J. ASEV Jpn., Vol. 31, No. 1, 31–40 (2020)

American Society for
Enology & Viticulture
JAPAN Chapter

【Research Note】

Aminoethoxyvinylglycine Combined with Indole-3-butyric Acid Stimulates Adventitious Root Formation of *Vitis kiusiana* Softwood Cuttings

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Effects of the exogenous application of aminoethoxyvinylglycine (AVG) in combination with indole-3-butyric acid (IBA) on the adventitious root formation of single node softwood cuttings were investigated in *Vitis kiusiana* grape (Kiusiana), a recalcitrant to root species. The pulse treatment of AVG after IBA treatment, in which 24 h 100 ppm IBA treatment followed by continuous or intermittent one time 24 h 5 ppm AVG treatments during 96 h after cuttings preparation, had no stimulative effect on the rooting, although the death of cuttings was inhibited by the pulse treatment. On the other hand, the combined treatment of AVG and IBA during night exhibited dramatic effect on the rooting of Kiusiana cutting. AVG of 5 ppm treatment in combination with 200 ppm IBA for 12 h during night increased the proportion of rooted cuttings per viable cuttings by 29 and 13 times more than that in the control and IBA alone, respectively. Callusing induced by IBA alone at the base of cuttings were inhibited by the addition of AVG. The basal end acute cut and the diagonally planting of Kiusiana soft wood cuttings had no effect on the adventitious root formation and the root growth affected by AVG treatment in combination with IBA.

Keywords: adventitious roots formation, aminoethoxyvinylglycine, indole-3-butyric acid, *Vitis kiusiana*

Introduction

Vitis kiusiana Momiyama (Kiusiana) is a dioecious wild grape that inhabits the southern region of Kyushu, Japan. Kiusiana is specified to be a critically endangered species in Red list of Japan (Ministry of the Environment of Japan, 2019). Not only for the preservation of Kiusiana but also for its future utilization as a grape resource, efficient propagation is needed for Kiusiana. Clonal propagation can multiply plants in short period of time and preserve true-to-type plants. In addition, clonal propagation can also multiply the superior individuals selected from natural populations. Propagation via stem cuttings is more efficient clonal propagation for woody plant compared with the other propagation, such as grafting and layering, for that matter of scale and technique. In general, grapes can be easily propagated by the cutting of stem, regardless of the softwood or hardwood (Weaver, 1976). However, Kiusiana is an exceptional grape whose cuttings of both types are recalcitrant to root (Mochioka et al., 2002).

Effective induction of adventitious root formation is essential for the success of propagation by cutting. Hormonal

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factor, as an endogenous factor, is crucial for the success of adventitious rooting, if the environment factors, such as temperature, light and moisture, are satisfied for the rooting. Among plant hormones, indole-3-acetic acid (IAA), the endogenous auxin in plants, plays essential role in the process of adventitious rooting of cuttings in most of plants (Pop et al., 2011). IAA originated from the leaves, growing apex and buds is basipetally transported through the polar auxin transport pathway and accumulated in the active site of cuttings, resulting in induction of adventitious roots formation at the site (Ford et al., 2002; Garrido et al., 2002; Sukumar et al., 2013). An increase of endogenous IAA prior to adventitious root formation was also confirmed in the cuttings of 'Muscat Bailey A' grape (Kawai, 1996). Exogenous auxin can also improve the adventitious root formation, and auxin, such as IBA, NAA (naphthalene acetic acid) and IAA, is practically used as a rooting stimulator in broad plant species including grapes (Smart et al., 2003).

