[Original Paper]

Relationship between Grape Ovule Formation and Ethylene

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Experiments were conducted to clarify the relationship between ovule differentiation in carpel and ethylene in 'Kyoho' (*Vitis*×*labruscana* Bailey×*V. vinifera* L.) and 'Takao' (a chance seedling of 'Kyoho') grape cultivars. In 'Kyoho', 67% of carpels had two ovules per carpel at 4 weeks before full bloom (WBFB) and this value increased to 98% at full bloom (FB). In 'Takao', the ovule number per carpel was mostly two at 4 WBFB, but increased from 3 WBFB to FB and 30.0% of the carpels had ≥ 3 ovules in a carpel at FB. 'Takao' ovules at FB were classified into primary and secondary ovules according to differentiation time. At FB, the width of the secondary ovule was almost the same as that of the primary ovule, but its length was smaller. 2-Chloroethylphosphonic acid (ethephon, CEPA) at 50 mg・L^{-1}, 1-aminocyclopropane-1-carboxylic acid (ACC) at 1,000 mg・L^{-1}, and aminoethoxyvinylglycine (AVG) at 500 mg・L^{-1} were used to treat flower clusters of both cultivars at 4, 3, and 2 WBFB. Then, the variations in ovule number in a carpel were recorded one week after FB. In untreated 'Kyoho' ovaries, 3.0% of carpels had ≥ 3 ovules per carpel. The percentage ranged from 0.5 to 2.9% in the case of 'Kyoho' ovaries treated with CEPA or ACC. AVG treatment significantly increased the percentage of carpels containing ≥ 3 ovules in a carpel from 6.4 to 8.9%. In untreated 'Takao' ovaries, 40.5% of carpels had ≥ 3 ovules per carpel. The percentage of carpels containing ≥ 3 ovules per carpel following CEPA and ACC treatment decreased significantly to 14.8 and 22.3% at 3 WBFB, while AVG treatment increased the percentage of carpels containing ≥ 3 ovules to 48.8% at 3 WBFB. The results show that ovule differentiation in grape carpel may be regulated by ethylene produced in the ovary.

Key words: 1-aminocyclopropane-1-carboxylic acid (ACC), aminoethoxyvinylglycine (AVG), carpel, 2-chloroethylphosphonic acid (ethephon, CEPA), ovule differentiation, Kyoho, Takao

Introduction

'Kyoho' (*Vitis*×*labruscana* Bailey×*V. vinifera* L.) has a tendency to abscise excessively florets and berries shortly after anthesis under an open culture in Japan. Komatsu (9) suggested that poor-berry setting in 'Kyoho' could not be attributed only to abnormal ovules, and that the development of embryo sacs at anthesis might be related to berry settings. On the other hand, 'Takao' (a chance seedling of 'Kyoho') is a seedless cultivar. Ashikawa (2) suggested that the seedlessness in 'Takao' probably depended upon the defective development of reproductive organs. Yamane et al. (13) showed that 'Takao' was a hypotetraploid (2n=4x-1=75), and suggested that the ploidy level might be associated with the defective development of female reproductive organs in 'Takao'.

Grape ovary is generally composed of two carpels originating from four ovules. However, Horiuchi (6) and Horiuchi et al. (7) reported that the number of carpels in an ovary and the number of ovules in a carpel differed among grape cultivars. For example, many grape cultivars including 'Kyoho' are the two-carpel, two-ovule/carpel type, while 'Takao', a two-carpel, polyovule/carpel type, has more than two ovules in a carpel. In contrast, 'Muscat Bailey A' and

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Revised manuscript received Oct. 6, 2009.

'July Muscat' are the polycarpel, two-ovule/carpel type.

