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Characteristics of Seedless Berries of Triploid Hybrid Grapes (*Vitis* complex) from Reciprocal Crosses between Diploid 'Muscat Bailey A' and Tetraploid 'Red Pearl'

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To evaluate fruit quality of triploid hybrid grapes obtained from reciprocal crosses between diploid 'Muscat Bailey A' and tetraploid 'Red Pearl', the flower clusters were immersed in a solution of GA_3 (100 mg/liter) at the full bloom stage and examined at the mature stage. Fruit set was observed in 55% of the 3x hybrids from 'Muscat Bailey A' × 'Red Pearl' and 68% of those from the reciprocal. GA_3 treatment resulted in fruit set in all the hybrids. GA_3–treated flower clusters of hybrids that showed no fruit set without GA_3 treatment set fruits with the average of 67 berries per cluster. In almost all hybrids that set fruits, GA_3 treatment increased the number of berries set per cluster remarkably, especially in those set small number of berries without GA_3 treatment. In hybrids that showed no fruit set without GA_3 treatment, average berry weight in GA_3–treated clusters ranged from 1 to 3 g with the average of 1.9 g. In almost all hybrids that set fruits without GA_3 treatment, GA_3 treatment to the flower clusters increased berry weight irrespective of that in non–treated flower clusters. In non–treated clusters, fertilized seeds were rarely detected in a few berries of hybrids from the reciprocal crosses (<0.3% per berry), whereas in GA_3–treated clusters the rates of berries with seeds decreased less than 0.1%. In non–treated clusters, a large number of unfertilized small seeds with hard seed coat (<0.4 small seed per berry in average) were often observed among the hybrids from the reciprocal crosses. In GA_3–treated clusters, the rate of small hard seeds in each hybrid was dramatically decreased less than 0.1 seed per berry. About 95% of hybrids showed moderate and low extent of lignification of pedicles in GA_3–treated clusters. Berry cracking was scarcely observed among the hybrids from the reciprocal crosses.

INTRODUCTION

Seedlessness is one of the most desirable characters in grape. In addition to breed stenospermocarpic seedless grapes (Winkler *et al.*, 1962; Einset and Pratt, 1975; Mullins

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et al., 1992), breeding of triploid grapes is seemed to have several superior points to that of stenospermocarpic grapes as follows. (1) Triploid grapes are highly male and female sterile and are almost seedless (Park *et al*, 2002). (2) All diploid and tetraploid grape cultivars with desirable characters are directly used for triploid breeding. (3) As compared with diploid grapes, triploid grapes are very vigorous and produce large-sized berries as tetraploid grapes do. (4) Tetraploid forms of grapes are easily produced by colchicine treatment *in vitro* (Notsuka *et al.*, 2000) and *in vivo*. (5) Several triploid seedless cultivars such as 'Osuzu', 'King Dela' and 'Polyvitis' have been bred and evaluated in Japan (Kawakami *et al.*, 1979) and Russia (Goldriga *et al.*, 1980).

Although triploid hybrid plants were scarcely obtained from interploid crosses between diploid and tetraploid cultivars (Yamashita *et al.*, 1993; Wakana *et al.*, 2002), they are recovered 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 producing with the aid of twice treatments of gibberellin (GA_3 100 ppm for diploid and 30 ppm for tetraploid cultivars) in seeded diploid cultivars 'Delaware' and 'Muscat Bailey A' and in seeded tetraploid cultivar 'Pione'.

From these reasons, we produced triploid hybrid plants from reciprocal crosses between diploid cultivar 'Muscat Bailey A' and tetraploid cultivar 'Red Pearl' to evaluated the characteristics of the fruits.