Ethylene, an endogenous plant hormone that control morphogenesis and development of plants, is another hormonal factor in adventitious root formation. Ethylene can closely relate to auxin in adventitious root formation in cuttings; the synthesis of ethylene can be stimulated by auxin in cuttings (Batten and Mullins, 1978; Sun and Bussuk, 1993), on the other hand ethylene can inhibit the polar transport of IAA (Negi et al., 2010). The effect of ethylene on the adventitious rooting, unlike auxin, depends on the plant species and organs. Positive effect of ethylene on adventitious root formation reported in a variety of plant tissues: mung bean hypocotyl cuttings (Pan et al., 2002), kiwifruit stem cuttings (Argita et al., 2003), tomato hypocotyl (Negi et al., 2010) and petunia (Druege et al., 2014). In contrast, ethylene has been shown to inhibit adventitious root formation in the stem cuttings of pea (Nordstrom and Eliasson, 1993) and faba bean (Kharafalla and Hattori, 2000). In Vitis species, an increase in ethylene was found in 1 cm stem cuttings in vitro before adventitious root formation (Moncousin et al., 1989). On the other hand, the negative effect of ethylene on the adventitious root formation of grape cuttings was demonstrated by the application of 2-chloroethylphosphonic acid (Ethephon), an ethylene release agent, *in vitro* (Soulie et al., 1993).

In our previous work with hardwood cuttings of grape, higher level of IAA in the cuttings was found in Kiusiana than in 'Campbell Early', an easy to root cultivar, at the beginning of cutting (Shiozaki et al., 2013). An excessive increase in IAA levels in hardwood cuttings was also reported in 'Muscat Bailey A' grape, when the rooting of cuttings was inhibited by the application of cytokinin (Kawai, 1997). In grapes, the excess level of IAA in the cuttings might trigger an ethylene increase at an undesirable level and result in negative impact on the adventitious root formation, as pointed out in eucalypt cuttings (Kilkenny et al., 2012).

Aside from the hormonal factors, physical factors such as basal end cut methods and orientation of cuttings into the rooting medium can influence adventitious root formation. Various procedures of the basal end cut (horizontal, oblique, cut back, and 2 or 3 sides trimming) and planting (vertical, diagonal, and horizontal (embedding)) have been empirically employed for the efficient propagation in each plant and organ (Horie, 1963). These methods can optimize the environment of the basal end of cuttings, with respect to the aeration and temperature, for its adventitious root formation. The broad section area of cutting base seems to be advantageous for its water uptake. The oblique cut and cut back end of cutting base treated with IBA resulted in good adventitious root formation, in comparison with the horizontal cut end, in peach hardwood cuttings (Gemma et al., 1982). In our previous study, the oblique cut of the base of cuttings and its diagonal planting in the rooting medium revealed an efficacy in the rooting and root growth of hardwood single node cuttings of Kiusiana without IBA treatment (Murai and Shiozaki, 2008).

The purpose of this study is to establish the optimum treatment of cuttings for adventitious root formation in Kiusiana grape, a difficult grape to propagate by cutting. The effect of aminoethoxyvinylglycine (AVG), an ethylene synthesis inhibitor, on adventitious root formation induced by IBA was investigated by treating AVG separately or simultaneously with IBA. The additive effect of physical factors (oblique cut of the cutting base and diagonal planting) was also investigated in the cuttings under the hormonal control.

Materials and Methods

Grape materials and softwood cuttings preparation

This experiment was conducted in 2011 and 2016 by using three vines of *Vitis kiusiana* Momiyama (hedgerow training; 13 to >20 years-old, through 2011 to 2016) grown in a research field at Osaka Prefecture University. The uniformly developed current shoots were taken from the grapes in July of each experiment year and single-node softwood cuttings of 10 cm with an intact leaf were prepared from nodes 5 to12 (distal to proximal). The average size of cuttings used in each experiment is shown in Table 1.

Preparation of IBA and AVG solution

IBA was dissolved in 0.1 N NaOH and then diluted in distilled water to the final concentration after the pH adjusting to 7.0 with 0.1 N HCl. AVG was dissolved in distilled water and prepared at the concentration of 5 ppm by diluting in distilled water, after adjusting the pH at 7.0 with 0.01 N HCl. In the case of mixture of IBA and AVG, AVG was dissolved in a solution in which IBA was preliminary dissolved and adjusted the pH roughly around neutral, and then diluted in distilled water to the final concentration after the pH adjusting to 7.0.

