Studies on Seedlessness of 'Kyoho' Grapes Induced by Gibberellin in Combination with Streptomycin

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Abstract

Studies were carried out to examine the practical use of GA_3 (20 ppm) with and without Streptomycin (SM 200-2000 ppm) for production of seedless 'Kyoho' grapes when applied at anthesis.

1. The percentages of seedless berry set were almost 100% by GA application at anthesis whether or not SM was added, and the later the application, the lower the percentages. The GA treated clusters with 2000 ppm SM produced almost 100 % seedless berry set even when applied 6 days after anthesis.
2. When the culsters were treated 4 days after anthesis seedless berry set was lowered in order of medium and large berries. This tendency was more true on weak terminals than vigorous ones. However, SM prevented from reduction of seedlessness on weak terminals.
3. Protection effect to physiological drop of berries was more pronounced in the GA treated clusters with SM than in those without SM.
4. The percentage of LL size berry obtained from the thinned cluster at harvest was greater when GA alone was applied later, and further enhanced by the 2nd application; it was 15.9 % with two applications applied 4 days after anthesis and later, and was 24.2 % with them 6 days after anthesis and later. On the other hand the addition of SM to GA solution resulted in the percentages of 0 – 3.7 % regardless of timing and number of application times, which demonstrated that this antibiotic markedly inhibited the berry growth.
5. Coloring of any treated berries was more hastened than control ones, and harvesting time of the former was about 10 days earlier than the latter.
6. Hardening of the treated fruit stalks was moderate, but berry loosening occurred when the clusters were formed in a light berry set type.
7. No cracking nor drought-spot like symptoms were observed in the seedless berries.

From these results further studies are necessary to establish certain practices to produce large size berries and to control berry loosening by examining appropriate concentration SM added to GA solution, mixed application with other chemicals and number of berries to remain attached to the cluster.

Introduction

'Kyoho' is a tetraploid grape brought about in our country, and one of the best cultivars popular in large berry size and its high flesh quality. This cultivar is characteristic of occurring severe flower shatter and seedless shot berries. Since the berries do not turn their color satisfactory as influenced by higher night temperatures before maturity, it has been thought to be a difficult cultivar to grow it under ordinary cultural conditions. On the other hand as 'Kyoho' has a nature of easily inducing "stenospermocarpy" – seedless berry set[1], the time has come to establish certain measures to obtain GA induced seedless 'Kyoho' grapes on a commercial basis. Studies conducted by Kishi et al[2], Yasunobu[3], Shiba[4] and others would greatly contribute to this accomplishment.

However, such practices as to induce seedless 'Kyoho' grapes by GA application result in problems of hardening of fruit stalks, berry looseing, lighter weighed and/or cracking berries, etc., which must be corrected for marketing quality. Above all, hardening of the fruit stalks and berry loosening have been serious problems to prevent this practice from commercial use. There are some control measures for them, which are to reduce the times of GA application (single application), the use of low GA solutions and cluster forming in a dense berry set type, etc. These measures should be carried out at the expense of berry size to some extent, thus losing popularity as a large size berry cultivar.

Recently, Ogasawara[5] by using streptomycin (SM), and Shimizu[6] by Kanamycin reported that seedless grapes could be induced. These antibiotics do not harden the fruit stem, but do not affect increasing the berry size either. Consequently, it is advisable to use them together with GA for a successful seedless 'Kyoho' production. Ogasawara suggested to apply SM to 'Kyoho' just prior to anthesis[5], and when SM was applied to weak terminals of 'Muscat Bailey A', it was more efficient in producing seedless berries at higher rates in contrast to GA application. Thus, it is possible to eliminate unstable seedless berry production due to different terminal vigor by the application of mix solution containing GA and SM[7]. Shiba showed that set percentage of seedless 'Kyoho' berries was greater in vigorous vines than moderate and weak vines when GA was applied to 'Kyoho' grapes in full bloom in combination with low concentration of PCPA (4-chlorophenoxy acetic acid)[4].

Based on these reports a preliminary experiment was carried out in 1986 to observe whether low GA would be efficient in producing seedless 'Kyoho' grapes and increasing their berry size when applied in combination with SM. Since some promising results were obtained from this experiment, further experiments were carried out to establish in respect to timing of the application and optimal concentration of the solution in 1987.