MATERIALS AND METHODS

Plant materials

Diploid cultivar 'Muscat Bailey A' and tetraploid cultivar 'Red Pearl' were used to produce triploid hybrid plants. Both the cultivars are complex intercontinental (European–American) hybrids with *Vitis vinifera* in their pedigrees. 'Red Pearl' is a tetraploid form of diploid cultivar 'Delaware'. Reciprocal crosses between 'Muscat Bailey A' and 'Red Pearl' were performed in 1989 and 1990 (Wakana *et al.*, 2002), and the triploid hybrid seedlings were derived through either embryo culture (Wakana *et al.*, 2003) or immature seed culture and subsequent embryo culture (Hiramatsu *et al.*, 2003). Eighteen triploid hybrid seedlings from 'Muscat Bailey A' × 'Red Pearl' and 60 ones from the reciprocal were used in this study. They were five– to six–year–old trees grown in a greenhouse located at Sasaguri orchard of the University Farm, Kyushu University, Fukuoka.

Cluster thinning and GA_3 treatment

The flower clusters of these triploid hybrid plants were adjusted so that one shoot has one flower cluster, and trimmed at the full bloom stage up to such extent that about 200 flower buds remained in each cluster. Then, the flower clusters were immersed in GA_3 solution (100 mg/l), which was made by dissolving commercial gibberellin (50 mg GA_3 supplemented with extension reagents; Kyowa Hakko Co.). In each triploid hybrid plant, ten clusters were immersed with the solution and another ten were not treated. No berry thinning was carried out during the development in both of the treated and untreated clusters.

Analysis of berry characters

The clusters with mature berries were harvested about 100 days post-anthesis or at September 1996 and 1997. The cluster weight, berry number per cluster, berry weight, berry cracking, Brix and number of seeds were examined as to the difference and relationships between GA_3-treated and non-treated clusters and between the reciprocal crosses. The extent of lignification of pedicles in the hybrids was observed in all of the clusters and divided into three categories, low, moderate and high, based on the lignification of pedicles in GA_3-treatd clusters in their parental cultivars.

RESULTS

Fruit set, cluster weight and berry weight

No fruits set without GA_3 treatment in 8 of 18 triploid hybrid plants from 'Muscat Bailey A' × 'Red Pearl' (M×R) and 19 of 60 those from the reciprocal (R×M), whereas fruits set with GA_3 treatment in all of the plants (Figs. 1, 2). In the triploid hybrids that

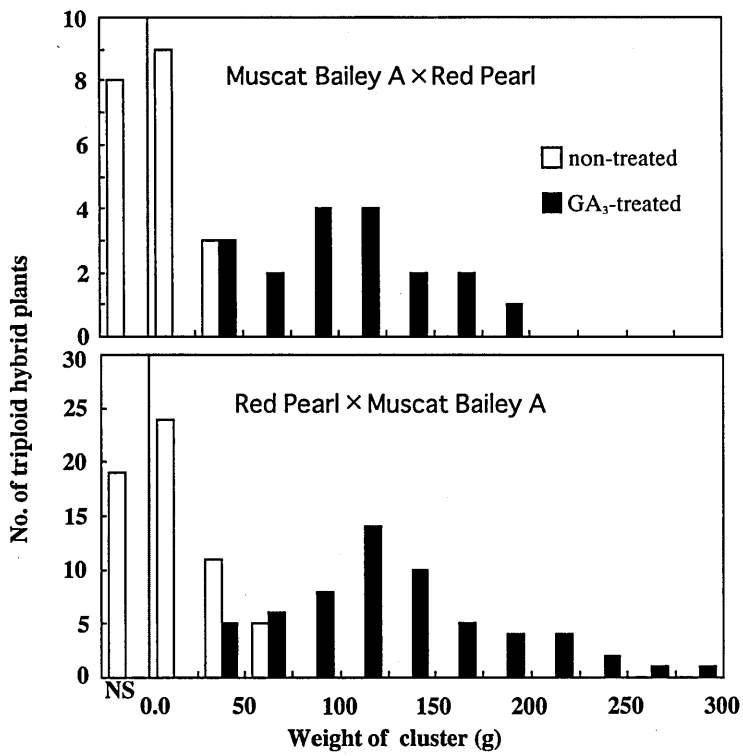

Fig. 1. Frequency distribution of triploid hybrid plants with clusters of different weight. White bars indicate the number of triploid plants without treatment, while black bars indicate the number of those treated the flowers with 100 mg/l GA_3. NS (non-setting) listed at the first lane indicates the triploid hybrid plants whose flower clusters dropped without setting.