There are numerous reports of the growth-modulating role of ethylene in in vitro cultures, and some studies have shown that it inhibited shoot regeneration (4, 5). Hu et al. (8) reported that the treatment of 'Kyoho' flower clusters at 1 to 3 weeks before full bloom (WBFB) with 50 to 300 mg・L^{-1} aminoethoxyvinylglycine (AVG) increased the rates of berry set and seeded berries, and this phenomenon was dependent on the increase in ovule number per carpel. It is well known that AVG is an inhibitor of 1-aminocyclopropane-1-carboxylic acid (ACC) synthesis in the ethylene biosynthesis pathway (3, 11) and can thus suppress ethylene production in many climacteric fruits (15). 2-Chloroethylphosphonic acid (ethephon, CEPA) gradually releases ethylene as a decomposition product in plant tissue and shows the same effect as ethylene gas (14). The objectives of this study were to observe the morphological differentiation and development of ovules from 4 WBFB to full bloom (FB) and to clarify the relationship between ovule differentiation in a carpel and ethylene in 'Kyoho' and 'Takao'.

Materials and Methods

Plant materials

The experiments were performed in 2001 and 2002. Eighteen-year-old 'Kyoho' grapevines growing in the Horticulture Farm of Chiba University, Chiba and 25-year-old 'Takao' grapevines growing in a commercial vineyard at Higashimurayama, Tokyo were used for the experiments.

Morphological observation

In 2001, ten inflorescences (flower clusters) on the shoots of both cultivars were collected at one-week intervals from 4 WBFB to FB. The florets taken from the inflorescences were fixed with 70% ethanol (EtOH) and stored. Then, they were dehydrated with EtOH and n-butanol series and embedded in paraffin. Longitudinal or transverse sections (15 µm thick) of 200 ovaries (20 ovaries per cluster) were cut with a microtome and stained with hematoxylin solution. Observations of ovule number per carpel, ovule size, and ovule differentiation position were conducted under a light microscope.

Treatment of chemicals related to ethylene synthesis

In 2002, flower clusters of 'Kyoho' and 'Takao' cultivars were treated with 50 mg・L^{-1} CEPA (Nissan Chemical Industries, Ltd.), 1,000 mg・L^{-1} ACC (Sigma-Aldrich Co.), and 500 mg・L^{-1} AVG (Abbott Laboratories) at 4, 3, and 2 WBFB. All solutions contained 0.05% Approach BI (Kao Co.) as surfactant. Ten flower clusters from each treatment were collected one week after full bloom (WAFB), and florets taken from the inflorescences were fixed with 70% EtOH. The ovaries of 200 florets (20 florets per cluster) from each treatment were cut transversely with a razor. The number of ovules in a carpel was counted under a stereoscopic microscope and recorded.

Results and Discussion

In 'Kyoho' (two-carpel, two-ovule/carpel type), 33% of the carpels had one or no ovule per carpel at 4 WBFB (Fig.

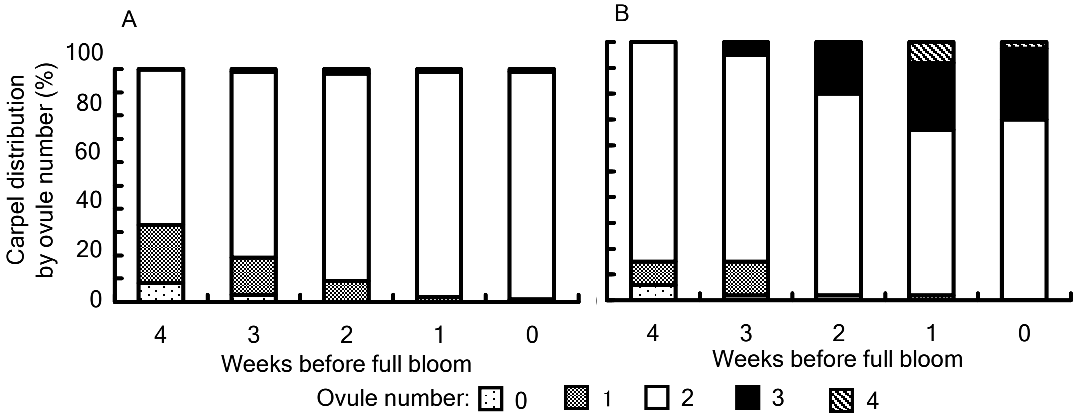

Fig. 1 Changes in number of ovules per carpel with ovary development in 'Kyoho' (A) and 'Takao' (B) grapes in 2001.

