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Effects of Plant Growth Regulators on the Growth and Tannin Accumulation in a Medicinal Plant, *Geranium thunbergii* SIEB. *et* ZUCC.

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SYNOPSIS

Geranium thunbergii was treated with three kinds of plant growth regulators. Abscisic acid (ABA) at 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} M, ethephon at 100, 200, and 300 ppm, and gibberellin A_3 (GA_3) at 50, 100, and 200 ppm were applied two months or one month before harvest in November 1992. Dry matter production and tannin concentration in leaves were determined. ABA and ethephon applications one month before harvest only a little decreased the dry weight of aerial parts, and these applications two months before harvest greatly reduced it. Application of 10^{-6} M ABA one month before harvest increased the tannin concentration in leaf blades more than that of control. Whereas, ethephon treatments one month before harvest decreased the tannin concentration. GA_3 treatments increased the number of leaves, but the tannin concentration was slightly decreased.

INTRODUCTION

Plant growth regulators are very important agents in the integration of developmental activities, and they also are involved in the plant response to the external physical environment. The capacities of each substance may control many physiological processes.[1] Thus, we examined the effect of different plant growth regulators on the growth and accumulation of tannin in a medicinal plant, *Geranium thunbergii*. Understanding how plants respond to various plant growth regulators may be useful to elucidate the mechanism of responses to various environmental conditions in this medicinal plant.

MATERIALS AND METHODS

Plant material and cultivation *Geranium thunbergii* plants were grown by the same method as mentioned in our previous paper, were used in this study. In May 1992, these two plants were transplanted into a rectangular plastic pot 15 cm wide, 32 cm long, and 14 cm deep, filled with a mixture of vermiculite and sandy loam soil (1 : 1 ; v/v). All plants were grown in a plastic greenhouse and watered twice or three times a week.

Experimental procedures Abscisic acid (ABA) at 10^{-3}, 10^{-4}, 10^{-5}, or 10^{-6} M, gibberellin A_3 (GA_3) at 50, 100, or 200 ppm, and ethephon at 100, 200, or 300 ppm were used. These all solutions contained 0.1% Tween 20 surfactant. In September these solutions were sprayed to the plants of

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one group and the same treatments were conducted on another group in October. The total volume of 25 ml per plant was applied to run off on the whole plants in the evening. Control plants were sprayed with clean water.

Measurement of plant growth All plants were harvested in November. The above-ground parts of plants were cut at the soil surface level. The numbers of leaves and mature pods were counted. To avoid mature pods cracking with subsequent loss of number, pods were harvested as soon as they turned to be brown or black, assumed to reach full maturity. Immature pods were also counted at harvest time. Dry weight of plants were weighed after oven-drying at 40°C for 72 h.

Assay of tannin concentration The tannin concentration was determined in leaf blades on the one-year-old stems, and on the new shoots emerged from the stems. Dried samples were grounded into a fine powder. The powder samples (150 mg) were extracted with 10 ml 60% acetone. The amount of tannin in extracts was determined by the colorimetric method of Okuda and co-workers.[2] The tannin concentration was expressed as a percentage of the original dry weight.

Statistical analysis Results were given as the means of three repeated experiments. Statistical analysis was based on a completely randomized experimental design using a Stat Flex statistical analysis package (View Flex Company, Tokyo[3]).

RESULTS

Figure 1. shows the dry weight of aerial parts of the plants sprayed with the growth regulators in September and October. ABA had little effect on the dry weight except at 10^{-3} M in October and at 10^{-6} M in September and October. The dry weight of plants were sprayed with the 10^{-3} M ABA in October was decreased to 64% of that the control. Application with 10^{-6} M ABA significantly decreased the dry matter production regardless of the application time, the difference was 23-25% less than that of control.

The dry weight was significantly decreased by spraying with ethephon in October. Ethephon at 300 ppm treatment in October reduced the dry weight to less than 50% of the control plants. However, spraying with ethephon in September did not affect the dry weight of aerial parts. Leaf chlorosis appeared on the leaves of plants 2-5 days after spraying with ABA and ethephon. The ethephon caused the tip burn at the same time. Any GA_3 treatments did not affect the dry weight of aerial parts.