Pulse treatment of AVG after IBA treatment

Cuttings were prepared as mentioned above at July 21, 2011. Treatment solution of IBA (100 ppm) and AVG (5 ppm) was prepared as the preparation of plant growth regulators, except using sterilize distilled water for their dilution, instead of distilled water. The treatment was conducted by dipping the base of each cutting in the 15 mL treatment solution contained in a 20 mL screw vial placed in a biotron (NK System Biotron, Nippon Medical & Chemical Instruments, Tokyo, Japan) controlled at 26°C, 85% relative humidity under a 16 h daylength. Cuttings were initially dipped in IBA for 24 h (day 1st), and then the cuttings were dipped in AVG for 24 h in day 2nd, 3rd or 4th. In non-treatment period, the cuttings were dipped in sterilize distilled water. The treated solution on the base of cuttings, except in the control, was wiped off with paper towel when the treatment was replaced. In the control, cuttings were dipped in new sterilize distilled water every 24 h during 96 h. In day 5th, cuttings were planted in watered vermiculite at ≥7 cm apart between each cutting, in 3 rows along the long side of a planting box (L 435 mm × W 320 mm × D 95 mm), and rooted in a mist house covered with black cheesecloth of 65% shading rate. Watering was conducted by intermittent mist operation for 1 min every 20 min from 6:00 AM to 9:00 PM.

Data on the rooting were collected at day 80 of planting. The cutting with no necrotic portion was considered as viable cuttings. All data on the rooting were collected on viable cuttings. Cuttings with root > 2 mm were classified as rooted cuttings, and those with the leaf were counted as cuttings with leaf. Root number was counted per a rooted cutting. The longest root length and total root fresh weight per a viable cutting was measured with a ruler and an electric balance (GF-200, A&D Company, Tokyo, Japan), respectively. Calli on the base of cuttings

Table 1. Soft wood cuttings size of *Vitis kiusiana* used in each experiment year.

Cuttings [z]		Basal end diameter of cutting [y]	Fresh weight of cutting [x]
experimental year	n	mm	g
2011	6	7.6 ± 0.4	5.8 ± 0.4
2016	26	7.1 ± 0.1	4.8 ± 0.2

Values are means ± SE.
[z] The cutting length was 10 cm.
[y] The average of minor and major diameter of cutting base.
[x] The data shows the fresh weight of cutting without the leaf.

were scraped off by using a scalpel and weighed with an electric balance (GF-200, A&D Company, Tokyo, Japan). Experiment was conducted in triplicate using 10 cuttings per the replication.

AVG + IBA dipping treatment and cutting preparation

The experiment was started at July 11, 2016. Softwood cuttings of Kiusiana were prepared at sunset in each experiment day. The effects of 200 ppm IBA, 5 ppm AVG and 5 ppm AVG + 200 ppm IBA on adventitious root formation were compared on the normal cuttings (horizontal cut end). In the experiment for the effect of basal end cut, acute cuttings, of which the basal portion along the bud was made acute ca 30° by using a grafting knife (Fig. 1.), were investigated by using the normal cuttings as the control. The basal end of cuttings was dipped in the 15 mL treatment solution in a 20 mL screw vial for 12 h at 25°C in a dark room. In the next morning (after 12 h treatment), cuttings were planted in rooting medium and rooted in the mist house as in the experiment of "Pulse treatment of AVG after IBA treatment". In the experiment for the basal end cut, the cuttings were dipped in AVG (5 ppm) + IBA (200 ppm) solution for 12 h and the treated cuttings were planted vertically or diagonally at an angle of 45° in the planting medium (Fig. 1). When the cuttings were planted diagonally, the leaves of cuttings were made to upward the adaxial side, and not to overlap one another as much as possible.

An experimental replication was conducted in one day and three replications were done in consecutive three days, in each experiment. Cuttings of 15 and 10 per the replication were respectively used for AVG + IBA treatment, and basal end cut and plant orientation. Each cutting was planted in watered vermiculite in a plastic polystyrene rooting pot (diameter: 105 mm, base diameter: 70 mm, depth: 95 mm) placed in a pot frame tray (TO Alphatray-20T, Takii, Kyoto). Management of cuttings, such as shading and watering, was conducted under the same condition of the experiment of 2011. Data were collected at day 55 and 50 of planting, respectively, for the experiment on the effect of IBA, AVG, and AVG + IBA and the experiment on the effect of basal end cut.

