Rachis Lignification and Seedless Berry Production in Seeded Grape Cultivar "Kyoho" as Influenced by Gibberellin and Quercetin

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Abstract

Rachis lignification and seedless berry production in seeded grape cultivar "Kyoho" as influenced by gibberellin and quercetin. M. NAKAMURA, E. TAKAHASHI and T. NODA, Faculty of Horticulture, Chiba University, Matsudo, Japan. *Tech. Bull. Fac. Hort. Chiba Univ.*, No. 22 : 7—12 1974.

PAL (phenylalanine ammonia-lyase) activity in Kyoho rachises and tendrils was increased by GA_{4+7} or GA_3 treatment with the concentration of 100 ppm, resulting in an increase of lignin cortent. GA treatment with quercetin resulted in a slight decrease in lignin content. Cellulose and hemicellulose contents in Kyoho rachises were also increased by GA treatment. GA applied twice more increased cluster weight and berry size, accompanying more increase in rachis weight, than those produced when GA treated once. GA produced approximately 100% seedless berries.

In a previous paper (TAKAHASHI et al., 1973), we reported that GA_{4+7} at 100 ppm was effective for the prevention of the shattering in Kyoho grapes but GA-induced hardening of the rachises caused mature berries to be liable to drop at the harvesting time. ITAKURA et al. (1965) observed that rachises of "Campbell Early" were thickened and hardened by GA treatment, and by means of histological observation found that the hardening was due to the lignification of the rachises of "Campbell Early". YASUNOBU (1972) reported that GA_3 promoted the lignification of Kyoho grapes markedly, though GA_{4+7} did a little, and that there was a close relation between lignification and hardening of the rachises.

The purpose of the experiments reported here was to study the effects of GA and quercetin on lignification of rachis related to fruit drop at harvest time and on seedless berry production in Kyoho grapes.

Materials and Methods

Three mature vines of *Vitis vinifera* (*V. Labrusca V. vinifera*) "Kyoho" grown in Chiba University vineyard at Matsudo were used. Two vines (A and B) were pruned moderately. Vine A was vigorous and vine B was not. Vine C was vigorous and pruned heavily. Two clusters per shoot were retained, and berry thinning was practiced in the clusters which overset berries. Gibberellin (GA_{4+7}) and quercetin were provided by Kyowa Hakko Kogyo Co., Ltd. and Wako Pure Chemical Industries, Ltd., respectively. GA_{4+7} contains about 30% of GA_4 and 70% of GA_7.

Field Experiments. Vines A and B were sprayed with GA_{4+7} at 100 ppm (GA_{4+7}), GA_{4+7} at 100 ppm + quercetin at 100 ppm (GA_{4+7}+Q) and GA_3 at 100 ppm + quercetin at 100 ppm (GA_3+Q), using Aerol OP at 100 ppm as a wetting agent. Each treatment above was applied to these clusters once (3 days after full bloom) and twice (3 and 21 days after full bloom). Clusters from the non-sprayed sides of the same vines were used for comparison. In vine C, GA_{4+7} at 100 ppm was sprayed to the clusters 3 days after full bloom, followed by application of GA_{4+7} at 100 ppm+quercetin at 100 ppm 21 days after full bloom. GA_{4+7} at 100 ppm+quercetin at 100 ppm was sprayed twice to the other clusters of vine C, 3 and 21 days after full bloom. At harvesting time (August 23), cluster weight, the number of berries per cluster, rachis weight and berry size were determined.

Phenylalanine ammonia-lyase (PAL) Assay. Tendrils and leaves of Kyoho grapes were used for the determination of PAL activity. GA_3 was sprayed at the concentration of 100 ppm to the shoots of Kyoho grapes, and after 4 days, tendrils and leaves of the same age were sampled together with control, and then stored in a freezer at −25°C.

Following the procedure of NEAL and KELLER (1970), crude enzyme was extracted from acetone powder which was prepared from 20 g of small pieces of the tissues of frozen tendrils and leaves. 2 g of the acetone powder were extracted with 23 *ml* 0.025 M phosphate (Na^+) buffer, pH 7.2 for 15 min with slow stirring. The suspension was centrifuged at 22,000 *g* for 12 min at 0°C and the supernatant was filtered through gauze.

