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'Bea–Kei', a New Triploid Seedless Grape Cultivar Derived from a 'Muscat Bailey A' × 'Kyoho' Cross

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To breed a seedless grape cultivar that produces large–sized berries with labor saving, 201 triploid hybrid plants were firstly derived from 24 interploid crosses with seven diploid and six tetraploid cultivars. A triploid grape BK9101 derived from a 'Muscat Bailey A' × 'Kyoho' cross was finally selected and given a cultivar name 'Bea–Kei'. 'Bea–Kei' showed high male and female sterility and produced small–sized seedless berries of about 2 g because of its low parthenocarpic ability. One time immersion treatment of the adequately thinned flower clusters with 100 ppm GA_3 at around full bloom, however, resulted in the formation of large–sized black berries of 8–11 g that stuck together. The trees showed high vigor and productivity, and, thus, required summer pruning for vigor control and thinning of flower clusters and flowers for high quality berry production. However, once the flower cluster thinning was carried out so that the each of the clusters had about 70 flowers, fruit thinning was unnecessary because of ideal fruit set and growth rates after the GA_3 treatment of the thinned flower clusters. The berries ripened from late August to early September in field conditions, and the berry quality did not deteriorate on trees by October. The berries were black–purple in rind color and contained high concentration of soluble solid sugars (19–21% in Brix). The rind of 'Bea–Kei' was not so slippery that the seed and pollen parents did. The flesh of 'Bea–Kei' was harder than the parents and showed superior taste than the parents. The estimation of the whole data obtained in this research led to the conclusion that 'Bea–Kei' has high potential as a new leading triploid cultivar that produces very high quality seedless berries with the aid of GA_3 at full bloom.

INTRODUCTION

Table grapes, which are consumed as fresh fruits, have attractive appearance: taste is said to be secondary, and good flavor may not be as important as production output, shipping tolerance and shelf life (Rieger, 2006). 'Thompson Seedless' producing small fruit is a major seedless cultivar in the world (Einset and Pratt, 1975; Ledbetter and Ramming, 1989), and 'Emperor', 'Italia' and 'Almeria', which produce large–sized berries, are major seeded cultivars in USA, Italy and Spain respectively, as well as 'Kyoho' in Japan. Large–sized seedless berries are produced in Japan mainly with the aid of twice treatments of GA_3 or with the aid of streptomycin and then GA_3 in cultivars such as 'Pione' and 'Kyoho'. In addition to the heavy work of GA_3 treatments to flower buds and young fruits, thinning of flowers and young fruits is the hardest and indispensable work in management of seeded cultivars whether GA_3 treatment is applied or not. Thus, production of large–sized seedless berries with labor saving is one of the desirable purposes in table grape breeding.

'Osuzu' derived from a 'Kyoho' × 'Super Hamburg' cross in 1963 and registered in 1980 is the first triploid seedless cultivars in Japan (NIFTS, 2002a) as well as 'Plolyvitis' reported in USSR (Goldriga *et al*., 1980), followed by 'King Dela' ('Red Pearl' × 'Muscat of Alexandria') registered in 1985 (NIFTS, 2002b), 'Haney Seedless' ('Kyoho' × 'Concord Seedless') registered in 1993 (Yamane *et al*., 1993), 'Kaimirei' ('Red Queen' × 'Kosyusanjyaku') registered in 2000 (Ozawa *et al*., 2000a), 'Summer Black' ('Kyoho' × 'Thompson Seedless') registered in 2000 (Ozawa *et al*., 2000b) and 'Nagano Purple' ('Kyoho' × 'Rosario Bianco') registered in 2004 (NIFTS, 2005). All these triploid cultivars often required twice GA_3 treatment to enhance sufficient fruit set and enlargement of fruit, and these, except 'Nagano Purple', produced small– to medium–sized berries even after GA_3 treatments. Thus, it is considered that production of a large number of triploid hybrids from various crosses is critical for the breeding of high quality triploid grapes with labor saving.

We started the breeding work about 20 years ago for the production of seedless triploid grapes that are expected to have several superior points than stenospermocarpic seedless grapes like 'Thompson Seedless' (Wakana *et al*., 2005). Although triploid hybrid plants were scarcely obtained *in vivo* from many interploid crosses between diploid and tetraploid cultivars (Wakana *et al*., 2002), they were produced with high frequencies through *in vitro* culture of abortive seeds and embryos (Yamashita *et al*., 1998; Wakana *et al*., 2003; Hiramatsu *et al*., 2003). Among the triploid hybrid plants derived through the *in vitro* culture, we found one that showed excellent characters, such as very high male and female sterility, appropriate number of fruits setting per cluster, uniformity in fruit size, large fruit size, early ripening but non–deterioration of fruit quality for about two months

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after ripening.