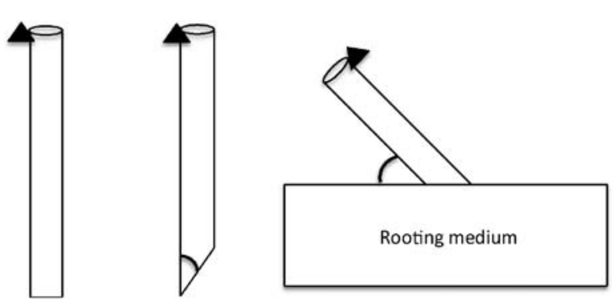

Fig. 1. Basal end preparation and putting orientation of cuttings. Left: Horizontal/Vertical, Middle: Acute/Vertical, Right: Acute/Diagonal Closed triangle shows bud.

Statistical analysis

All data was analyzed by ANOVA and the means were compared by Fisher's PLSD test at <5% using StatView 5.0 (SAS Institute Inc.). Percent data were arcsine transformed before analysis.

Results

Effect of pulse treatment of AVG after IBA treatment

The proportion of viable cuttings of Kiusiana softwood was 16.7% in the control (putting in sterilize distilled water during 96 h with changing the water every 24 h) and IBA (100 ppm) alone treatment (Table 2). The highest proportion of viable cuttings was found in the pulse treatment of 72-96 h AVG. There were no significant differences in the proportion of rooted cuttings per viable cuttings between the treatments. In the control, the root number of the only one rooted cutting was 2 and length of longest root was 20.5 cm. Average root number in pulse treatment of AVG after IBA treatment was ranging from 2 to 4.4 times greater than that in the control. The length of longest root in pulse treatment of AVG after IBA treatment (18.5 to 22.5 cm) was almost around that in the control. The average root number, length of longest root and average root weight in the three pulse treatments were not significantly different from one another. There was no significant difference in average callus weight between the treatments.

Table 2. Effect of 24 h pulse treatment of aminoethoxyvinylglycine (5 ppm) after indole-3-butylic acid (100 ppm) treatement on rooting of *Vitis kiusianan* softwood cuttings.

Treatment (h)		Viable cutting	Cutting with leaf	Rooted cutting	Average root number	Longest root length	Average root weight per cutting	Average callus weight per cutting
IBA	AVG	(%)	(%)	(%)	(n)	(cm)	(g)	(g)
—	—	16.7 ± 6.7 b[z]	44.4 ± 29.4 a	33.3 ± 33.3 a	2	20.5	0.6	1.0 ± 0.5 a
0–24	—	16.7 ± 6.7 b	100 a	44.4 ± 29.4 a	5	17.7	2.0	1.4 ± 0.8 a
0–24	24–48	23.3 ± 3.3 b	66.7 ± 33.3 a	88.9 ± 11.1 a	7.8 ± 2.8 a	18.5 ± 1.5 a	2.4 ± 0.3 a	0.6 ± 0.3 a
0–24	48–72	40.0 ± 11.5 ab	100 a	72.2 ± 14.7 a	4.1 ± 2.2 a	21.8 ± 1.9 a	1.6 ± 0.2 a	0.9 ± 0.3 a
0–24	72–96	56.7 ± 16.7 a	91.7 ± 8.3 a	43.5 ± 22.5 a	8.9 ± 3.2 a	22.5 ± 1.5 a	3.9 ± 0.3 a	0.9 ± 0.3 a

Data were collected at 80 days after planting.
Values are means of 3 replications ± SE (10 cuttings/one replication).
[z] Values with different letters in each column are significantly different by Fisher's PLSD test at <5%.

Table 3. Effect of dipping treatment of aminoethoxyvinylglycine (5 ppm) and indole-3-butyric acid (200 ppm) for 12 h at night on the rooting of *Vitis kiusiana* softwood cuttings.