The number of leaves per plant affected by plant growth regulators are shown in Fig. 2. The plants sprayed with ABA did not show any significant difference from the control. However, spraying in October tended to decrease the leaf number more than treatments in September. The plants sprayed with ethephon in October had leaves 34-69% fewer than that of control, those sprayed with 300 ppm having the fewest leaves. However, spraying with ethephon at 200 and 300 ppm in September increased significantly the number of leaves about 55-129% more than in the control plants. Most GA_3 treatments increased the leaf number to 1-fold of the control.

The number of pods was little affected with ABA treatments (Table 1). Ethephon treatments restrained the pod setting (Table 2). Spraying with GA_3 in September decreased the pod number. Moreover, pod development was delayed by spraying with GA_3 in October, the number of immature pods increased compared with that of control (Table 3).

Spraying with ABA at 10^{-6} M in October increased the tannin concentration in leaf blades to about 105% on the one-year-old stems, and 110% on the new shoots (Fig. 3). Other ABA treatments had little effect on the accumulation of tannin within different leaves. Ethephon treatments suppressed the tannin accumulation. Especially, spraying with ethephon in October

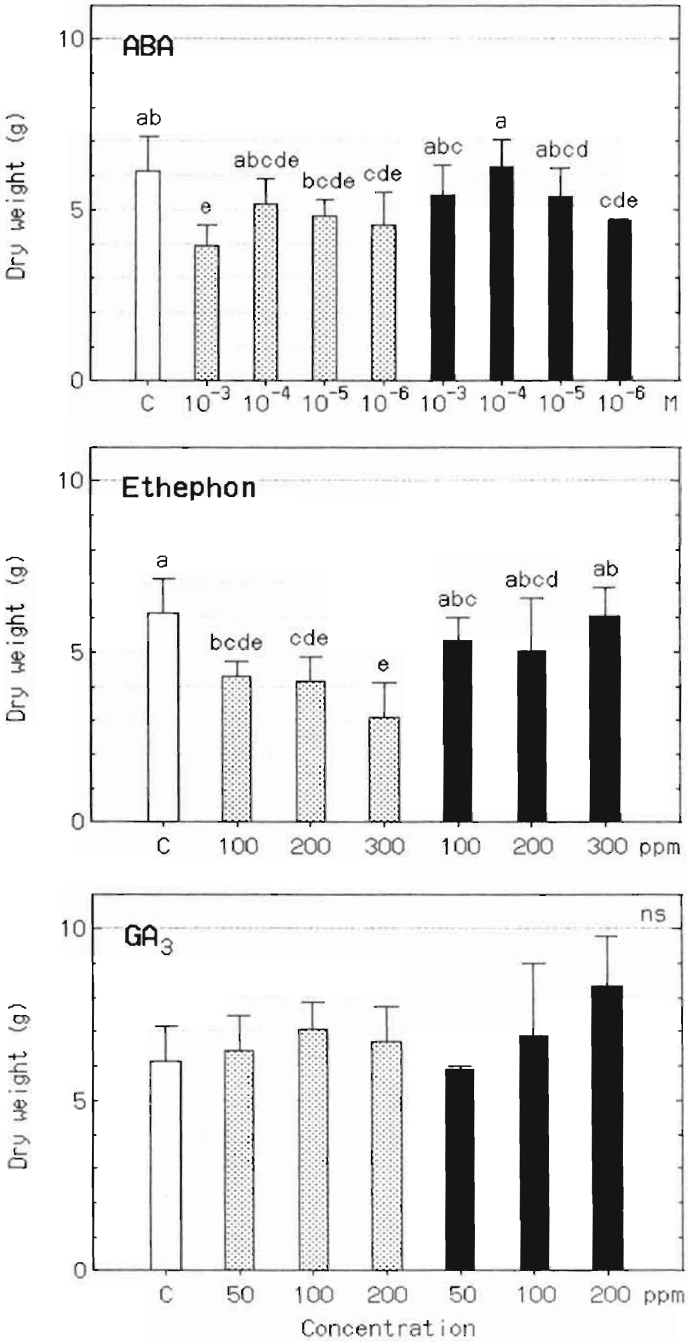

Fig. 1 Effects of plant growth regulators on dry weight of aerial parts spraying in september (closed bars), october (dotted bars), and control (open bars)
Data are means ± SD of three replicates. Bars with different letters show significant differences by Duncan's multiple range test at p=0.05. ns, not significant.

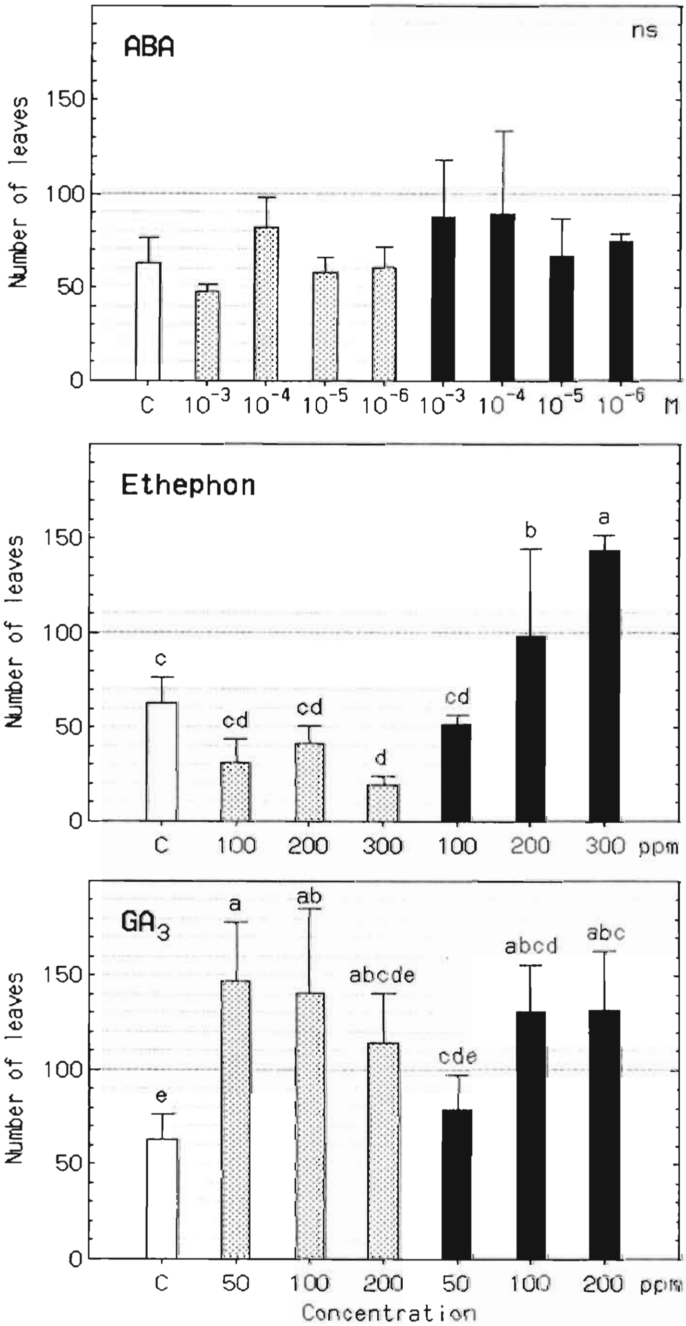

Fig. 2 Effects of plant growth regulators on number of leaves spraying in september (closed bars), october (dotted bars), and control (open bars)
For details, see the legend of Fig. 1.