We studied for 18 years about this excellent triploid hybrid that will be registered and released as a new seedless grape cultivar.

MATERIALS AND METHODS

Plant materials

Seven diploid cultivars and six tetraploid cultivars were used to produce triploid hybrid plants (Wakana *et al*., 2007). Interploid crosses between these diploid and tetraploid cultivars were made from 1985 to 1999 (Wakana *et al*., 2002). The triploid hybrid seedlings were derived either by seed sowing, embryo culture (Wakana *et al*., 2003) or by immature seed culture and subsequent embryo culture (Hiramatsu *et al*., 2003). A total of 201 triploid hybrid seedlings derived from 24 interploid crosses were examined to detect excellent characters as commercial cultivars. Nine of the 201 triploid hybrids were firstly appreciated as candidates for new seedless cultivars, and then BK9101, a triploid seedling derived from a 'Muscat Bailey A' × 'Kyoho' cross in 1990, was finally selected in 1999 as a candidate for the commercial cultivar producing large–sized seedless berries (Table 1). The triploid seedling was propagated through grafting on Teleki 5BB and Hybrid Fran rootstocks in 1999, and planted in 2000. Appreciated were the grafted plants and the original seedling that were grown in a greenhouse located at Sasaguri orchard of the University Farm, Kyushu University. The characteristics of 'Bea–Kei' trees and berries were examined mainly with six–year–old trees based on 'Syubyo Tokusei Bunrui Chosa Hokokusyo (report on assessment and classification of characters of seeds and saplings)'(Yamanasi Prefectural Fruit Experiment Station, 1978).

Pollen germination test

Pollen germination test on agar medium followed the previous report (Park *et al*., 2002).

Cluster thinning and GA_3 treatment

Each of the flower clusters at full bloom were adjusted so that they had 70 flowers in each year except 1995: first, the cluster branches with more than eleven flowers were thinned out, second, seven consecutive branches with ten flower buds each were used for berry production, and third, branches with less than nine flowers that located at the terminal part of the flower cluster were also thinned out. In the year of 1995, the flower clusters were trimmed so that they had 80 flowers each: eight consecutive branches with ten flower buds each were used for berry production.

The flower clusters at full bloom were immersed with 100 ppm GA_3 solution supplemented with adjuvant (Kyowahakko). The other methods of GA_3 treatment followed those described previously (Wakana *et al*., 2005).

Analysis of berry characters

In each year, either ten or fifteen clusters with rip-

Table 1. The crosses used for the breeding of triploid grapes

Cross	No. of 3x hybrids obtained	No. of 3x hybrids in first selection
2x × 4x		
Delaware × Cannon Hall Muscat	5	0
Delaware × Kyoho	5	0
Delaware × Yufu	12	0
Muscat of Alexandria × Kyoho	4	1
Muscat of Alexandria × Red Pearl	1	0
Muscat Bailey A × Kyoho	22	1
Muscat Bailey A × Cannon Hall Muscat	6	1
Muscat Bailey A × Red Pearl	18	0
Muscat Bailey A × Rizamato(4x)	1	0
Neo Muscat × Kyoho	1	0
Neo Muscat × Red Pearl	1	0
Rosario Bianco × Kyoho	2	0
Rosario Bianco × Yufu	2	0
Sekirei × Yufu	2	0
Sekirei × Red Pearl	4	0
Rizamat × Yufu	1	1
Total	88	4
4x × 2x		
Red Pearl × Muscat Bailey A	68	1
Red Pearl × Neo Muscat	2	0
Red Pearl × Rizamat	3	0
Yufu × Delaware	5	0
Yufu × Muscat of Alexandria	2	0
Yufu × Rizamat	5	1
Yufu × Rosario Bianco	7	2
Yufu × Sekirei	21	1
Total	113	5

ened berries were randomly harvested about 100 days post–anthesis or in early September. To estimate the difference of berry characteristics between GA_3–treated and non–treated clusters, the cluster weight, berry number per cluster, berry weight, number of fertilized large and unfertilized small seeds, berry cracking and soluble solid content were examined. The soluble solid content (sugar content) was examined with a refractometer for ten berries in each of the ten clusters and recorded the refractive index (Brix). The seed was divided into two categories, i.e., fertilized large seeds that contained endosperm and/or embryo or the traces of them, and unfertilized small seeds that were empty and less than one–half in length (Wakana *et al*., 2005; Sarikhani, 2007).

RESULTS AND DISCUSSION

Pedigree of 'Bea–Kei'

Based on the description of grape cultivars by Hedrick (1908), 'Bea–Kei' selected from 201 triploid hybrid grapes was classified into a so–called European–American hybrid grape, which has cytoplasm of *V. vinifera* cv. 'Black Hamburg' and nuclear DNA consisting of 11 parts of *V. vinifera*, 3 parts of *V. labrusca* and 2 parts of *V. lincecumii* (Fig. 1).

