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Effects of GA_3 and ABA Application on After-ripening of *Panax quinquefolium* Seeds during Stratification

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Abstract : The effects of gibberilin (GA_3) on levels of endogenous indole-3-acetic acid (IAA) and zeatin in both fresh and stratified American ginseng (*Panax quinquefolium*) seeds were investigated. In our first experiment, the fresh seeds were stratified after soaked in 80 ppm GA_3 solution for 24 hours. We found that the IAA concentration in embryo increased by 50.7% and 82.1% respectively at the 120th day and the 188th day of stratification, and the zeatin concentration also increased by 3.8% and 51.6% respectively. In our second experiment, we treated the seeds after 134 days stratification with 80 ppm GA_3 for 24 hours and then continued to stratify them. We found that the IAA concentration in embryo increased by 32.9% and 17.7% respectively at the 164th day and the 208th day of stratification while zeatin concentration increased by 22.7% and 30.6% respectively. In our another experiment, we studied the effects of GA_3, abscisic acid (ABA) and GA_3 plus ABA on germination rate of seeds treated with these plant hormones during stratification. We found that when the stratified seeds whose ratio of embryo had reached 75% were treated with 80 ppm GA_3 for 24 hours and then were allowed to be stratified for another 88 days, the weight and length of embryo ($p < 0.05$), and germination rate ($p < 0.01$) increased. In contrast, the 25 ppm ABA treated with for 24 hours was found to inhibit the growth of embryo ($p < 0.05$) and reduce the germination rate ($p < 0.05$). The experiment of combination treatment of GA_3 and ABA showed that GA_3 could relieve the inhibitory effects of the ABA on the development of the seeds.

Key words : *Panax quinquefolium*, seeds, IAA, zeatin, GA_3, ABA, germination.

Introduction

When American ginseng (*Panax quinquefolium*) seeds are harvested, the immature embryos continue to grow during the subsequent afterripening period. This period is usually as long as about 20 months if the seeds lie in stratification outdoors. To reduce this period, many approaches have been carried out.[1-4] It is generally known that GA_3 could stimulate the embryo growth and shorten the afterripening period, and this stimulant effect of GA_3 on embryo growth of *Panax quinqueforlium* seeds has been verified by other authors.[1-4] However, the ways through which the exogenous GA_3 can stimulate the development of embryo have not been studied yet. As we know, hormones usually involve in the development of seeds. Our previous work[5] showed that the long afterripening period of American ginseng seeds may be attributed to the low levels of endogenous growth hormone contents of the seeds at the earlier stage of stratification. Thus it is very reasonable for us to deduce that the promotion effects of GA_3 on embryo growth may work through influencing the endogenous hormone levels. In this report, we studied the interaction between exogenous GA_3 and endogenous IAA and zeatin as well as their effects on em-

bryo growth of American ginseng seeds during stratification. Several other reports are also available about effects of exogenous plant hormones on plant growth and development. For example, zeatin could promote cell division in certain plant tissue,[6] ABA could inhibit germination of barley seeds, and GA_3 could relieve the inhibition effect of ABA.[7] But the combined effect of exogenous GA_3 and ABA on embryo development of American ginseng seeds has not been reported in the literature. In this paper, we also studied the effects of exogenous hormones mentioned above on embryo growth of American ginseng seeds.

Materials and Methods

1. Plant Materials

The seeds used in all our experiments were harvested in September, 1992, in Zuojia District, Jilin City, P. R. China.

2. Treatment Procedures

The fresh seeds were divided into three groups. The first group was soaked with 80 ppm GA_3 for 24 hours and then stratified successively at 18~20℃ for 80 days, at 8~13℃ for 54 days, and finally at 0~5℃ for 88 days. The second group was firstly stratified at 18~20℃ for 80 days, at 8~13℃ for 54 days in proper order and then was taken out and soaked with 80 ppm GA_3 solution for 24 hours. After this treatment, stratification continued at 0~5℃ for 88 days. The third group, which is used as control, was soaked with water at room temperature for 24 hours at first and then was stratified in the same way as described in the first group. The water contents of stratified medium (sand) in all the above treatments were about 10%.

3. Sampling and Analyses

Samples from the first group and the control were taken at the 96th, the 120th and the 188th day in sequence. Samples from the second group and the control were taken at the 164th and the 208th day respectively.

For the analyses of IAA and zeatin contents in the seeds in the experiments, a half gram of embryo or endosperm (fresh weight) was homogenized with pre-cooled methanol (−10℃). The homogenized tissues were transferred to a 150 m*l* conical flask, mixed with 100 m*l* pre-cooled methanol, stirred for 4 hours and then filtered. The solution was kept at 0℃ for further use. The residues were mixed with 50 m*l* pre-cooled methanol (−10℃) and the mixture was kept at 0℃ for 12 hours. Then it was shaken at 5℃ for 2 hours and filtered. The two filtered solutions were combined and then evaporated in a vacuum. The residue was resolved in methanol and fixed to a volume of 5 m*l* for further HPLC analyses.