Table 1. Number of pods in the plants sprayed with abscisic acid one month or two months after applications

Treatment	conc.	Number of pods per pot (n=3)		
		mature	immature	total
Control		68.3	3.2	71.5
one month[a]	10^{-3} M	51.0	3.0	54.0
	10^{-4} M	53.8	1.7	55.5
	10^{-5} M	47.3	1.7	49.0
	10^{-6} M	54.7	0.2	54.8
two months[a]	10^{-3} M	60.2	2.5	62.5
	10^{-4} M	70.0	0.7	70.7
	10^{-5} M	65.0	1.5	66.5
	10^{-6} M	49.3	2.8	52.2
				all ns

ns, not significant.
[a] after applications.

Table 2. Number of pods in the plants sprayed with ethephon one month or two months after applications

Treatment	conc.	Number of pods per pot (n=3)		
		mature	immature	total
Control		68.3 a	3.2 cd	71.5 a
one month[a]	100 ppm	60.2 ab	0.0 d	60.2 ab
	200 ppm	38.5 bc	0.0 d	38.5 bcd
	300 ppm	30.8 cde	0.0 d	30.8 cde
two months[a]	100 ppm	33.0 cd	7.5 b	40.5 bc
	200 ppm	15.3 cdef	14.3 a	29.7 cde
	300 ppm	0.7 f	7.0 bc	7.7 e

Means with different letters in the same column show significant differences by Duncan's multiple range test at $p=0.05$.
[a] after applications.

Table 3. Number of pods in the plants sprayed with gibberellin A_3 one month or two months after applications

Treatment	conc.	Number of pods per pot (n=3)		
		mature	immature	total
Control		68.3 a	3.2 bc	71.5 a
one month[a]	50 ppm	43.2 b	6.3 bc	49.5 abc
	100 ppm	39.3 b	11.0 a	50.3 abc
	200 ppm	48.3 ab	11.2 a	59.5 ab
two months[a]	50 ppm	35.7 b	6.7 b	42.3 bc
	100 ppm	37.8 b	2.2 c	40.0 bc
	200 ppm	33.7 b	2.2 bc	35.8 c

Means with different letters in the same column show significant differences by Duncan's multiple range test at $p=0.05$
[a] after applications.