Characteristics of trees

The characteristics of 'Bea–Kei' trees in Sasaguri orchard of Kyushu University Farm locating in Fukuoka prefecture were summarized in Table 2. As has been reported in the other triploid cultivars (Yamane *et al*., 1993) and plants (Wakana *et al*., 2007), triploid 'Bea–Kei' trees showed very higher vigor than 'Kyoho' and 'Muscat Bailey A'. The shoots grew very vigorously along with vigorous secondary shoot growth and the growth continued by the end of rainy season in July. Because of its very high vigor, it was often observed that one compound bud of 'Bea–Kei' produced more than three shoots. All buds of 'Bea–Kei' steadily produced shoots

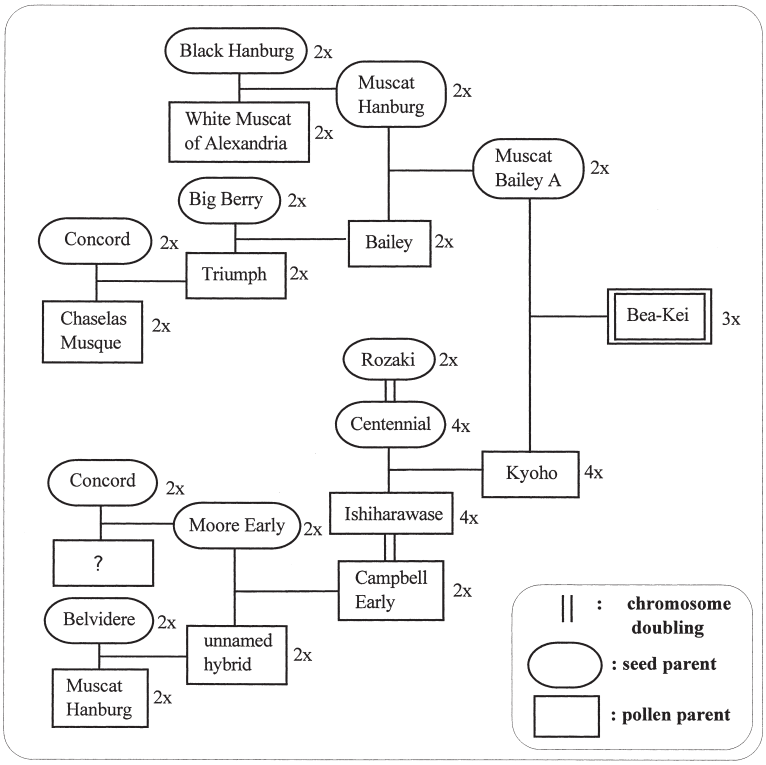

Fig. 1. Pedigree of triploid seedless grape 'Bea–Kei'.

Table 2. Characteristics of 'Bea–Kei', 'Kyoho' and 'Muscat Bailey A' trees

Cultivar	Tree age	Rootstock	Tree vigor	Canopy	Shoot length	Cane color
Bea–Kei [a]	6	Own roots	Very vigorous	Very wide	Very long	Reddish brown
Bea–Kei [b]	6	Teleki 5BB	Very vigorous	Very wide	Very long	Reddish brown
Bea–Kei [b]	6	Teleki 5BB	Very vigorous	Very wide	Very long	Reddish brown
Bea–Kei [c]	6	Hybrid Fran	Very vigorous	Very wide	Very long	Reddish brown
Kyoho [b]	6	Hybrid Fran	Vigorous	Wide	Long	Dark brown
Muscat Bailey A [b]	6	Hybrid Fran	Medium	Wide	Long	Reddish brown

[a] A mother tree growing in a side–netted plastic film house; [b] Trees growing in a side–netted plastic film house; [c] Trees growing under protection plastic film cover from rain in open field.

with one to three flower clusters irrespective of their position on the cane. The flower cluster of 'Bea–Kei' often had a large accessory cluster in the first and second clusters counted from the base of shoot, but had a small secondary cluster in the third clusters (Fig. 2). The flowers of 'Bea–Kei' were hermaphrodite as observed in its parents, and flowered almost simultaneously when its parents did. The pollen of 'Bea–Kei' was very highly sterile: none of 3000 pollen grains germinated on the agar medium of pollen germination test. The leaves were large–sized and three–lobed.

'Bea–Key' trees showed relatively high disease resistance to powdery mildew and botrytis, but relatively low resistance to anthracnose (bird's eye rot) and downy mildew in comparison with vinifera cultivars and European–American hybrid cultivars. Thus, it is recommended that the trees are cultivated under plastic film to protect them from rain.