The conditions for analyses are as follows:

• Equipment	Waters 224 HPLC
• Column	Novapak C_{18}
• Mobile phase	25% CH_3OH, 35%CH_3CN, 40%H_2O
• Flow rate	0.7 m*l*/min
• Detector	UV 254 nm, 0.1 AUFS
• Linear correlation range	0.01 μg~0.01 mg
• Rate of recovery	IAA 83.0%, zeatin 84.5%
• Internal standard	IAA, zeatin

4. Field Test

Field test was carried out to examine the exogenous hormones and their interactions on embryo growth of the fresh seeds were mentioned above. The seeds were allowed to be stratified as soon as they were harvested in September, 1992. The method for stratification has been described above which was at 18~20℃ for 80 days and at 8~13℃ for 54 days in sequence. When ratio of embryo reached 75% in February, 1993, the seeds were taken out and divided into 4 groups, and then were treated with exogenous hormones in the way as follows:

Group 1 : soaked with 80 ppm GA_3 for 24 hours

Group 2 : soaked with 25 ppm ABA for 24 hours

Group 3 : soaked with 80 ppm GA_3 plus 25 ppm ABA for 24 hours

Group 4 (control) : soaked with water for 24 hours

After the above treatment, the seeds continued to be stratified at 0~5℃ for 88 days till April, 1993. Then some of the seeds were sampled for measuring embryo ratio and weight. The values were the mean of 30 samples. Most of them were sown in an area of 24 m^2 experimental field for field test. The seeds in each of the four groups were sown in 3 duplications. Germination rate was measured in June, 1993 after 52 days of sowing. The values were the mean of 3 duplications. The statistical analysis of the data was done using the modified LSD(Bonferroni) test.

Results and Discussion

The period of the seed stratification in our experiments was 222 days altogether which could be divided into two stages: the first stage was 134 days and the second stage was 88 days (134~222 days). The first stage was defined when the embryo ratio reached 75% after 134 days stratification. Usually, an embryo ratio of 75% is required for subsequent stratification at lower temperature (0~5℃), as a lower embryo ratio may result in poor germination.

1. Effects of Exogenous GA_3 on IAA and Zeatin Contents in Seeds

(1) Seeds treated with GA_3 in first stage

Table 1 indicated that when seeds were treated with GA_3, content of IAA and zeatin in both embryo and endosperm increased. At the 96th day, content of IAA in endosperm of seeds treated with GA_3 was 99.8% higher than that of control, and content of zeatin in treatment is 36.2% higher than that of control. The similar results in

Table 1. Effects of GA_3 used in first stage on contents of IAA and zeatin of seeds[a]

	IAA (μg/g)											
	Embryo			Endosperm			Endosperm			Embryo		
	Treatment	Control	Δ%	Treatment	Control	Δ%	Treatment	Control	Δ%	Treatment	Control	Δ%
96th day				1.077	0.539	99.8				0.256	0.188	36.2
120th day	1.159	0.769	50.7	0.922	0.548	68.2	0.370	0.268	3.8	0.268	0.244	9.8
188th day	0.872	0.479	82.1	0.605	0.406	49.0	0.923	0.609	51.6	0.701	0.545	28.6

Treatment : The first group in *treatment procedures*, Control : The third group in *treatment procedures*.

[a] Δ% (IAA)=[(IAA content in treatment − IAA content in control) / IAA content in control]×100%.

Δ% (Zeatin)=[(zeatin content in treatment − zeatin content in control) / zeatin content in control]×100%.

Table 2. Effects of GA_3 used in second stage on contents of IAA and zeatin of seeds[a]

	IAA (μg/g)											
	Embryo			Endosperm			Embryo			Endosperm		
	Treatment	Control	Δ%	Treatment	Control	Δ%	Treatment	Control	Δ%	Treatment	Control	Δ%
164th day	0.888	0.668	32.9	0.667	0.575	16.0	0.933	0.744	25.4	0.778	0.634	22.7
208th day	2.502	2.126	17.7	1.280	1.162	10.2	0.946	0.567	66.8	0.384	0.294	30.66

Treatment : The second group in *treatment procedures*, Control : The third group in *treatment procedures*.

[a] Δ% (IAA)=[(IAA content in treatment − IAA content in control) / IAA content in control]×100%.

Δ% (Zeatin)=[(zeatin content in treatment − zeatin content in control) / zeatin content in control]×100%.

both embryo and endosperm occurred at the 120th day and the 188th day respectively.