Materials and Methods

Three vines of 20-yr-old 'Kyoho' grape on Hybrid Franc growing in University farm, College of Agriculture, University of Osaka Prefecture were used for this experiment. Such practices as pruning, fertilization, pest control, etc. were carried out conventionally.

In 1987, blooming time of this cultivar was May 28, and reached full bloom on May 30. Forming of the clusters and thinning berries were practiced on June 13 (14 days after anthesis), only medium size berries remaining attached confining to 30-40 berries per cluster.

Both GA and SM applied were those for commercial use; GA_3 for grapes and SM for shot hole disease. The application days of each treatment and the concentration of the mix soulution were shown in Table 1. The First treatment was done on any day of May 28, at anthesis; June 1, 4 days after anthesis and June 3, 6 days after anthesis, and 2nd application was done to all the treated clusters on June 10. The GA Concentration for 1st treatment was 20 ppm, and it was combined with 200, 400 or 2000 ppm of SM, while that for 2nd treatment was 10 ppm without addition of SM. The severity of flower shatter and set percentage of the seedless berries observed prior to thinning berries. After the grapes were harvested during the period from August 12 to 16, set percentage of seedless berries, berry weight, number of set berries, skin color, soluble solid and acid content (%) were determined.

Table 1. Design of experiment for inducing seedless 'Kyoho' grapes by GA in combination with SM.

Treatment		1st treatment			2nd treatment	
GA conc	SM conc	At anth 5/28	4 days 6/1	6 days 6/3	Trt 6/10	Cont
20 ppm	0	Yes	Yes	Yes	Yes*	Yes
20	200	Yes	Yes	Yes		
20	400	Yes	Yes	Yes		
20	2000	No	No	Yes		

Remarks : 1. The concentration of surfactant was not adjusted by the different treatments
2. Yes = Treatment practiced,
No = Treatment not practiced,
Yes*= 10 ppm GA.
3. Liquid form of 20 % SM (Streptomycin) was used for 1st treatment, and GA (gibberellin) alone was applied for 2nd treatment.

Results

1. *Number of set berries and set percentage of seedless berries after flower shatter as influenced by the vigorousness of fruiting - terminals*

The data obtained from GA treatments in combination with different SM concentrations were shown in Table 2.

The percentage of berry set per cluster in each treatment was satisfactory, and flower shatter was very light. When berry density was expressed by the berry number per unit length of fruit stalk, the value on weak control terminals was 3.4, that on vigorous terminals treated by GA at anthesis 3.6 and those on other terminals were more than 4.9, the highest on being 8.5. Generally, berry set seemed to be more dense on vigorous terminals than weak terminals. Higher berry density, 5.1 even on vigorous control terminals was probably due to fine and mild weather during blooming period. However, the ratio of smaller berries set was higher by GA application as observed preliminary experiment. This was especially true on the clusters treated at anthesis, and shot berry set was also higher on them.

Seedless berry set was nearly 100 % or 100 % on the clusters of any terminals when GA with and without SM were applied at anthesis. However this percentage became lower in medium and especially large size berries when they were applied 4 days after anthesis. This tendency was more obvious on the vigorous terminals than weak ones (Fig. 1).

Seedless fruit set was improved by the addition of SM to the GA solution, and the effect was more pronounced at 400 ppm than 200 ppm. This tendency was also naturally observed in the previous experiment.

Table 2. Number of set berries and percentage of seedless berries on 'Kyoho' grapes after physiological drop.