1A). However, the value decreased as the flowers approached FB, and 98% of the carpels had two ovules per carpel at FB. A very small percentage of carpels had three ovules per carpel. In 'Takao' (two-carpel, polyovule/carpel type), 15% of the carpels had one or no ovule per carpel at 4 WBFB (Fig. 1B). Although the value was lower than that in 'Kyoho', it decreased in a manner similar to 'Kyoho' thereafter. However, the percentage of carpels that had ≥ 3 ovules per carpel increased from 3 WBFB to FB, reaching 30% at FB. From these observations, ovules in the carpels at FB were classified into primary ovules that differentiated early on (at 4 WBFB) and secondary ovules that differentiated later. The results also showed that the differentiation and development of ovules in 'Kyoho' and 'Takao' cultivars occurred even at 4 or 3 WBFB. However, the differentiation of primary ovules in 'Kyoho' occurred later than that in 'Takao', and the differentiation ability of the secondary ovules in 'Takao' was higher than that in 'Kyoho'. In this respect, 'Takao' may be a special cultivar in terms of ovule differentiation.

In 'Takao', the longitudinal growth (length) of primary ovules in carpel differentiating around 4 WBFB continued at a constant rate until FB (Fig. 2). Meanwhile, the transverse growth (width) of primary ovules leveled off at 1 WBFB. On the other hand, the length of secondary ovules differentiating around 4 to 3 WBFB continued at a constant rate until FB, similar to the length of primary ovules. However, the length of the secondary ovules at FB was only

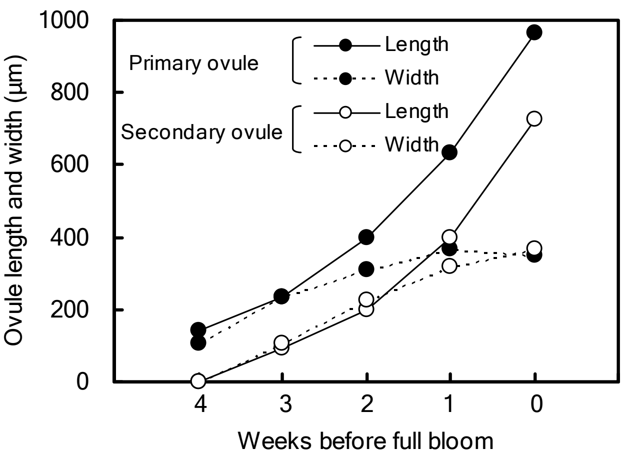

Fig. 2 Changes in longitudinal and transverse growth (length and width) of primary and secondary ovules of 'Takao' grape in 2001.

70% of that of the primary ovules at the same period. The widths of the primary and secondary ovules at FB were almost the same. Together, the results show that the secondary ovules might not have reached maturity at FB, and the period for differentiation of secondary 'Takao' ovules in carpels was 4 to 3 WBFB.

