Preliminary Studies on Breaking of Dormancy and Germination of *Panax ginseng* Seeds

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人蔘種子의 休眠打破 및 發芽에 關한 基礎硏究

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ABSTRACT

The studies were carried to know the effects of GA_3, Ethrel and H_2O_2 on dormancy and germination in ginseng seeds. GA_3 stimulated the embryo growth and increased dehiscent (Kaekapp) ratio of the seeds for more than Ethrel and H_2O_2

GA_3 not only increased germination ratio but also shortened the period of germination. Ethrel and H_2O_2 showed no effects on the germination and there were no significant differences among the treatment levels of GA_3.

The slow germination of ginseng seeds seemed to be mainly due to the dormancy of endosperm or seed coat rather than of embryo.

INTRODUCTION

Ginseng is one of the most important agricultural export crop in Korea. It has been a valuable medicine since ancient time. Through many ages, Korea people have used it as a medicine of highly nutritious value to cure chronic diseases, such as nevros-thenia, decrease of sexual desire, weakness of body, diabetes, etc[4)12)].

According to past observation, ginseng seeds ripen in the fall, but generally do not germinate until the following fall without stratification of the seeds for more than 3 months[12)16)28)]. Much labor, time and cost are needed for this stratification and so it is useful to find out and develop new method to hasten the breakage of dormancy and stimulate germination of the seeds, as an alternative to the laborious method.

References about shortening the duration of stratification and then stimulating the germination of the seeds are limited[3)9)10)11)24)25)]. Among these Ohosumi and Miyasawa[24)25)], Grushivitskü[11)] and Kim[16)] showed an increases of embryo(seed coat dehiscence) ratio and germination ratio when the seeds treated with GA_3.

It seemed of interest to know what plant regulators would act as a stimulator for breaking the dormancy and stimulating germination of ginseng seeds[3)8)13)15)16)]. In this experiment, the main purpose was to study the effects of GA_3, Ethrel, H_2O_2 and chilling on stratification and germination[14)].

MATERIALS AND METHODS

Ginseng seeds obtained from the Kwacheun Ginseng Experiment Station were used in this experiments. Seeds were divided into 2 parts of A and B. A part's seeds were soaked with 0(water), 100, 200 and 400 ppm solution of GA_3, Ethrel and H_2O_2 for 24 hours respectively. The treated seeds of each level were divided with 3 replications and then they were kept in a pot (15cm diam.×25cm height) in which the seeds were arranged as shown Fig.1. Pots were placed under ground for 3 months from Aug.8

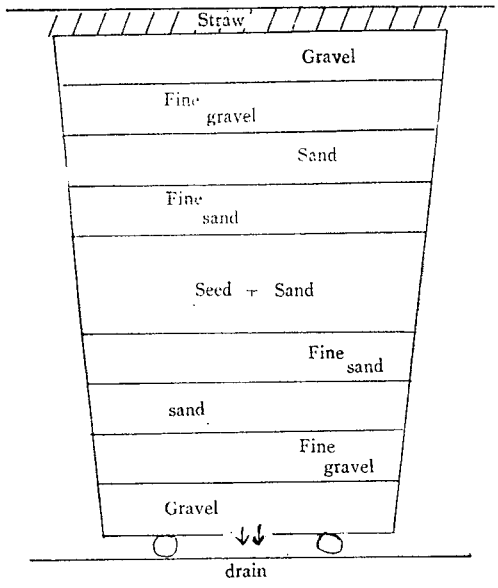

Fig. 1. Pot in which each level of soaked ginseng seed were arranged for the stratification.

to Nov.8 and maintained in a moist condition by frequent watering. B part's seeds were kept at a laboratory room without stratification treatment. The effects of chemicals on stratification were estimated twice on Sept. 20 and Nov. 8 by measuring the rates of seed coat dehiscence and the embryo growth.