PAL activity was assayed by measuring the amount of *trans*-cinnamic acid produced. The standard reaction mixture (5.0 *ml*) contained L-phenylalanine (10.0 μmoles), tris-HCl buffer, pH 8.55 (100 μmoles), 2-mercaptoethanol (10.0 μmoles) and the enzyme. A blank was employed in which phenylalanine was absent until the reaction had been ended. The reaction mixture was incubated for 60 min at 37°C in centrifuge tubes, without agitation. The reaction was terminated by the addition of 0.4 *ml* of 1.0 N trichloroacetic acid, and the volume adjusted to 6.0 *ml* with water. After standing at 37°C for 15 min the tubes were centrifuged for 20 min at 28,000 *g* to remove the precipitated protein. The supernatants were then decanted and retained and the cinnamic acid produced was assayed by measuring absorption at 280 nm against a control in which phenylalanine was not added until after the reaction was terminated. A unit of phenylalanine ammonia-lyase is defined as the amount of enzyme catalyzing the synthesis of 1.0 μM trans-cinnamic acid per min under the conditions employed. Protein concentration was determined by the procedure of LOWRY *et al.* (1951), using a bovine serum albumin standard.

Cell Wall Substance Analysis. Rachises stored at −25°C after the weight measurements and tendrils used for enzyme assay were used for the cell wall substance analysis. Lignin, cellulose and hemicellulose contents of the rachises and tendrils were determined by the methods of MACHIDA and TASHIRO (1968).

Results

Effect of GA and Quercetin on Lignin, Cellulose and Hemicellulose. GA increased lignin content (dry weight basis) about 2-fold and lignin (g) per rachis about 6-fold in Kyoho rachises compared with control (Table 1). As shown in Table 1, quercetin had a little effect on the repression of GA-induced lignin increase of Kyoho rachises. Cellulose and hemicellulose contents in GA-treated rachises were higher than control. Especially, the cellulose and hemicellulose contents in GA_3-treated rachises and tendrils were richer than those treated with GA_{4+7}.

Effect of GA on PAL Activity of Rachises, Tendrils and Leaves. The result of PAL extracted from rachises, tendrils and leaves in Kyoho grapes is shown in Fig. 1 and Table 2. In rachises (Fig. 1), GA_{4+7} applied twice with the concentration of 100 ppm induced rapid increase of PAL activity after June 21 until July 1 and maintained the high PAL activity until after

Table 1. Effect of GA treatments with quercetin on lignin, cellulose and hemicellulose contents of Kyoho rachis

Treatment	Lignin per dry wt (%)	Lignin per rachis (g)	Cellu. per dry wt (%)	Cellu. per rachis (g)	Hemi. per dry wt (%)	Hemi. per rachis (g)
GA_{4+7} (1)***	58.1	1.47	11.1	0.28	18.1	0.46
GA_{4+7} (2)	60.2	2.23	17.6	0.65	17.4	0.64
GA_{4+7}+Q**(1)	55.8	1.37	12.7	0.32	19.2	0.48
GA_{4+7}+Q (2)	58.5	1.68	13.9	0.40	22.5	0.64
GA_3+Q (1)	35.5	1.73	16.8	0.82	21.9	1.06
GA_3+Q (2)	64.0	3.27	17.8	0.91	25.3	1.29
Control	25.0	0.25	10.9	0.11	14.8	0.15
Treated tendrils* (GA_3)	20.1	—	21.3	—	27.5	—
Untreated tendrils*	12.1	—	12.1	—	19.8	—

* Used for enzyme assay.
* * Quercetin.
* * * Times of GA application.

Table 2. Effect of GA treatment on L-phenylalanine ammonia-lyase activity of Kyoho tendrils and leaves

Treatment	Total protein (mg)	Total m Units	mU/mg protein
K-treated tendrils (GA_3)	22.88	16.25	0.71
K-untreated tendrils	20.30	7.67	0.38
K-treated leaves (GA_3)	16.80	6.69	0.40
K-untreated leaves	13.97	4.60	0.33

K Kyoho.