Treatment			No. of set berris					No. of s.b	Seedless berry set (%)			
			L	M	S	Tot.	per cm of f.s		L	M	S	Ave.
Control		VT	15	14	41	70	5.1	3	0.0	15.3	39.8	32.6
		MVT	23	28	7	58	5.1	0	0.0	0.0	5.6	1.6
		WT	18	21	2	41	3.4	0	0.0	0.0	2.5	1.5
At anthesis	GA + SM 0 ppm	VT	5	25	28	58	3.6	13	75.8	100.0	100.0	98.3
		WT	8	44	24	76	5.7	22	100.0	88.6	100.0	93.4
	GA + SM 200 ppm	VT	10	24	33	67	5.0	18	100.0	100.0	100.0	100.0
		WT	–	–	–	–	–	–	–	–	–	–
	GA + SM 400 ppm	VT	18	38	24	80	8.5	16	100.0	100.0	100.0	100.0
		WT	6	35	21	62	4.9	18	100.0	100.0	100.0	100.0
4 days after anthesis	GA + SM 0 ppm	VT	29	28	13	70	5.7	7	72.2	97.5	100.0	87.4
		WT	29	15	11	55	5.0	8	5.8	63.9	100.0	47.1
	GA + SM 200 ppm	VT	13	29	27	69	7.2	14	78.9	86.4	92.7	87.5
		WT	17	29	13	59	6.2	8	41.3	73.0	94.6	70.3
	GA + SM 400 ppm	VT	28	30	12	70	7.0	6	90.9	100.0	100.0	96.3
		WT	15	27	11	53	5.9	17	65.9	84.9	100.0	83.3

Remarks :
1. Observation was done on June 15 before thinning (17 days after anthesis).
2. Average of 5 clusters.
3. L ; more than 13 mm in diam.
   M; 10 - 13 mm in diam.
   S ; less than 10 mm in diam.
4. Values of fruit stalk were the length from the highest branch to the lowest one attaching to the stalk.
5. f.s = fruit stal, s.b = shot berries.
6. VT = Vigorous termianls, MVT = Moderately vigorous terminals, WT = Weak terminals

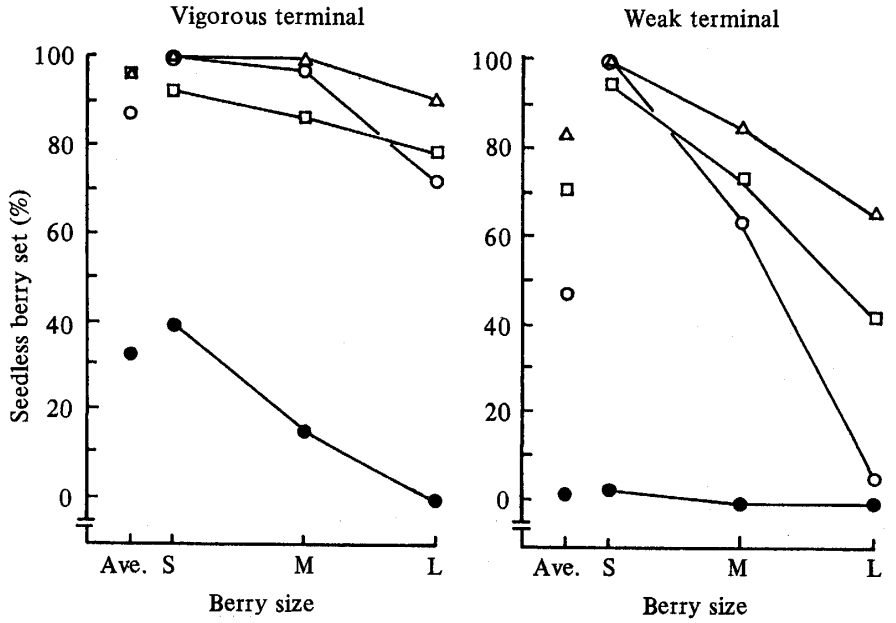

Fig. 1. The relationship between vigorousness of terminals and seedless 'Kyoho' berry set when GA solution with and without SM were applied 4 days after anthesis. ● ; Control, ○ : GA, □ : GA + SM (200 ppm), △ : GA + SM (400 ppm).

2. *Percentage of seedless berry set, berry weight, berry color, sugars and acids of the harvested grapes*

The cluster formed and thinned after petal fall was examined at harvest and the result was shown in Fig. 2-4.

As shown in Fig. 2, without any exception GA application with SM at 200 ppm and 400 ppm at anthesis induced 100 % of seedless berry set, and even that without SM was high at 94 %. The percentage became lower when GA was applied 4 and 6 days after anthesis and fell in ranges of 82.3 - 86.3 (%) with SM and 71.3 - 75.4 (%) without SM.