In untreated 'Kyoho' ovaries, 3.0% of carpels contained ≥ 3 ovules per carpel at 1 WAFB (Table 1). In 'Kyoho' ovaries treated with CEPA (50 $mg \cdot L^{-1}$) or ACC (1,000 $mg \cdot L^{-1}$), 0.5 to 2.9% of carpels contained ≥ 3 ovules per carpel. Treatment with AVG (500 $mg \cdot L^{-1}$) significantly increased the percentage of carpels containing ≥ 3 ovules per carpel , and the value ranged from 6.4 to 8.9%. CEPA and ACC treatment at 3 WBFB and 4 WBFB, respectively, decreased considerably the percentage of carpels containing ≥ 3 ovules per carpel. In untreated 'Takao' ovaries, 40.5% of carpels contained ≥ 3 ovules per carpel at 1 WAFB. Treatment with CEPA and ACC decreased significantly the percentage of carpels containing ≥ 3 ovules, and the value ranged from 14.8% to 33.9%. Treatment with CEPA and ACC at 3 WBFB effectively decreased the percentage of carpels containing ≥ 3 ovules. AVG treatment at 3 WBFB increased the percentage of carpels containing ≥ 3 ovules to 48.8%, while AVG treatment at 4 and 2 WBFB decreased the percentage to 35.8% and 37.1%, respectively (Table 1). In 'Takao', the percentage of carpels containing ≥ 3 ovules at around FB was different between 2001 and 2002. In 2001, 30% of the carpels contained ≥ 3 ovules per carpel at FB (Fig. 1B), while in 2002, the value increased to 40.5% at 1 WAFB (Table 1). This difference was probably due to the different climatic conditions during the growth period of florets before full bloom (BFB).

AVG is an inhibitor of pyridoxal phosphate-requiring enzymes including ACC synthase (1). Inhibition of ACC synthase activity usually results in decreased ethylene production. CEPA that is degraded in plant tissue releases ethylene near the degradation site. Released ethylene effects are similar to endogenous ethylene effects on fruit ripening, abscission, and other growth phenomena (12). Therefore, the results of AVG, ACC, and CEPA treatment suggest that ovule differentiation in a carpel may be regulated by ethylene content in grape ovary. Hu et al. (8) reported that

Table 1 Effects of CEPA, ACC, and AVG treatment on number of ovules in a carpel of 'Kyoho' and 'Takao' grapes at one week after full bloom in 2002.

	Treatment			Carpel distribution by ovule number (%)					
Cultivar	Chemical	Conc. (mg・L^{-1})	Time (WBFB^{z})	1≥	2	3	4	5≤	3≤
Kyoho	CEPA	50	4	2.5±0.2^{y}	95.5±0.1	2.0±0.1	0.0	0.0	2.0±0.1
			3	1.4±0.1	97.7±0.1	0.9±0.2	0.0	0.0	0.9±0.2
			2	1.0±0.1	96.1±0.1	2.9±0.1	0.0	0.0	2.9±0.1
	ACC	1000	4	0.0	99.5±0.3	0.5±0.1	0.0	0.0	0.5±0.1
			3	2.0±0.2	96.5±0.2	1.5±0.1	0.0	0.0	1.5±0.1
			2	0.0	97.6±0.1	2.4±0.1	0.0	0.0	2.4±0.1
	AVG	500	4	0.5±0.1	93.0±0.2	6.4±0.1	0.0	0.0	6.4±0.1
			3	1.5±0.1	90.6±0.1	7.9±0.1	0.0	0.0	7.9±0.1
			2	1.0±0.1	90.1±0.1	8.9±0.1	0.0	0.0	8.9±0.1
	Untreated			1.0±0.1	96.0±0.1	3.0±0.1	0.0	0.0	3.0±0.1
Takao	CEPA	50	4	1.5±0.1	64.8±0.2	31.7±0.1	2.0±0.1	0.0	33.7±0.1
			3	1.5±0.1	83.7±0.2	13.8±0.1	1.0±0.1	0.0	14.8±0.1
			2	2.5±0.1	65.4±0.1	29.1±0.1	3.0±0.4	0.0	32.1±0.2
	ACC	1000	4	5.1±0.2	61.0±0.1	32.9±0.1	1.0±0.2	0.0	33.9±0.1
			3	6.1±0.4	71.6±0.4	20.4±0.1	1.9±0.1	0.0	22.3±0.1
			2	1.5±0.1	71.8±0.2	25.2±0.2	1.5±0.2	0.0	27.0±0.2
	AVG	500	4	0.5±0.1	63.7±0.3	33.8±0.1	2.0±0.1	0.0	35.8±0.1
			3	0.0	51.3±0.2	43.8±0.2	4.9±0.3	0.0	48.8±0.2
			2	1.5±0.1	61.4±0.1	35.6±0.1	1.5±0.4	0.0	37.1±0.2
	Untreated			1.5±0.1	58.0±0.2	35.6±0.1	4.9±0.1	0.0	40.5±0.1

^{z} Weeks before full bloom. ^{y} Mean±S.E.