Treatment	Viable cutting	Cutting with leaf	Rooted cutting	Average root number	Longest root length	Average root weight per cutting	Average callus weight per cutting
	(%)	(%)	(%)	(n)	(cm)	(g)	(g)
Cont.	73.3 ± 7.7 a[z]	83.2 ± 8.5 a	3.0 ± 3.0 b	0.7 ± 0.7 b	0.5 ± 0.5 b	0.7 ± 0.7 a	1.10 ± 0.10 ab
IBA	28.9 ± 2.2 b	85.0 ± 7.6 a	6.7 ± 6.7 b	1.0 ± 1.0 b	5.8 ± 5.8 ab	1.7 ± 1.7 a	1.20 ± 0.20 a
AVG	77.8 ± 8.0 a	97.6 ± 2.4 a	7.8 ± 4.2 b	0.8 ± 0.4 b	7.5 ± 4.1 ab	0.6 ± 0.5 a	0.82 ± 0.10 ab
AVG + IBA	77.8 ± 9.7 a	100 a	88.0 ± 7.2 a	6.7 ± 0.5 a	16.5 ± 0.5 a	3.2 ± 0.1 a	0.77 ± 0.10 b

Data were collected at 55 days after planting.
Values are means of 3 replications ± SE (15 cuttings/one replication).
[z] Values with different letters in each column are significantly different by Fisher's PLSD test at <5%.

Effect of AVG + IBA dipping treatment

AVG (5 ppm) alone treatment had no effect on the rooting of cuttings (Table 3). The proportion of viable cuttings of Kiusiana softwood significantly deceased in IBA (200 ppm) alone. AVG + IBA treatment increased the proportion of rooted cuttings per viable cuttings by 29 times greater than that of the control. The average root number was 6 to 9 times greater than the control and other treatments. Cuttings of AVG + IBA had the longest root in length of longest root, among the treatment. Average callus weight per cutting was heaviest in IBA alone and lightest in AVG + IBA. Figure 2 shows the representative cuttings in the experiment of AVG + IBA; development of fine roots as well as main roots was observed.

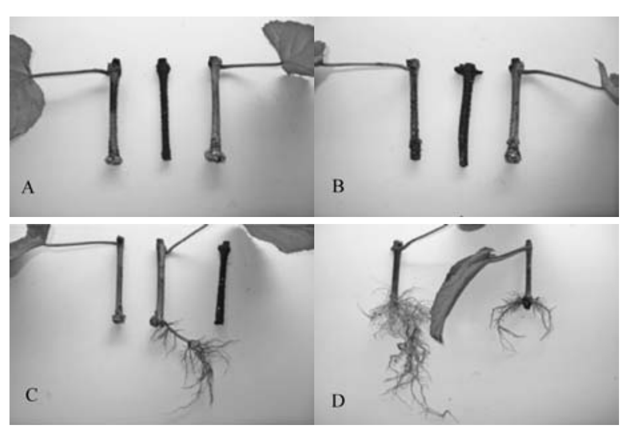

Fig. 2. Kiusiana softwood cuttings 55 days after planting (2016). A: Cont., B: IBA 200 ppm, C: AVG 5 ppm, D: AVG 5ppm + IBA 200 ppm

Table 4. Effects of the basal end cut angle of cuttings and its putting orientation into rooting medium on the rooting of *Vitis kiusiana* treated with aminoethoxyvinylglycine (5 ppm) + indole-3-butyric acid (200 ppm).