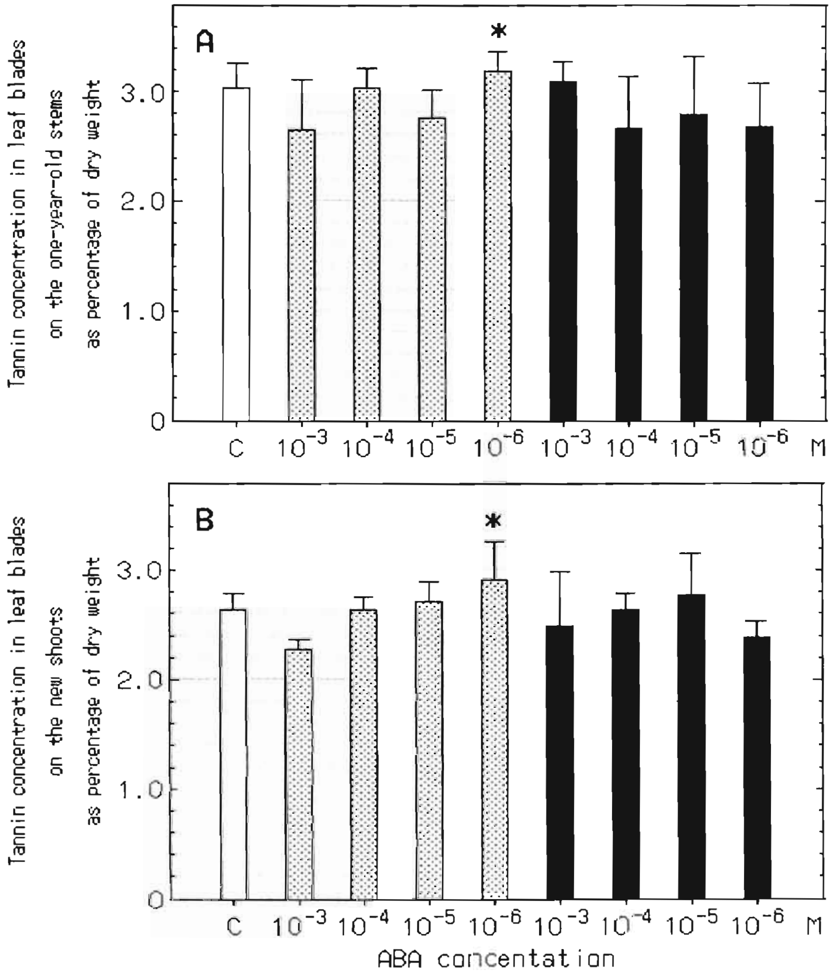

Fig. 3 Changes in tannin concentration in leaf blades on the one-year-old stems (A), and on the new shoots (B) spraying with abscisic acid in september (closed bars), october (dotted bars), and control (open bars)
Bars represent means ± SD of three replicates.

led to a large decrease in tannin concentration on the new shoots which was 21-27% less than that of control (Fig. 4). Changes in tannin concentration by GA_3 treatments had a little decrease (Fig. 5).

DISCUSSION

The spraying with ABA and ethephon before harvest suppressed the dry weight of aerial parts in *Geranium thunbergii*. Ethephon treatments one month before harvest in particular damaged the leaves and shoot tip, which decreased the dry weight. This was presumably due to either that *Geranium thunbergii* is very sensitive to ethylene, or the concentration of ethephon used in this study was too high. This indicates that ethephon can not be used practically for the usefulness

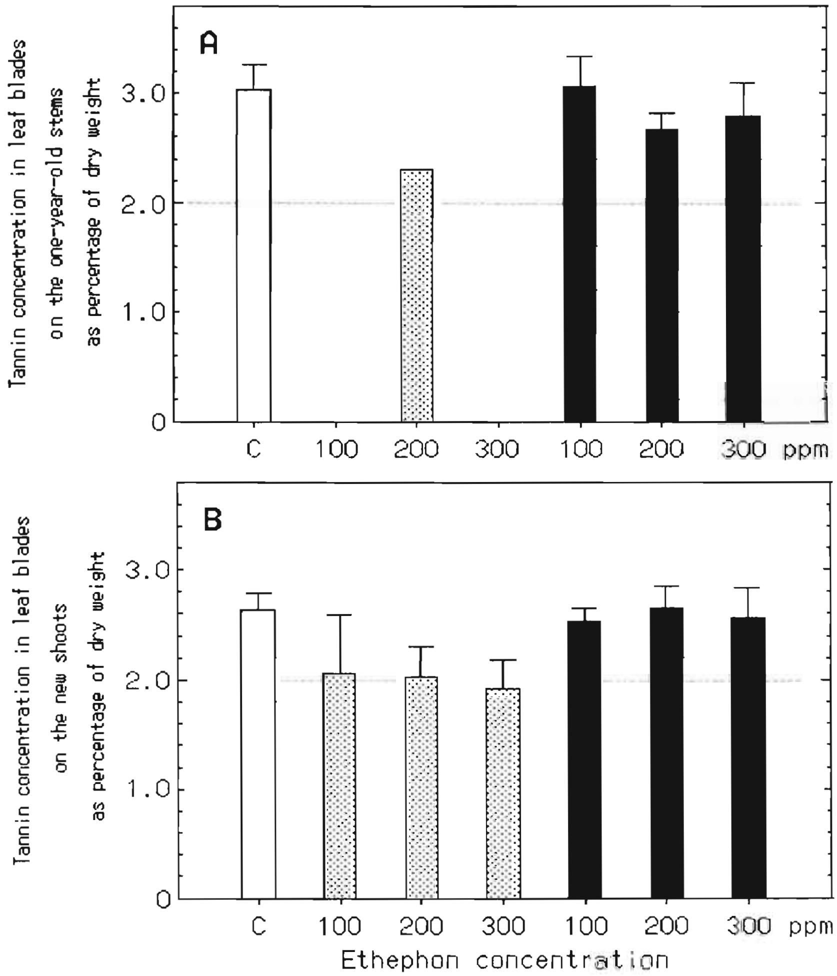

Fig. 4 Changes in tannin concentration in leaf blades on the one-year-old stems (A), and on the new shoots (B) spraying with ethephon in september (closed bars), october (dotted bars), and control (open bars)
For details, see the legend of Fig. 3.