set fruits without GA_3 treatment, number of fruits per cluster was less than 80 with the average of 15 for M×R hybrids and 21 for R×M hybrids (Fig. 2), while the weight of cluster was below 50 g for M×R hybrids and below 75 g for R×M hybrids (Fig. 1). In the triploid hybrids that set fruits with GA_3 treatment, however, number of fruits per cluster ranged from 9 to 100 for M×R hybrids with the average of 60 and 15 to 120 for R×M hybrids with the average of 52 (Fig. 2), while the weight of cluster ranged from 25 to 200 g for M×R hybrids and 25 to 300 g for R×M hybrids (Fig. 1).

In all the hybrids from the reciprocal crosses, the berry weight was remarkably higher in GA_3–treated clusters than non–treated ones (Fig. 3). Namely, the berry weight in the non–treated clusters of M×R hybrids was below 2.5 g, whereas that in the GA_3–treated clusters ranged from 1 to 5 g. The M×R hybrid that produced 2.5 g berries in average contained large berries with seeds. When the hybrid was deleted from the data, the berry weight in the non–treated clusters of M×R hybrids was below 1 g. On the other hand, the berry weight in the non–treated clusters of R×M hybrids was below 2 g, whereas that in their GA_3–treated clusters ranged from 1 to 4 g with the average of 2.2 g. The average

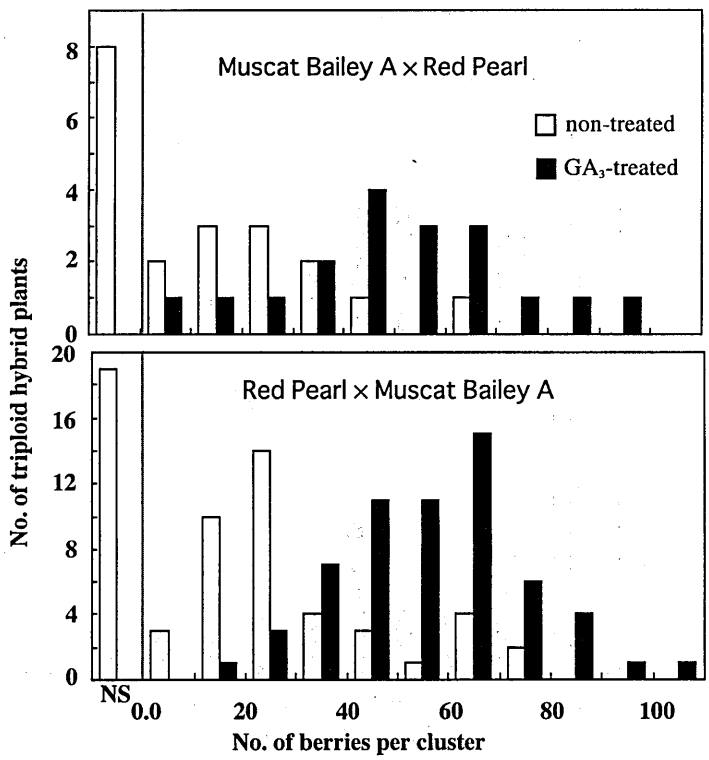

Fig. 2. Frequency distribution of triploid hybrid plants producing different number of berries per cluster treated with or without GA_3. White bars indicate the number of triploid plants without treatment. Black bars indicate the number of those treated the flowers with 100 mg/l GA_3. NS (non-setting): see Fig. 1.