(2) Seeds treated with GA3 in second stage

As shown in Table 2, GA_3 used in second stage could also promote IAA and zeatin levels in seeds. At the 164th day, contents of IAA and zeatin in embryo of seeds treated with GA_3 were respectively 32.9% and 25.4% higher than those of the control, the similar results were observed in the endosperm. At the 208th day, contents of IAA and zeatin in both embryo and endosperm after treated with GA_3 were also higher than those of the control.

Table 1 and 2 indicated that exogenous GA_3 promotes IAA and zeatin in both the first stage and the second stage, and the increments of IAA and zeatin in first stage were higher than those in second stage. Generally embryos grow from 0.4 mm to 5.0 mm in length during afterripening period. In nature, the growth rate of embryo is very low and the levels of IAA and zeatin are also very low at the same stage.[5] Therefore, we assume that the low growth rate of embryo is partially due to the low levels of IAA and zeatin and that the mechanism for GA_3 to stimulate embryo growth may work through promoting endogenous levels of IAA and zeatin in seeds.

2. Effects of GA_3, ABA and GA_3 Plus ABA on Development of American Ginseng Seeds

Our experiments showed that GA_3 could cause the increments of weight and length of embryo and increase the germination rate of the seeds tested.

(1) Effects of exogenous hormones on length of embryo (Table 3)

Table 3 showed that GA_3 stimulated growth of embryo ($p < 0.05$), ABA could inhibit embryo extending (no statistic difference), and GA_3 could partially reduce the inhibition effect of ABA.

(2) Effects of hormones on weight of embryo

Results in Table 4 indicated that GA_3 accelerated the increase in embryo weight, but without any statistic differences; ABA inhibited the increase in embryo weight ($p < 0.05$); and GA_3 could partially relieve the inhibitory effect

Table 3. Effects of hormones on length of embryo[a]

Treatment	Length of embryo (mm)	p=0.05	p=0.01
GA_3	6.5	a	A
GA_3+ABA	6	ab	A
Control	5.4	b	A
ABA	5.2	b	A

[a] Using modified LSD (Bonferroni) test (n=30); Values that corresponded by one same letter (e.g. a and ab, or b and ab) in each column were not significantly different from each other at the level indicated by the p values. Values that corresponded by different letters (e.g. a and b) were significantly different at the level indicated by the p values.

Table 4. Effects of hormones on weight of embryo[a]

Treatment	Weight of embryo (mg)	p=0.05	p=0.01
GA_3	5.7	a	A
Control	5.4	a	A
GA_3+ABA	4.5	b	A
ABA	4.2	b	A

[a] Using modified LSD (Bonferroni) test (n=30); Values that corresponded by one same letter (e.g. a and ab, or b and b) in each column were not significantly different from each other at the level indicated by the p values. Values that corresponded by different letters (e.g. a and b) were significantly different at the level indicated by the p values.

Table 5. Effects of hormones on germination rate[a]

Treatment	Germination rate (%)	p=0.05	p=0.01
GA_3	85	a	A
GA_3+ABA	77.3	b	AB
Control	74	b	B
ABA	63.7	c	C

[a] Using modified LSD (Bonferroni) test (n=30); Values that corresponded by one same letter (e.g. b and b) in each column were not significantly different from each other at the level indicated by the p values. Values that corresponded by different letters (e.g. a and b, or b and c) were significantly different at the level indicated by the p values.

of ABA.

(3) Effect of hormones on germination rate

When seeds were treated with GA_3, germination rate increased ($p < 0.01$). When seeds were treated with ABA, germination rate decreased ($p < 0.01$). GA_3 could counteract the effect of ABA (Table 5).

From Table 3~5, we found that GA_3 could stimulate embryo growth and increase germination rate. This result was the same as that of previous work on American ginseng.[1,4] We also knew that ABA could inhibit embryo growth and its inhibitory effect could be relieved by GA_3. This result was similar to that of previous work on other seeds.[7] As GA_3 treatment increased the activity of amylase of American ginseng seeds,[4] and ABA treatment decreased that of barley seeds,[7] we may try to draw some conclusions from these seemingly unrelated facts. Our results clearly showed that the promotion effects of GA_3 on embryo growth and germination rate was associated with the increase of the contents of endogenous IAA and zeatin of American ginseng seeds during strastification. Further more, we, for the first time, proved the inhibitory effect of ABA on embryo growth and germination rate of American ginseng seeds and this inhibitory effect can be counteracted by combination treatment of GA_3 and ABA. We may deduce that ① the promotion effect of GA_3 on the embryo growth and germi nation rate may work through increasing the activity of amylase of the seeds of the American ginseng; ② the inhibitory effect of ABA on the activity of amylase of barley seeds may be applicable on ginseng seeds which needs to be verified by further experiments; and ③ the interactive effects between GA_3 and ABA on American ginseng seeds may process through affecting the activities of amylase and other related enzymes.

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