Cuttings treatment		Viable cutting	Cutting with leaf	Rooted cutting	Average root numbe	Longest root length	Average root weight per cutting	Average callus weight per cutting
Base shape	Orientation	(%)	(%)	(%)	(n)	(cm)	(g)	(g)
Horizonal	Vertical	60.0 ± 10.0 ns	100 ns	89.2 ± 5.8 ns	7.1 ± 0.4 ns	16.8 ± 0.9 ns	3.1 ± 0.3 ns	0.5 ± 0.3 ns
Acute	Vertical	60.0 ± 5.8	84.9 ± 8.3	81.9 ± 11.7	4.9 ± 0.11	4.5 ± 1.4	2.3 ± 0.3	0.8 ± 0.2
Acute	Diagonal	60.0 ± 5.8	83.8 ± 8.5	6.0 ± 2.2	17.8 ± 1.7	2.5 ± 0.5	0.8 ± 0.2	0.9 ± 0.3

Data were collected at 50 days after planting.
Values are means of 3 replications ± SE (10 cuttings/one replication).
[ns] Not significantly different by Fisher's PLSD test at <5%.

Combination effect of cutting preparation and AVG + IBA treatment

No marked differences, except of the percentage of viable cuttings, was found between the control (horizontal + vertical) in table 4 and AVG + IBA in table 3, which are essentially the same treatment, in two experiment of 2016. No additive effect of cutting base cut in acute angle and its putting orientation into rooting medium was found in the rooting of Kiusiana softwood cuttings induced by AVG + IBA treatment (Table 4).

Discussion

Non effectiveness IBA alone on the adventitious root formation of Kiusiana cuttings should be noted as the results in this line of experiments. This non-effectiveness of IBA alone was also reported in the hardwood cuttings of Kiusiana (Mochioka et al., 2002). In general, the rooting of grape vine cuttings can be improved by the application of IBA (Kracke and Cristoferi, 1983; Ohkawa et al., 2007); in the former, the rooting of grape cuttings was stimulated by the flash treatment of IBA at 4000 ppm, and the long time dipping treatment of IBA in the range of 10 to 100 ppm, in the latter. The response of Kiusiana cuttings to IBA for stimulation of adventitious root formation seems to be inherently, irrespective of the developmental and physiological differences of the cuttings (either softwood or hardwood cuttings), and is unique in grapes.

The inhibitory effect of Ethepon, ethylene release agent, on the viability and rooting of softwood cuttings of Kiusiana was found in our preliminary experiment (personal communication; March, 2010). Exogenous auxin including IBA can stimulate ethylene production in plant tissue including cuttings, as Sun and Bassuk (1993) reported. Thus, we hypothesized that the invalid effect of exogenous IBA in the adventitious root formation of Kiusiana cuttings is probably brought by the elevated ethylene induced by IBA, and the control of ethylene in the cuttings is the key to successful propagation of Kiusiana.

In the experiment of the pulse treatment of AVG (5 ppm) after IBA (100 ppm) treatment, the viability of control cuttings, which dipped in sterilize distilled water for 96 h, was extremely lower than that in the other experiment of this work (Table 2). However, the pulse treatment of AVG, especially in 72-96 h treatment, improved the viability of cuttings. In the experiment of July of 2009, water up-take of one node softwood cutting with a leaf for 24 h was 3.1 ± 0.2 mL (average of 20 cuttings) in Kiusiana and this was 40% less than that in ‘Campbell Early’, an easy to root species (personal communication: March, 2010). In the present study (2016), the average of water up-take of 26 softwood cuttings of Kiusiana (horizontal cut end) for 12 h during night was 1.7 ± 0.1 mL (data not shown). Poor water up-take of cuttings is probably related to the water stress sensitivity of the cuttings. Kiusiana softwood cuttings might be inherently sensitive to water stress in comparison with the other grapes such as ‘Campbell Early’. Although the pulse treatment of AVG for consecutive 4 days was conducted under controlled relative humidity in a biotron, the cuttings might be subjected to water stress in the biotron with constantly air-flowing for the temperature control. One of the responses to water stress of plants is an increased ethylene production (McKeon et al., 1995). An increase in ethylene induced by water stress may cause the death of Kiusiana cuttings.

The relation between treatment timing and the effectiveness on the viability might point out an importance that the effect of AVG, probably IBA being no exception, is transient rather than continuing after the treatment. Although the viability of Kiusiana cuttings was significantly higher in the late pulse treatment of AVG (72-96 h) than that in the early treatment, the proportion of rooted cuttings was tend to be higher in the early treatment than the late treatment. Considering this result and other results (Table 3), treatment of AVG seems to be more efficient for the rooting when the treatment is conducted at the same time of IBA treatment.