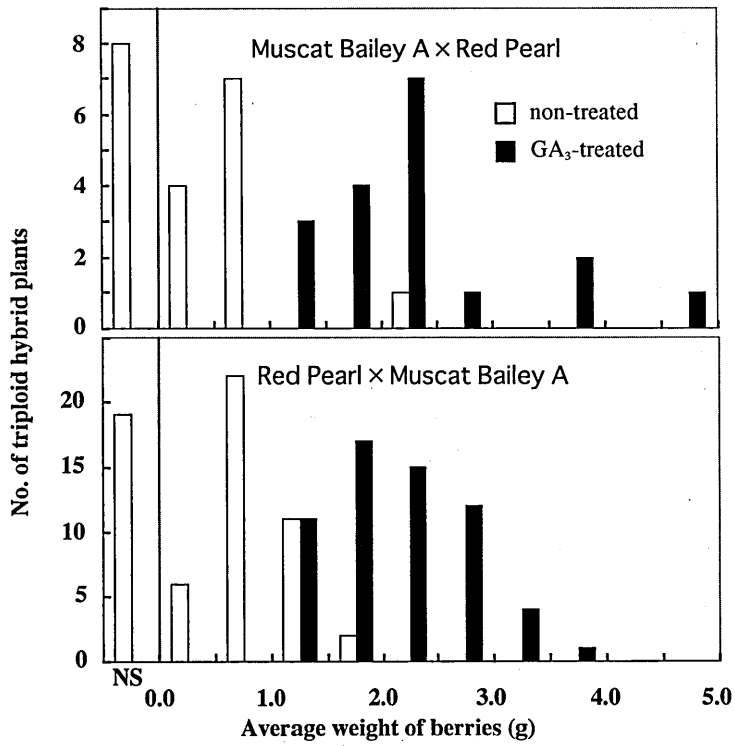

Fig. 3. Frequency distribution of triploid hybrids showing different berry weights. White bars indicate the number of triploid plants in non-treatment, while black bars indicate those treated the flowers with 100mg/l GA_3. NS (non-setting): see Fig. 1.

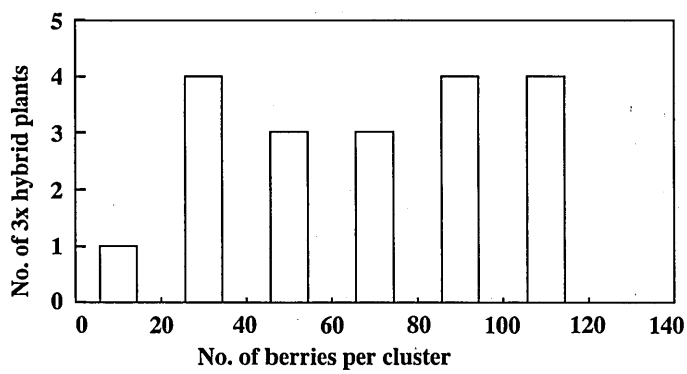

Fig. 4. Frequency distribution of triploid hybrids that set berries only after GA_3-treatment to the flower clusters in 'Red Pearl' × 'Muscat Bailey A'. Their flower clusters were immersed in GA_3 (100 mg/l) solution and the number of berries per cluster was examined at the ripening stage.

weight of berries in GA_3–treated clusters of 60 R×M hybrids was almost the same as that in GA_3–treated clusters of 18 M×R hybrids, i.e., 2.3 g.

All of the GA_3–treated flower clusters of hybrids that showed no fruit set in non–treatment set fruits with the range from >10 to <120 berries per cluster (Fig. 4). The average number of berries per cluster was 67. In almost all hybrids that set fruits without