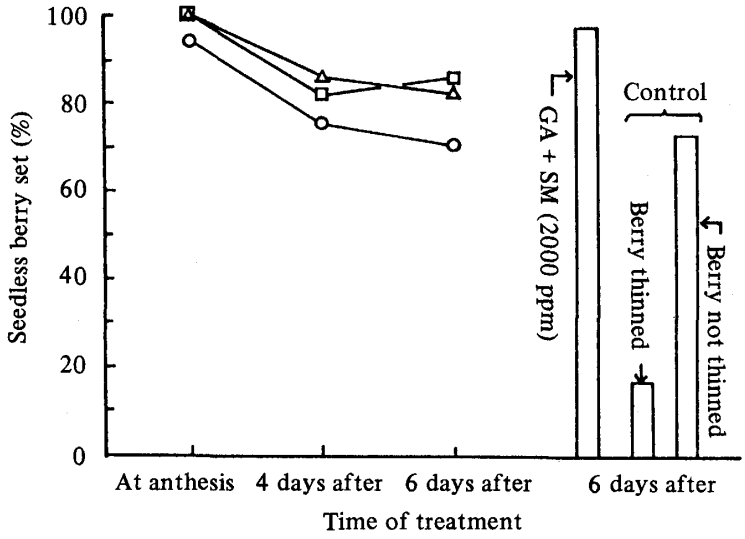

Fig. 2. Seedless berry set of 'Kyoho' grapes at harvest.
○ : GA, □ : GA + SM (200 ppm), △ : GA + SM (400 ppm).

High percentages of berry set at harvest as compared to those after petal fall were attributable to the fact that larger berries suspected as containing seed were thinned long before harvest. GA solution containing 2000 ppm SM was effective in producting 100 % seedless berry set even when applied 6 days after anthesis. Since most of shot berries observed after petal fall naturally abscised thereafter, there remained little problem for harvested grapes.

As data showing berry enlargement varied so greatly and difficult to compare among treatments only average values were shown in Table 3 and Fig. 3.

The size of berry treated with GA alone was larger when applied 4 and 6 days after anthesis than applied at anthesis, and LL size berries were increased when 2nd application was done on June 10.

GA application with 200 - 400 ppm SM also produced higher rates of L size berries, however LL size berries were hardly induced by the single application (×1). The effect of GA application with 2000 ppm SM was similar to that with 400 ppm SM when applied on the same days, and produced only 0.8 % L size berries even when 2nd application was done. In addition such a high rate of SM (2000 ppm) caused etiolation of berries, and it took about 3 weeks before full recovery, and such berries seemed to cease their growth during etiolation. This disorder confined to only berries, but not appearing in the leaves.

Thus GA application with SM increased set percentage of seedless berries but decreased berry size as compared to those treated with GA alone. From these results SM

Table 3. The ratios graded by berry size and the percentages of seedless berry set in each grade.

	Trt*		Berry size (%)				Seedless berry set (%)				
			LL	L	M	S	LL	L	M	S	Per cluster
GA-0	0d	×1	0.6	27.6	60.6	11.2	0.0	64.8	100.0	100.0	94.7
		×2	2.5	36.3	57.2	4.0	50.0	85.0	100.0	100.0	93.3
	4d	×1	6.2	48.8	43.5	1.5	0.0	66.8	100.0	100.0	80.5
		×2	15.9	50.9	31.7	1.5	6.3	67.8	98.5	100.0	70.3
	6d	×1	14.0	59.0	27.0	0.0	18.1	75.1	100.0	–	80.3
		×2	24.2	52.0	23.8	0.0	27.6	65.3	96.9	–	62.2
GA-1	0d	×1	0.0	35.1	61.0	3.9	–	100.0	100.0	100.0	100.0
		×2	0.0	26.0	70.4	3.6	–	100.0	100.0	100.0	100.0
	4d	×1	0.0	28.4	70.4	1.2	–	66.2	89.7	100.0	83.0
		×2	0.0	22.7	73.7	3.6	–	62.3	89.0	100.0	81.5
	6d	×1	1.0	69.7	29.3	0.0	50.0	82.3	96.9	–	85.9
		×2	2.5	62.7	34.1	0.7	66.7	73.4	93.0	100.0	85.6
GA-2	0d	×1	0.0	10.0	87.8	2.2	–	100.0	100.0	100.0	100.0
		×2	0.8	37.6	61.7	0.0	100.0	100.0	100.0	–	100.0
	4d	×1	0.0	40.7	58.7	0.6	–	76.3	99.4	100.0	93.7
		×2	3.7	73.2	23.1	0.0	21.0	73.9	93.2	–	78.6
	6d	×1	0.0	32.1	61.7	6.2	–	64.7	92.1	100.0	81.1
		×2	1.1	55.1	43.2	0.6	0.0	74.2	96.7	100.0	83.6
GA-3	6d	×1	0.0	38.1	57.0	5.0	–	95.3	100.0	100.0	97.5
		×2	0.8	49.1	42.0	8.1	0.0	94.7	100.0	100.0	96.9
Control		Th	35.3	45.0	7.8	11.9	1.2	0.0	40.0	95.8	16.8
		N-Th	17.8	6.2	11.6	64.4	4.6	7.2	80.0	99.0	74.0