July 30 compared to rapid decrease of that of control after July 1. The rapid increase of PAL activity occurred around June 21 in both GA-treated and control rachises. On July 30, the level of PAL activity of rachises treated with GA was about 6 times as high as that of control. In tendrils (Table 2), which considered to be homologous organ of rachises, PAL activity was increased about 2-fold by GA treatment. In leaves, it was also increased. As shown in Table 2, protein concentrations in tendrils and leaves were increased by GA treatment.

Effect of GA on Cluster Weight and Number of Berries per Cluster. GA increased the cluster weight and the number of berries per cluster by about 2-fold, respectively, and GA applied twice made clusters larger than those applied once (Table 3). A comparison of cluster weight produced by GA treatment among vines A, B and C suggests that when GA is applied vines or shoots of which vegetative growth is vigorous can produce larger clusters without shattering than those produced in weak vines or shoots.

Effect of GA and Quercetin on Rachis and Berry Weights and Seedlessness. Rachis weight of GA-treated cluster increased 2- or 3-fold compared with that of control (Table 4). Rachis weight of GA_{4+7}+quercetin-treated clusters decreased by about 1 g (average) compared with that of GA_{4+7} −treated clusters. GA applied once and twice increased the seedless berries weight

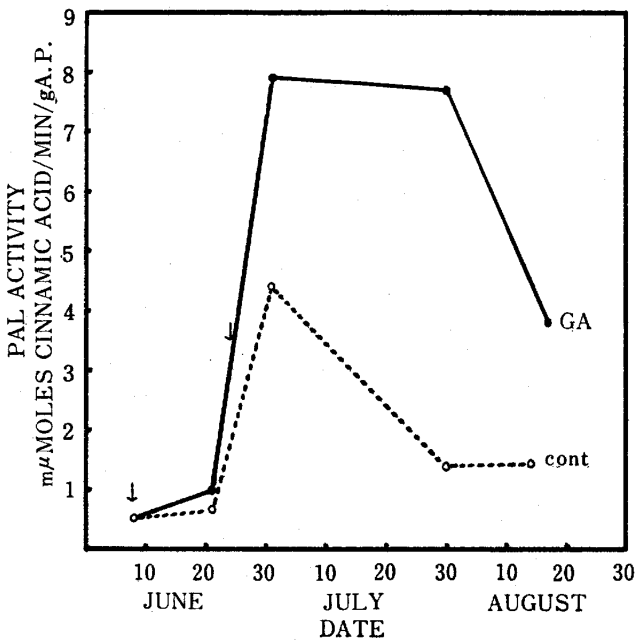

Fig. 1. Effect of GA treatment on phenylalanine ammonia-lyase activity of Kyoho rachises. ↓ designates times when GA_{4+7} at 100 ppm was applied. Full bloom was on June 5. Crude enzyme was extracted from acetone powder with borate buffer, pH 8. 8.

Effect of GA on Berry Size and Shape. Both length and width of berry were enlarged by GA applied twice about 1 to 2 mm (average) than GA applied once (Table 5). The growth rate of length was greater than that of width in the berries treated with GA as well as in the seeded berries of control. The berry shape of the clusters treated twice with GA approached that of control (seeded). The rate of length / width increased in the berries treated twice with GA rather than in the berries treated once with GA.

Discussion

It has been reported that GA application to "Kyoho", seeded cultivar, causes the rachises hardening resulting in fruit drop at harvest (KISHI *et al.*, 1962; YASUNOBU, 1972), and this hardening and thickening of rachises would be attributed to the increase of lignin in rachises by GA treatment (YASUNOBU, 1972). In this work, inc-

Table 3. Effect of GA treatments on cluster weight and number of berries of Kyoho grapes

Treatment	Cluster wt g				No. of berries per cluster			
	A**	B	C	Ave. (A, B)	A	B	C	Ave. (A, B)
GA_{4+7} (1)	308.8*	208.0	—	258.4	44	24	—	34
GA_{4+7} (2)	479.4	247.0	—	363.2	59	29	—	44
GA_{4+7}+Q (1)	342.6	284 4	—	313.5	51	37	—	44
GA_{4+7}+Q (2)	440.0	217.1	557.5	328.6	53	27	66	40
GA_3+Q (1)	373.0	262.1	—	317.6	55	35	—	45
GA_3+Q (2)	398.1	302.2	—	350.2	46	30	—	38
GA_{4+7}+(GA_3+Q) (2)	—	—	388.6	—	—	—	37	—
Control	158.7	174.0	—	166.4	21	24	—	23