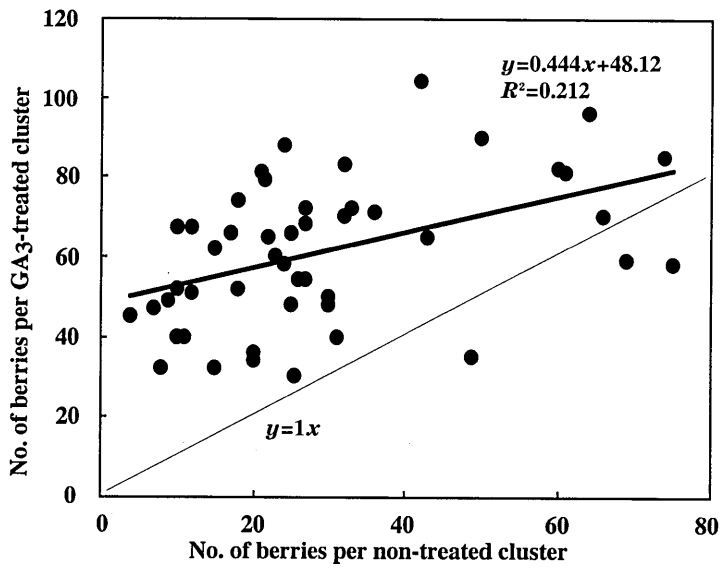

Fig. 5. Correlation of berry number between GA_3–treated and non–treated clusters in triploid hybrids between 'Muscat Bailey A' and 'Red Pearl'.

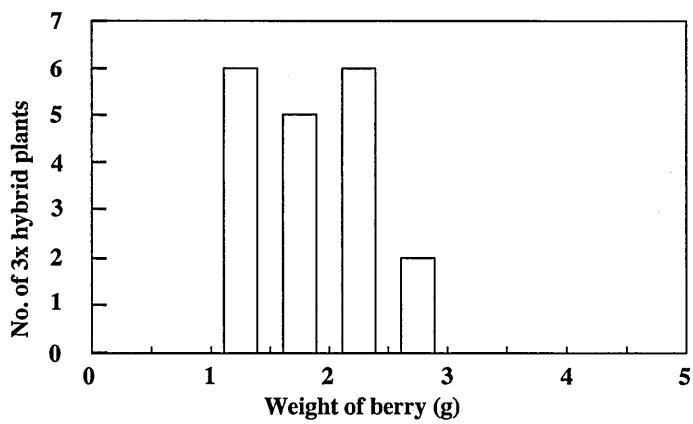

Fig. 6. Frequency distribution of triploid hybrids that set berries only after GA_3–treatment to the flower clusters in 'Red Pearl' × 'Muscat Bailey A'. Their flower clusters were immersed with GA_3 (100 mg/l) solution and the weight of berries was examined at the ripening stage.

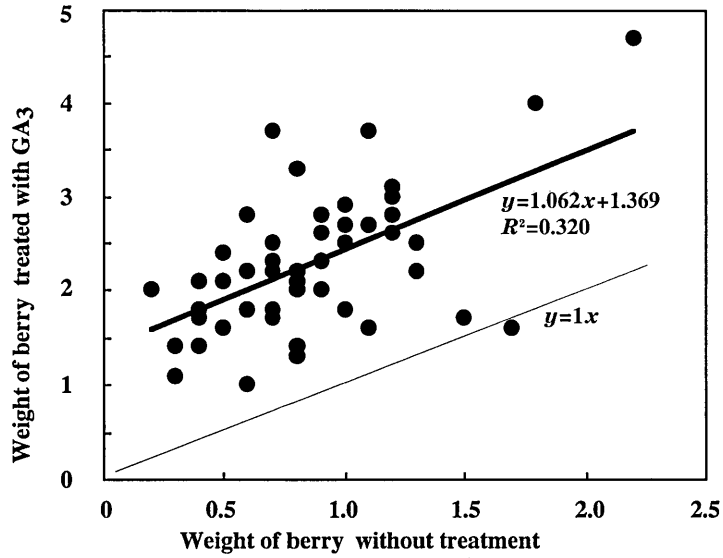

Fig. 7. Correlation of berry weight between GA_3–treated and non–treated clusters in triploid hybrids between 'Muscat Bailey A' and 'Red Pearl'.

GA_3 treatment, GA_3–treatment increased the number of berries per cluster remarkably; especially in those showing low fruit set rates (Fig. 5).

In hybrids that showed no fruit set without GA_3 treatment, weight of GA_3–treated berries ranged from 1 to 3 g with the average of 1.9 g (Fig. 6). In almost all hybrids that set fruits without GA_3 treatment, berry weight in GA_3–treated clusters increased irrespective of that in non–treated flower clusters (Fig. 7). The significant positive correlation with a correlation ratio 1.062 was found between average berry weight of GA_3–treated and that of non–treated berries in the hybrids (Fig. 7).