of this plant because there is a danger of reducing markedly the plant growth. Mattoo and Suttle[4] extensively reviewed the inhibitory effects of ethylene on vegetative shoot growth. The dry weight of the plants treated with ethephon in September was little affected. This result was resulted from the increase in the number of leaves on newly emerged shoots. Our findings showed that GA_3 treatments enhance dry weight production, either by increasing the number of leaves, or elongation of the stems (data not shown). Pankov[5] reported that GA_3 promotes the vegetative growth of strawberry plants.

Response of the pod development to plant growth regulators varied. ABA treatments did not affect the number of pods, but ethephon treatments decreased it. The timing of GA_3 application influenced the development of pods. Spraying with GA_3 in September reduced markedly the number of pods, and the treatments in October delayed pod maturity. These findings suggested that the effect of plant growth regulators on the reproductive development of *Geranium thunber-*

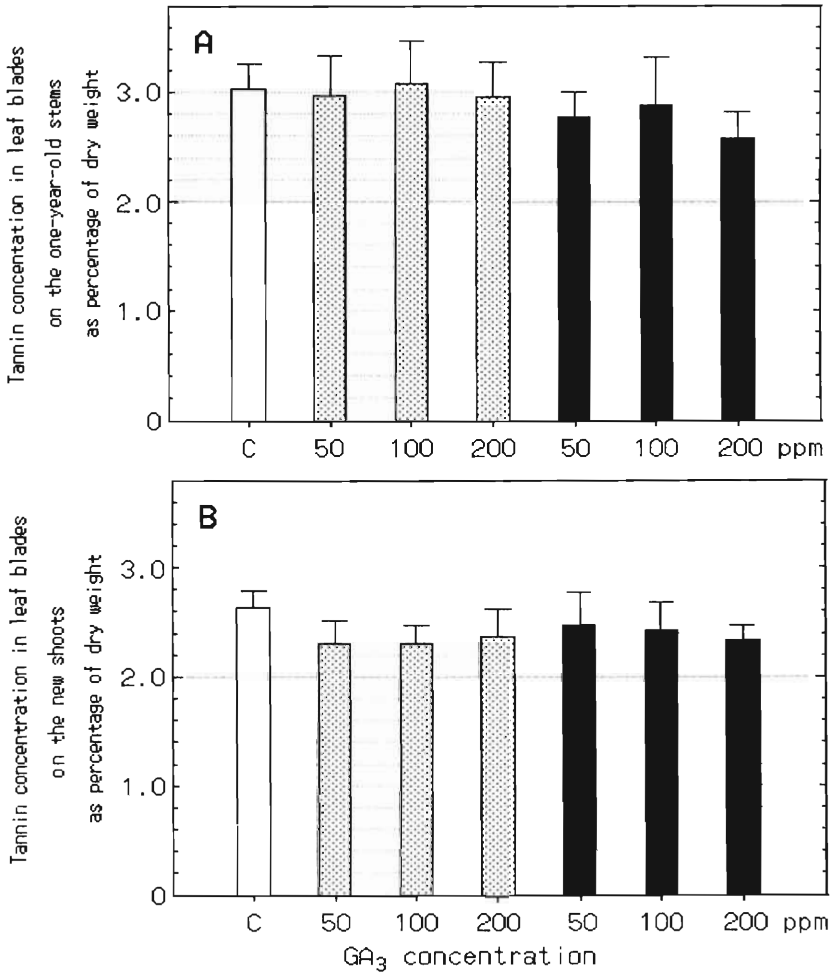

Fig. 5 Changes in tannin concentration in leaf blades on the one-year-old stems (A), and on the new shoots (B) spraying with gibberellin A_3 in september (closed bars), october (dotted bars), and control (open bars)
For details, see the legend of Fig. 3.

gii depends on the kind of regulators, or the time of application.