* Average of 5 to 12 clusters.
* * A, B and C refer to GA-treated vines with different vigour.

about 1 and 3 g respectively compared with 5. 5 g (seedless) of control (Table 4). However, the berry weight increased by GA applications did not reach 10. 6 g (seeded) of control. By GA applications, vines produced over 99 % seedless berries (Table 4).

rease of lignin, together with cellulose and hemicellulose increases in Kyoho rachises was observed with applications of GA_3 and GA_{4+7} (Table 1). This finding confirms that GA increases cell wall constituents, such as lignin, cellulose and hemicellulose, as reported in peas

Table 4. Effect of GA treatments with quercetin on rachis and berry weights and seedless berries production of Kyoho grapes

	Rachis wt g				Berry wt g				% of seedless berries*
Treatment	A	B	C	Ave. (A, B)	A	B	C	Ave. (A, B)	Ave. (A, B)
GA_{4+7} (1)	11.5**	8.6	—	10.1	7.1***	7.4	—	7.3	99.7
GA_{4+7} (2)	13.7	8.0	—	10.9	9.2	8.2	—	8.7	99.7
GA_{4+7}+Q (1)	10.3	7.2	—	8.8	8.0	7.3	—	7.7	99.9
GA_{4+7}+Q (2)	10.7	8.4	16.8	9.6	9.2	7.6	9.0	8.4	99.8
GA_3+Q (1)	17.5	12.0	—	14.8	8.1	6.9	—	7.5	100.0
GA_3+Q (2)	18.8	14.6	—	16.7	8.7	9.4	—	9.1	99.8
GA_{4+7}+(GA_3+Q) (2)	—	—	11.3	—	—	—	9.7	—	—
Control	5.5	5.2	—	5.4					70.2
Seeded					10.8	10.3	—	10.6	
Seedless					5.0	6.0	—	5.5	

* Calculated by 100·(total berries-seeded berries)/total berries in plot.
* * Average of 5 to 12 clusters.
* * * Average of 10 berries.

Table 5. Effect of GA treatments on berry size

	Berry size (mm)								Len./wid.
	Length				Width				
Treatment	A	B	C	Ave. (A, B)	A	B	C	Ave. (A, B)	Ave. (A, B)
GA_{4+7} (1)	22.6*	23.8	—	23.2	21.9	22.7	—	22.3	1.04
GA_{4+7} (2)	25.8	25.2	—	25.5	23.7	23.4	—	23.6	1.08
GA_{4+7}+Q (1)	24.0	22.9	—	23.5	23.0	22.4	—	22.7	1.04
GA_{4+7}+Q (2)	25.6	23.3	25.1	24.5	23.9	22.6	24.1	23.3	1.05
GA_3+Q (1)	23.8	23.5	—	23.7	22.9	22.2	—	22.6	1.05
GA_3+Q (2)	24.7	25.6	—	25.2	23.0	24.4	—	23.7	1.06
GA_{4+7}+(GA_3+Q) (2)	—	—	26.1	—	—	—	24.8	—	—
Cont. Seeded	27.0	27.2	—	27.1	25.2	25.3	—	25.3	1.07
Seedless	18.9	21.1	—	20.0	20.2	21.5	—	20.9	0.96

* Average of 10 berries.

by McComb (1966). The increases of these substances, especially lignin, in rachis may mechanically induce fruit drop at harvest or after harvest.

Higuchi (1966) indicated that in bamboo shoots PAL (L-phenylalanine ammonia-lyase) and TAL (L-tyrosine ammonia-lyase) were synthesized progressively just before the lignification of the bamboos and by the enzymes, L-phenylalanine and L-tyrosine were deaminated to cinnamic acid derivatives and incorporated into lignin. As found in the bamboo lignification, in this study, the results of Fig. 1, Table 2 and Table 1 cleary indicates that PAL activity in Kyoho rachises and tendrils was increased by GA treatment, resulting in an increase of lignin content. A similar result was reported by Cheng and Marsh (1968) with dwarf peas. Furthermore, from the result of Fig. 1 it may be found that in rachises rapid PAL synthesis or activation occurred around June 21 and second GA application on June 24 more stimulated the increase of PAL activity and the duration of this high PAL activity led to following lignification of rachises.