Seed formation

In non–treated clusters, fertilized seeds were rarely detected in a few berries of hybrids from the reciprocal crosses ($<$0.3% per berry; $<$0.1% per flower), whereas in GA_3–treated clusters the rates of berries with seeds decreased less than 0.1% (0.03% per flower) and more than 70% of hybrids were completely seedless. In non–treated clusters, a large number of unfertilized small seeds with hard seed coat ($<$0.4 small seed per berry) were often observed among the hybrids from the reciprocal crosses. In GA_3–treated clusters, the rate of small hard seeds in each hybrid that produced many small seeds in control was dramatically decreased less than 0.1 seed per berry. Compared with fertilized seeds, the length and width of small seeds were less than one–half. They contained neither embryo nor endosperm and were sinkers. No significant differences were found in both of the fertilized seed production rates and unfertilized small seed pro-

duction rates between M×R and R×M hybrids.

Brix, berry cracking and lignification

No significant differences in soluble solid content (Brix) between the M×R hybrids

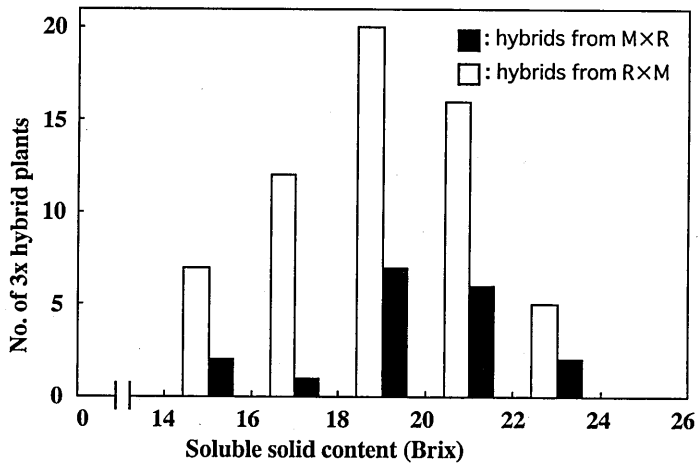

Fig. 8. Frequency distribution of 3x hybrid plants showing different concentrations of soluble solids (Brix) in the berries. White bars indicate the 3x hybrid plants derived from 'Red Pearl' × 'Muscat Bailey A' (R×M), while solid bars indicate those derived from the reciprocal (M×R).

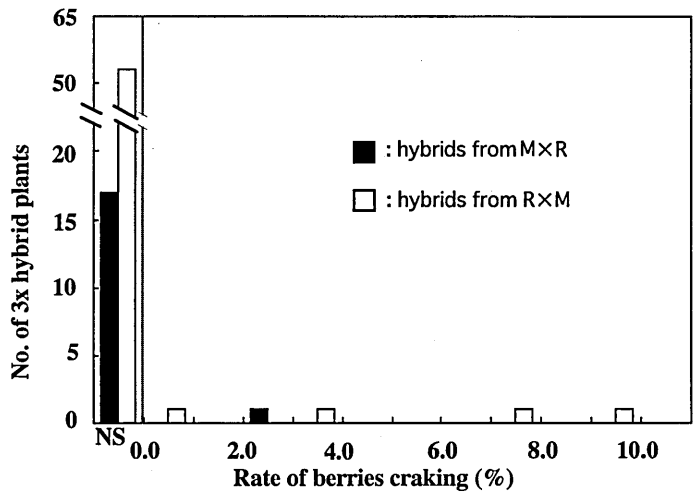

Fig. 9. Frequency distribution of 3x hybrid plants showing different rates of berry cracking. White bars indicate the 3x hybrid plants derived from 'Red Pearl' × 'Muscat Bailey A' (R×M), while solid bars indicate those derived from the reciprocal (M×R).

and R×M hybrids(Fig. 8). The average Brix in both hybrids was about 19. Berry cracking was rarely observed among the hybrids from the reciprocal crosses (Fig. 9). Of 18 M×R hybrids, only one showed 2% berry cracking, while four of 60 R×M hybrids showed 0.5–10.0% berry cracking.

