thinning they produced desirable clusters autonomously. The two hybrids will be propagated and their characteristics will be estimated again for commercial cultivar release.

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