It is known that PAL is the key enzyme between shikimic acid pathway and pathways leading to lignins, coumarins, flavonoids, etc. in dicotyledonous plants (NEISH, 1964;1965;1968). NEAL and KELLER (1970) reported that the PAL activity was inhibited by quercetin, one of the flavonols, *in vitro* experiment. This fact was evidenced by the results obtained in this work that there was a slight decrease in lignin content of rachises in Kyoho grapes when quercetin was applied with GA. However, as reported by KISHI (1970), quercetin effect was too small to prevent severe hardening of rachises causing fruit drop at harvest or after harvest.

In GA applications in grapes to induce seedless berry development or to prevent young fruit shattering, it must be noticed that the time of GA application is important to obtain the best results depending on the cultivars used. From this study with Kyoho grapes, it appears that later application of GA before shattering may be effective to prevent the shattering and increase fruit set.

It was found that size of GA-induced seedless berries was larger than shot berries of control but slightly smaller than seeded berries of control. This result may be due to the function of exogenous GA as "sink" for the deposition of translocated carbohydrates, as well as endogenous GA in seed, as demonstrated by WEAVER et al. (1969).

Literature cited

1) CHENG, C. K.-C. and H. V. MARSH, JR. 1968: Plant Physiol., **43**: 1755
2) HIGUCHI, T. 1966: Agr. Biol. Chem., **30**(7): 667
3) ITAKURA, T., K. OZAKI and H. MACHIDA 1965: Bull. Hort. Res. Sta., Japan, Ser. A, No. **4**: 67
4) KISHI, T., M. TAZAKI and T. AMEMIYA 1962: Bulletin of the Yamanashi prefectural Agricultural Experiment Station, **7**: 16
5) KISHI, T. 1973: Studies on the Utilization of Gibberellin in Grapes, 67
6) LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL 1951: J. Biol. Chem., **193**: 265
7) MACHIDA, H. and T. TASHIRO 1968: Bull. Hort. Res. Sta., Japan, Ser. A., No. **4**: 93
8) McCOMB, A. J. 1966: Ann. Bot., **30**: 155
9) NEAL, D. O'. and C. J. KELLER 1970: Phytochem., **9**: 1373
10) NEISH, A. C. 1964: Biochemistry of Phenolic Compounds, ed. by J. B. HARBORNE, Academic Press, p. 295
11) ―――――― 1965: Plant Biochemistry, ed. by J. BONNER and J. E. VARNERE, Academic Press, p. 601
12) ―――――― 1968: Constitution and Biosynthesis of Lignin, ed. by K. FREUDENBERG and A. C. NEISH, Springer-Verlag, p. 30
13) TAKAHASHI, E., T. KIKITSU, M. NAKAMURA and K. NAGASAWA 1973: Tech. Bull. Fac. Hort Chiba Univ., **21**: 19
14) WEAVER, R. J., W. SHINDY and W. M. KLIEWER 1969: Plant Physiol., **44**: 183
15) YASUNOBU, Y. 1972: Rakuyō Kaju Kankei Seiiku Chōsetsuzai Shiken Seiseki Shuroku: 138

摘　要

ブドウ巨峰の果梗の木化と無核果作出におよぼすジベレリンおよびケルセチンの影響

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巨峰の果梗と巻きひげにおける PAL (phenylalanine ammonia-lyase) 活性は GA_{4+7}, GA_3 100 ppm の処理によって増加し，その結果リグニン含量が増加した．ケルセチンを伴ったGA処理はリグニン量の減少にわずかな効果を示した．果梗中のセルロース，ヘミセルロースもGA 処理によって増加した．

GA の2回処理によって，1回処理よりも果房重，果粒径が更に増加し，果梗重もまた2～3倍に増加した．GA 処理区はほぼ 100%の無核果率であった．