Compared with the parental cultivars, about 95% of hybrids showed moderate and low extent of lignification of pedicles in GA_3–treated clusters, and only a few showed high extent (Table 1). Lignification was also observed in the peduncles, primary branches, secondary branches and rachis of GA_3–treated clusters.

Table 1. The degree of lignification in pedicles of flower clusters treated with GA_3 (100 mg/l) solution in triploid hybrid plants derived from reciprocal crosses between 'Muscat Bailey A' and 'Red Pearl'.

Cross	No. of triploid hybrid plants in indicated degree of lignification in the pedicle[a] (%)			
	Low	Moderate	High	Total
Muscat Bailey A × Red Pearl	6 (33)	11 (61)	1 (6)	18
Red Pearl × Muscat Bailey A	24 (40)	34 (56)	2 (3)	60
Total	30 (39)	45 (58)	3 (4)	78

[a] Low: lower degree of lignification of pedicles than that in 'Muscat Bailey A' and 'Red Pearl'. Moderate: intermediate degree of lignification of pedicles between low and high. This degree almost correspond to that of 'Muscat Bailey A' and 'Red Pearl'. High: higher degree of lignification of pedicles than that in 'Muscat Bailey A' and 'Red Pearl'.

DISCUSSION

Park *et al.* (2002) studied the fertility of 187 triploid grape hybrids including those from the same reciprocal crosses as this study and they found that pollen germination rates examined on agar medium ranged from 0 to 5.9% with the average of 0.24%. They (2002) have also reported that, in 3x × 2x and 3x × 4x crosses, the percentage of ovules developing into seeds varied in different triploid hybrid plants and ranged from 0.1 to 2.3%. In the present study, triploid grapes derived from reciprocal crosses between 'Muscat Bailey A' and 'Red Pearl' produced only a few seeded berries (<0.1% per flower), indicating very low male and female fertility. The very low rate of ovules developing into seeds (0.025%) in each triploid hybrid results from their self–pollination and/or pollination with different triploid hybrids.

Since the degree of male and female sterility is triploid–hybrid–specific characters (Park *et al.*, 2002), it is necessary for breeding to select such triploid hybrids that might have extremely low male and female fertility. In addition to the selection, GA_3 treatment at the full bloom stage of clusters reduces further the fertilized seed formation in the clusters. Thus, it is suggested that some of the excellent triploid hybrids derived from the present reciprocal crosses have high potential to become new cultivars.

In some of the triploid hybrids, on the other hand, formation of unfertilized small seeds with hard seed coat was observed. The extent of small seed formation seems to be

under genetic control, since the rate of small seeds per berry was stable in each triploid hybrid and was different in different hybrids. Since the formation of small hard seeds results in loss of consumer's interest, we must exclude such hybrids that small seeds are produced even after GA_3 treatment at the full bloom stage of clusters.

The average weight of berries in non-treated clusters was less than 1 g. Moreover, more than 30% of the hybrids did not set berries. These suggest that the hybrids have very low parthenocarpic ability and that triploid hybrids with high parthenocarpic ability will not be expected to produce from the present reciprocal crosses. The berry sizes and set rates were, however, recovered by GA_3 treatment in the triploid hybrids. Thus, the immersion treatment of flower clusters in GA_3 solution is essential to produce clusters with high quality seedless berries.

Between M×R and R×M triploid hybrids, there were no significant differences in cluster weight, number of berries per cluster, average weight of berry, berry cracking rate, Brix and seed formation in both of the non-treated and GA_3-treated clusters. So far as this study concerned, we may not consider the direction of cross in interploid reciprocal crosses.

The triploid hybrids derived from the present crosses have a problem on berry size. Production of small berries in the triploid hybrids may be due to the usage of 'Red Pearl' as a parent for the reciprocal crosses. However, the present study indicates that the reciprocal crosses produce many excellent triploid hybrid plants from which some new commercial cultivars may be selected. Usage of cultivars producing large-sized berries for interploid crosses may be effective for breeding triploid cultivars producing larger berries than the present triploid hybrids.

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