* Treatment, ×1 = Single application, ×2 = 2 applications**, 0d = Treatment at anthesis, 4d = Treatment 4 days after anthesis, 6d = Treatment 6 days after anthesis, GA-0 = GA (20 ppm) + SM (0 ppm), GA-1 = GA (20 ppm) + SM (200 ppm), GA-2 = GA (20 ppm) + SM (400 ppm), GA-3 = GA (20 ppm) + SM (2000 ppm). Th = Berry thinned, N-Th = Berry not thinned. ** GA (10 ppm) alone was used for 2nd application.

itself might have some influence to inhibit grape berry growth.

When changes in berry weight per cluster were compared among treatments, they showed similar trend to those in berry size (Fig. 3). The weight of the berries treated with GA alone was greatest, and the later the day of application, the greater the weight. The 2nd application naturally increased the weight, however, the addition to SM produced less weighed berries, and 2nd application did not improve the weight as observed in berry size.

The relationships between terminal vigorousness and berry weight were observed in different groups of terminals having berry set higher than 95% and lower than 90 % (Fig. 4). In the former group average berry weight was about 7 g regardless of terminal vigorousness whether or not SM was added to the GA solution at the rate of 200 - 2000 ppm, if the 2nd application was omitted (×1), whereas it was 7.4 - 8.0 g if the 2nd one was carried (×2). In the latter group berry weight of the cluster treated with GA alone was 9.5 - 10.0 g, and it became lower when SM was added to the solution (8.0 - 8.6 g). Weak terminals carried greater weighed berries when GA alone was applied, while