Characteristics of berries

In all flower clusters, fruit set occurred and resulted in the formation of a great number of small berries and a few large berries (Table 3). The small berry formation resulted from stimulative parthenocarpy with highly sterile pollen of 'Bea–Kei', since unpollinated flowers dropped within 2weeks after anthesis. The large seeded berries rarely produced with the rate of one berry per five clusters. The fruit clusters of 'Bea–Kei' were large, shouldered and set fruits loosely. When flower cluster thinning and trimming were made, the fruit cluster set fruits tightly. The berries were around 2 g, sherical, seedless and purple–black in skin color at ripening. Unfertilized small seed formation (Wakana *et al*., 2005) occurred very scarcely with the rate of less than five berries per cluster.

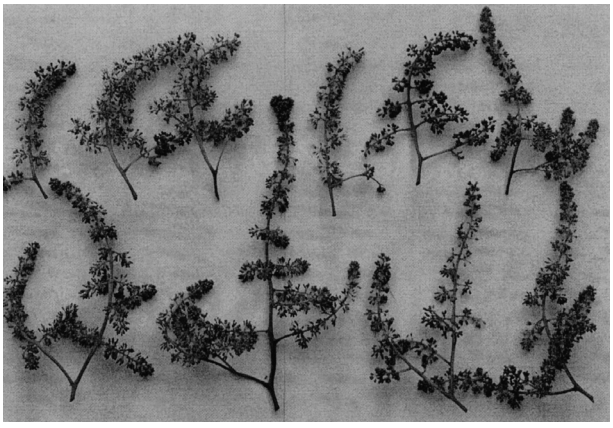

Fig. 2. Flower clusters of 'Bea–Kei' at anthesis.

Characteristics of GA_3–treated clusters

When the trimmed flower clusters of 'Bea–Kei' were immersed once with a solution of GA_3 at full bloom, the clusters and ovaries enlarged and reached 330–440 g and 8–11 g in cluster and berry weight respectively (Fig. 3 and Table 3). The berry shape was ovoid to cylindrical. The hardening of cluster axes and pedicles was not recognized, i.e., they were very flexible and elastic. The purple–black–colored fruit skin was medium in thickness and showed the tendency to adhere to the flesh to some extent: a little bit difficult to peel by finger press. The flesh texture was meaty; this character was seemed to be suitable for processing. The Brix value (sugar content) ranged from 19.1 to 20.3%, suggesting there is little year–to–year variation and the sugar content is as high as that of 'Kyoho'. Although the titratable acidity was not examined in the juice, the content is seemed to be medium by tasting.

Prospect

Triploid grape 'Bea–Kei' derived from 'Muscat Bailey A' × 'Kyoho' has superior characteristics for tree vigor, productivity, berry characters, disease resistance and labor saving in comparison with 'Kyoho' and 'Muscat

Table 3. Characteristics of berries treated with 100 ppm GA_3 at full bloom in triploid hybrid 'Bea–Kei' derived from a 'Muscat Bailey A' × 'Kyoho' cross

year	No. of clusters examined	Mean weight of berry clusters (g)[a]	Mean No. of berries	Mean weight of berries	Brix	No. of seeded berries per cluster	No. of large seeds per cluster	No. of small seeds per cluster
Non–treated clusters								
1994	10	43.1	38.6	1.4	–	4.1	0	4.7
1995	10	113.9	58.0	2.1[b]	–	2.2	0.5	1.7
1996	10	17.3	9.0	1.5[c]	–	0.8	0.8	0
GA_3–treated clusters								
1994	10 [d]	358.6	44.1	8.5	19.8	0	0	0
1995	10 [d]	539.0	54.4	10.7	19.1	0	0	0
1996	10 [d]	328.6	43.0	7.8	20.3	0	0	0
2002	10 [d]	375.0	42.1	9.1	19.3	0	0	0
2002	15 [e]	438.3	47.2	9.3	20.1	0	0	0

[a] Seedless berries without fertilized seeds. [b] The weight of berries with large seeds ranged from 4.2–10.2 g with the average of 7.5 g. [c] The weight of berries with large seeds ranged from 3.4–6.2 g with the average of 4.4 g. [d] Clusters were harvested from a tree with own roots. [e] Clusters were harvested from a tree grafted on a Teleki5BB root stock.

Fig. 3. Fruits of 'Bea–Kei' grape cultivar at the end of August just before full ripening.
a: Clusters were treated with 100 ppm GA_3 solution at full bloom. b: Typical example of 400 g clusters with about 40 berries each.

Bailey A'. Although the cluster thinning and trimming are necessary, berry thinning is not, i.e., after the appropriate cluster thinning and trimming they produced desirable clusters with desirable number of berries of equal size autonomously. The present result suggest that 70 flowers per cluster is the best trimming for the production of 350–400 g berry clusters consisting of about 40 berries with high quality. 'Bea–Kei' is recommended to cultivate under film and is adaptable to the area where 'Kyoho' and 'Muscat Bailey A' are possible to cultivate.

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