ACC content and ethylene evolution rate in AVG-treated florets of 'Kyoho' grapes were lower than untreated control. As ethylene production in 'Kyoho' ovary is high, differentiation of the secondary ovules is inhibited. On the other hand, the opposite is seen in 'Takao'. However, relationship between ovule formation and endogenous ethylene synthesis was unclear, though total ACC content, ethylene evolution rate, and ACC oxidase activity in AVG, ACC, and CEPA-treated florets of 'Kyoho' and 'Takao' were investigated in this experiment.

Hu et al. (8) reported that the treatment of 'Kyoho' flower clusters BFB with 50 to 300 mg・L^{-1} AVG increased the rates of berry set and seeded berries. They also reported that the amounts of water-soluble inhibitors of pollen tube growth in the pistils were decreased significantly after treatment with AVG, compared with the untreated control. These results might be related to the increase in ovule number per carpel. The reasons for the poor berry set of 'Kyoho' are predicted as follows: 1) there is a gap between fertilization timing and ovule growth promotion, and 2) the increase in the amounts of water-soluble inhibitors of pollen tube growth in pistils (9, 10). AVG treatment also increased the number of seeds per berry (8). Therefore, the differentiation of secondary ovules maybe related to the improvement of poor berry set of 'Kyoho'.

Additional data are necessary to confirm these results, especially the relationship between ethylene content and the activities of ethylene-related enzymes, such as ACC synthase and ACC oxidase activities, for certain grape cultivars, including 'Kyoho' and 'Takao'.

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［研　究　報　文］

ブドウの胚珠形成とエチレンとの関係

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要　約

ブドウ‘巨峰’および‘高尾’を用いて、心皮内の胚珠分化とエチレンとの関係を明らかにするために実験を行った。2001 年、‘巨峰’では 1 心皮当たり 2 個の胚珠の割合は満開 4 週前では 67%､満開期では 98%であった。一方、‘高尾’では 1 心皮当たりの胚珠数は満開 4 週前ではほとんど 2 個であったが、3 週前から満開期にかけて増加し、満開期では 1 心皮における 3 個以上の胚珠数の割合が 30.0%であった。‘高尾’の満開期の心皮では分化の時期の違いによる 1 次胚珠および 2 次胚珠が観察され、2 次胚珠の大きさを 1 次胚珠と比較すると、横径はほとんど同じであったが、縦径は小さかった。2002 年、50 mg･L^{-1} 濃度の 2-クロロエチルホスホン酸（エテホン、CEPA）、1,000 mg･L^{-1} 濃度の 1-アミノシクロプロパン-1-カルボン酸（ACC）および 500 mg･L^{-1} 濃度のアミノエトキシビニルグリシン（AVG）を満開 4 週前、3 週前および 2 週前に両品種の花穂にそれぞれ処理し、満開 1 週後の心皮における胚珠数を調査した。その結果、‘巨峰’では 1 心皮における 3 個以上の胚珠数の割合は無処理子房では 3.0%であり、処理時期により差異はあるものの CEPA および ACC 処理では 0.5-2.9%であったのに対して、AVG 処理は 6.4-8.9%まで有意に増加させた。‘高尾’では 1 心皮における 3 個以上の胚珠数の割合は無処理子房では 40.5%であったが、CEPA および ACC の満開 3 週前処理では、それぞれ 14.8%、22.3%と有意に減少したのに対して、AVG の満開 3 週前処理では 48.8%に増加させた。以上の結果から、ブドウの心皮における胚珠分化は子房内で生成されるエチレンにより制御されている可能性が示された。