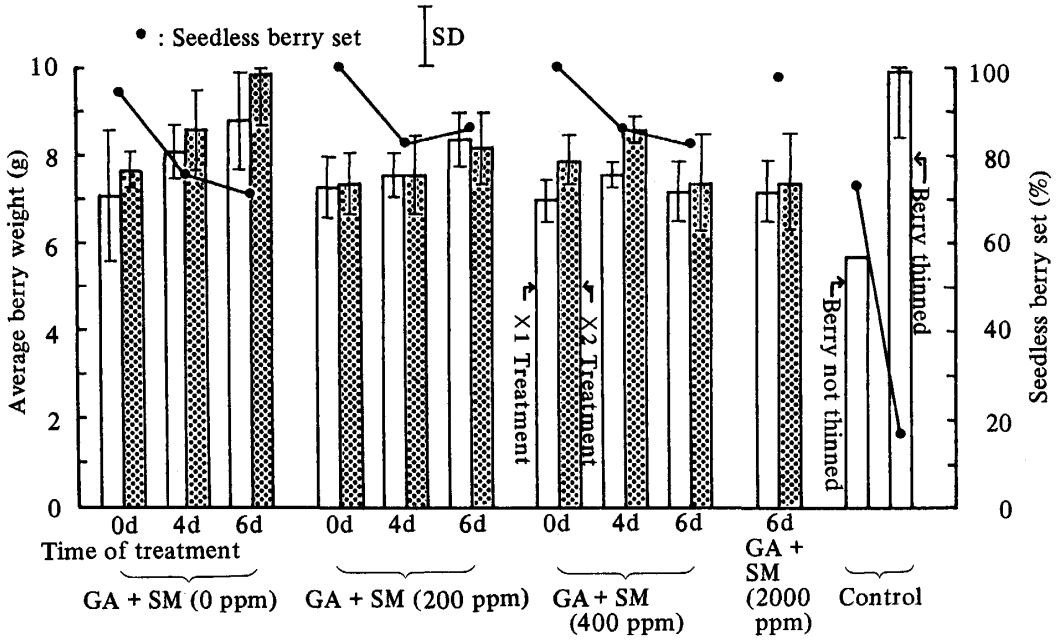

Fig. 3 The Percentage of seedless 'Kyoho' berry set and the average berry weight induced by GA application with and without SM.
SD = Standard deviation, 0d = At anthesis, 4d = 4 days after anthesis, 6d = 6 days after anthesis

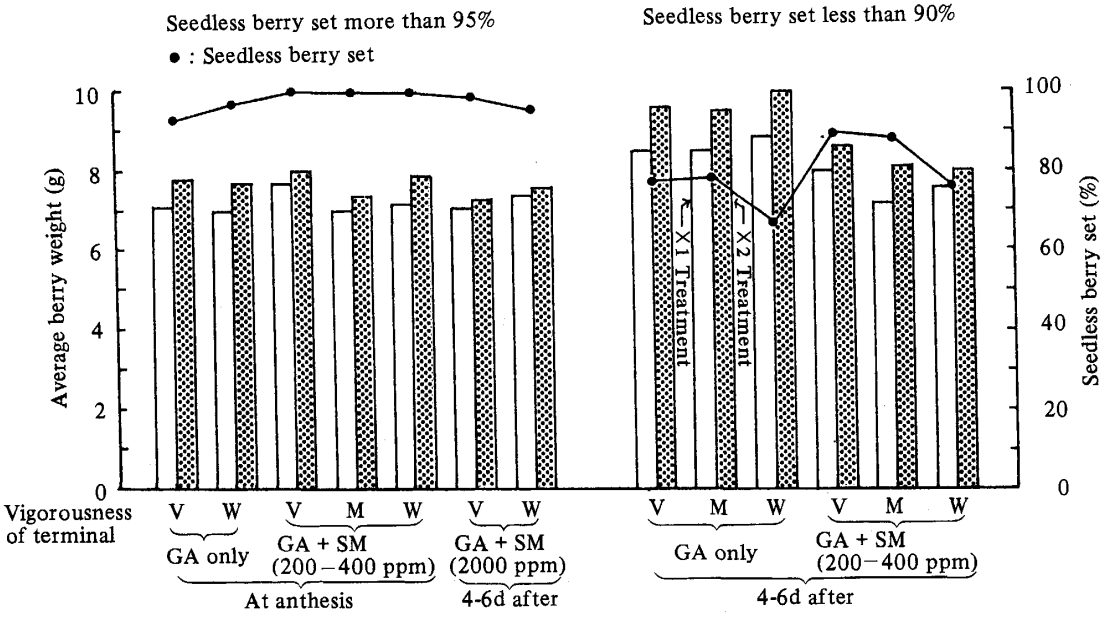

Fig. 4 The effect of vigorousness of terminals on the percentage of seedless 'Kyoho' berry set and the average berry weight when GA was applied in combination with SM.
V = Vigorous, M = Moderately vigorous, W = Weak, 4-6d after = 4-6 days after anthesis.

vigorous terminas carried them when GA was applied in combination with SM.

As previously mentioned, SM proved to have a reverse effect in respect to increasing berry size. In this study larger size berries were thinned after physiological drop to prevent from including seeded berries. This might be one of the reasons why many less-weighed smaller size berries occurred in those treatments.

Coloring of berries hastened by GA application regardless of presence of SM. Coloring of the treated berries started on July 8, while that of control berries of large and medium size did not turn the color even on July 21. Coloring seemed to be somewhat enhanced in the GA treated cluster with 2000 ppm SM. These treated grapes were ready to be harvested on August 10, while the seeded control grapes are usually harvested at the end of August in our University farm. Thus, GA treatment with and without SM would enhance harvesting time for more than 10 days. Average soluble solid content in the berries treated with GA regardless of SM was 17.7 and slightly lower than control. While average acid content was 0.6 and slightly higher than control (Table is not included in the text). Although hardening of fruit stalk due to GA application was moderate, berry loosening was severe when over ripened. No symptom of cracking nor drought-like berries were observed in the harvested grapes treated with GA regardless presence of SM.