Spraying with ABA at 10^{-6} M in October had a beneficial effect which increased the tannin concentration. ABA which is considered to be a stress hormone may be related to the tannin accumulation in this plant. Aurisano *et al.*[6] suggested that the ABA action involves plant in the development of tolerance to various stress conditions. Plant growth is mildly inhibited under stress regimes.[7] Inhibition of plant growth under some stress conditions, may be related to increased tannin concentration.

Ethephon treatments reduced the tannin accumulation in the plants with markedly decreasing the dry weight. Cvikrová *et al.*[8] suggested that ethephon elevates the accumulation of phenolic acids in alfalfa *in vitro*. However, they showed that ethephon enhances transiently the total contents of phenolic acids that are metabolized during subsequent 5-6 days. Ethephon as a ethylene-releasing agent, increases respiration, induces enzyme activity, and modifies metabolic activities.[6]

GA_3 treatments caused the little increase of plant dry weight, but reduced tannin concentration in the leaves. Since various conditions which stimulate plant growth showed reverse responses to impair tannin accumulation, the reduction in tannin concentration may be related to the promotion of plant growth.

The results of this study indicated that the plant growth responses to regulators is different. Dry matter production was slightly suppressed by ABA but markedly by ethephon treatments in this study, whereas GA_3 treatments stimulated number of leaves. Therefore, application of 10^{-6} M ABA one month before harvest was appropriate method to increase the tannin concentration in leaves with mild stress effects.

REFERENCES AND NOTES

1) R.J. Weaver, "Plant Growth Substances in Agriculture," W.H. Freeman and Company, San Francisco, 1972, p. 594.
2) T. Okuda, K. Mori, T. Hatano, *Chem. Pharm. Bull.*, **33**, 1424 (1985).
3) Address: 4-19-17-205, Sanda, Minatoku, Tokyo 108, Japan.
4) A.K. Mattoo, J.C. Suttle, "The Plant Hormone Ethylene," CRC Press, Inc., Florida, 1991, p. 337.
5) V.V. Pankov, *Scientia Hortic.*, **52**, 157 (1992).
6) N. Aurisano, A. Bertani, M. Mattana, R. Reggiani, *Physiol. Plant.*, **89**, 687 (1993).
7) T.C. Moore, "Biochemistry and Physiology of Plant Hormones," Springer-Verlag, Inc., New York, 1989, p. 330.
8) M. Cvikrová, M. Hrubcová, M. Vágner, I. Macháčková, J. Eder, *Physiol. Plant.*, **91**, 226 (1994).

植物成長調整剤が薬用植物，ゲンノショウコ，の成育とタンニン生産に及ぼす影響

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要　　約

高等植物の成育に対して顕著な影響を及ぼすことが知られている3種類の成長調整剤，アブサイシン酸（ABA）10^{-3}〜10^{-6} M，エスレル（ethephon）100〜300 ppmおよびジベレリン（GA_3）50〜200 ppmの濃度範囲の溶液を本植物の収穫1か月前または2か月前に植物全体に噴霧した。収穫後植物の成育量および葉のタンニン含量を測定した。

それらの結果，ABAとエスレルの1か月前処理区では無処理区に比べて植物体乾重がわずかに，2か月前処理区では著しく減少させられた。ジベレリンは特に2か月前処理区で葉数の増加をうながした。葉のタンニン含量については，ジベレリンとエスレル処理区では対照区よりも，それぞれおよそ14および27%減少したが，低濃度(10^{-6} M)のABAの収穫1か月まえ処理区では約5〜10%の増加がみられた。したがって，このような植物調節剤の利用にあたっては，薬剤濃度と噴霧時期，などの十分な検討が必要であろう。