Preparation of cuttings at sunset and dipping treatment for 12 h in the dark were conducted to relieve water stress as much as possible, in the experiment of 2016. In this experimental condition, more than 60% of the cuttings in each treatment, except for 200 ppm IBA treatment, survived for 50 or 55 days of cutting period (Table 3 and 4). There was no significant different in the rate of rooted cutting per viable cutting between the control and IBA alone (Table 3). In addition to this non-effectiveness of IBA alone, decrease in the rate of viable cuttings was found in IBA alone; the viable cutting rate was 44.4% less than that in the control. This indicates that IBA alone treatment is not practical for stimulation of adventitious root formation of Kiusiana cuttings even in this experimental condition considering water stress of the cuttings.

Callus weight per cutting was highest in IBA alone and lowest in AVG + IBA (Table 3). Similar trend in the callus weight was found in the experiment of pulse treatment of AVG (Table 2). Reducing callus formation at the base of shoots with increasing root number was reported in *in vitro* shoot culture of apple treated with AVG on a basal rooting medium containing IBA (Ma et al., 1998). They also found that 1-aminocyclopropane -1- carboxylic acid, a precursor of ethylene, increased callus formation. Grapes have no preformed adventitious root primordia in the canes, and the adventitious roots directly develop from cambial cells, not from callus (Smart et al., 2003). This may indicate that excess callusing in the base of cuttings induced by increased ethylene is inappropriate for the induction of adventitious root formation.

Kiusiana cuttings dipped in AVG (5 ppm) + IBA (200 ppm) for 12 h during night rooted 11 times more than that in other treatments including AVG alone. Lack of the stimulatory effect of not only IBA alone but also AVG alone on the rooting, and dramatic effectiveness of AVG + IBA indicates that elimination of the adverse effects of IBA-induced ethylene is necessary to exert the inherently stimulatory effect of IBA on the adventitious root formation in Kuisiana softwood cuttings.

Cutting base cut in acute angle and its planting orientation into the rooting medium did not stimulate the adventitious root formation in Kiusiana cuttings treated with AVG + IBA. This high proportion of both the viable cuttings, and rooted cuttings per the viable cuttings indicate that the application of IBA in combination with AVG, under releasing from water stress such as the dipping application during night, can efficiently induction of rooting of the soft wood cuttings of *Vitis kiusiana* grape irrespective of the physical factors such as the base cutting method or putting orientation of cuttings. This efficient rooting method facilitates the propagation, and results in contribution for the preserve and utilization of *Vitis kiusiana* grape.

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アミノエトキシビニルグリシンとインドール-3-酪酸の混用処理はクマガワブドウ（*Vitis kiusiana* Momiyama）の緑枝挿しの発根を促進する

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摘　要

挿し木発根が困難なクマガワブドウ（*Vitis kiusiana*）において、アミノエトキシビニルグリシン(AVG)とインドール-3-酪酸（IBA）の混用処理が緑枝一芽挿しの不定根形成に及ぼす影響を調査した。IBA（100 ppm）処理後のAVG（5 ppm）のパルス処理（最初の24時間にIBAを処理し、それに続く24～48時間、48～72時間もしくは72～96時間のいずれかでAVGを24時間処理する）は、挿し穂の枯死を抑制したが、不定根形成には影響しなかった。一方、AVGとIBAの混用処理は、クマガワブドウの挿し木発根において劇的な効果を示した。挿し穂調製後の夜間12時間の200 ppm IBAと5 ppm AVGの混用処理は、生存した挿し穂あたりの発根率を対照区およびIBA単用区のそれぞれ29倍および13倍に増加させた。挿し穂基部のカルス重量はIBA単用区でもっとも高かったが、AVG + IBA区でカルス形成は抑制された。挿し穂基部の斜め切断と用土への斜め挿しは、AVG + IBAが誘起する不定根形成と根の成長に影響しなかった。