From these data obtained, though it must be true that GA_3 application in combination with SM would disturb the normal berry growth and result in hardening of fruit stalks and berry loosening to some extent, it surely has a satisfactory effect in inducing seedless berries at higher rates with good coloring and no cracking. Therefore, GA solution containing SM would be one of very promising practices to induce seedless 'Kyoho' grapes with commercial values, if timing of its application and appropriate concentrations of GA and SM to increase berry size sufficiently are thoroughly studied.

Discussion

Two studies have been reported to be efficient in preventing GA treated seedless 'Kyoho' grapes from hardening of their fruit stalks and berry looseing. The first one is by Yasunobu's work[3], showing that single application of GA solution at 100 ppm in full bloom in contrast to two applications of it before and after bloom was able to prevent from berry loosening if the cluster was thinned to 70 berries forming a dense berry set type. In this practice 100 % seedless berry set would be expected, but the berry weight should not be 7 g at most.

The second one is the work done by Shiba[4]. He was able to induce 100 % seedless 'Kyoho' berry set by two applications of GA solution in a range of 10-25 ppm with PCPA before and after bloom. This practice seemed not only to lessen hardening of fruit stalk but also to bring the berry weight up to 9-10 g. Further improvement of hardening in fruit stalk was possible if berry set was confined to lower part of the cluster. Even in such a case berry loosening and drought-likeberries as well would occur if the cluster was formed in a light berry set type. Shiba noticed that light flower shatter would ocurr if the application was done in full bloom, and seedless berry set fell in a range of 70 - 80 % when moderate or less vigorous vines were treated. Therefore, it is practical to apply GA solution before or after bloom in combination with 10 - 25 ppm PCPA, which would increase the berry size to a satisfactory extent.

The following advantages in respect to the practice of GA application with SM at

anthesis were confirmed in our experiment; 1) the addition of SM to GA solution definitely improved berry set, especially on those weak terminals, 2) 100 % seedless berry set was obtained by the application at anthesis, 3) flower shatter and hardening of fruit stalks were reduced, 4) coloring being characteristic of this cultivar occurred smoothly. In contrast to these advantages, there were some disadvantages as follows; seedless berry weight did not go over 8.0 - 8.6 g even two GA applications, the addition of SM to the GA solution apparently caused to inhibit berry growth and berry loosening occurred when the clusters were formed in a light berry set type. In fact, the percentage of LL size berries obtained was very low when GA was applied to the clusters in combination with SM as compared to when GA alone was applied. The percentage of LL size berries obtained in the clusters receiving the application 4-6 days after bloom and additional one (×2), which were lower in seedless berry set, 15.9 - 24.2 % for GA alone and 0 - 3.7 % for GA with 200 - 400 ppm of SM.

The increase in seedless berry set by the addition of SM to GA solution would probably be due to inhibitory effect of SM to seed growth and development. While this antibiotic might bring about inhibitory effect for ovary growth, cell division and enlargement in ovary wall tissues.

Concerning the induction of seedlessness by SM, Ogasawara suggested that principal action of SM should be to cause ovule abnormality and thus some different mechanism to cause seedlessness by GA based on the fact that SM carries sterilizing power against bacteria (inhibitory action of protein synthesis). Since GA is effective in producing seedless berry set on vigorous vines, while SM is so on moderate and less vigorous ones, the application of GA in combination with SM regulates the inconsistency of seedlessness due to vine vigorousness[5, 7].

In this experiment the application of GA with SM at anthesis was able to induce 100 % seedless 'Kyoho' grape berries and to prevent from flower shatter as well. However, these is no doubt that SM carries inhibitory action for berry growth. Therefore, further studies are necessary to establish certain practices to produce large size seedless 'Kyoho' berries by examining appropriate concentration of SM to be added to GA solution and mix use of such a chemical to improve berry growth as PCPA.

Acknowledgments

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