In the germination test, the stratified seeds of A part were resoaked for 24 hours with the same levels of the same chemical solutions which were used for the stratification treatments. The rest of stratified seeds of A part were chilled at +2(as check), ±0, −5, and −10°C for 10 days respectively[1)7)17)]. Control seeds of A part (stratified seeds soaked in water as check) were also soaked with 0(water as check), 200, 400 and 800 ppm solution of GA_3, Ethrel and H_2O_2 for 24 hours respectively. All those 3 groups of treated seeds were placed on moist filter in petri dishes maintained at 19-20°C under dark, and counting and removing the rooting seeds were made for more than 50 days (Fig.2). In general, results were expressed by means of 3 replicates, 70 seeds each.

On the other hand to know whether the low germination seeds is mainly due to a dormancy of the embryo itself, endosperm or seed coat of the seeds, the seeds and endosperm of A part which stratified and the seeds of B part non-stratified were

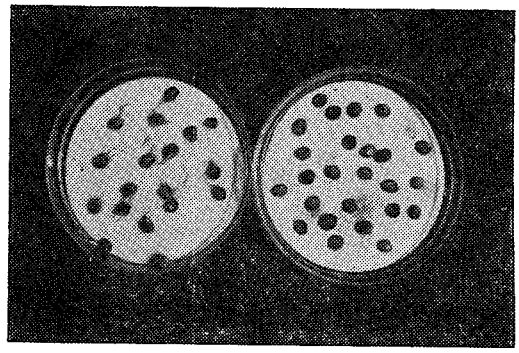

Fig. 2. Ginseng seeds placed on moist filter paper in petri dishes, left: rooting, right: no rooting

sterilized and soaked for 2 days in distilled water and kept under 20°C. Their respiration ratios of 100 seeds at 20°C were measured using a Hartmann and Braun Infrared CO_2 absorption[22)23)26)29)30)31)].

The embryos were excised from the seed or endosperm under moderately sterile conditions in a sterile chamber by using a scalpe or razor avoiding cutting the embryo[5)18)19)] and placed in test tube on the several kinds of medium[20)] containing 10ppm GA_3, 5ppm IAA, 1 IAA+Kinetin and IAA+0.5ppm 2-iP.

The basal medium consisted of salts and organic compounds accordings Linsmaier and Skoog (1965). Some of the tubes were kept at 26°C and others at 4°C for 8 day and then their growth were observed.

Abbreviations: IAA=indole acetic acid
GA_3=gibberellic acid A_3
2-ip =6−(r,r-Dimethyl allyl'amind) purin

RESULTS AND DISCUSSION

The results presented in table 1 show that the embryo ratio and dehiscent (Kaekapp) percentage of seed coat were significantly increased by soaking in chemical solutions of GA_3, Ethrel and H_2O_2, especially in GA_3 treatment. At the first measurement (on Sept. 20), the embryo ratio of 33% and 24% and the dehiscent ratio of 16% and 47% were shown only in the 200ppm and 400ppm of GA_3 treatment. On the other hand, however, at the second measurement (on Nov. 8) the GA_3 treatment showed the average embryo ratio of 75% and the average dehiscent ratio of 93%; while in the Ethrel and H_2O_2 treatments the average embryo ratio of 73% and

69% and the average dehiscent ratio ranged between 91% and 89% respectively. No significant differences were measured among the concentration levels of each chemical treatment. Accordingly, it was considered that in the beginning of treatment, the GA_3 treatment stimulated the embryo growth and then increased the dehiscent ratio, but the effect diminished as the stratification was progressed; otherwise, and dehiscent ratio might have been increased by accelerating the embryo growth, as some thing like hormone substance was synthesized for the seeds[27]. The average embryo ratio and the average dehiscent ratio have been increased by 22% and 13% respectively in the GA_3 treatment compared with those in control. It is evident from above results that GA_3 treatment hasten the breakage of dormancy and stimulates germination of ginseng seeds for more than Ethrel and H_2O_2 and that the seeds started dehiscing when the embryo ratio reached around 30%.

The test ginseng seeds carried out revealed a very low percentage of germination, in general. The germination was significantly accelerated by GA_3. The highest percentage and earlier germination were oberved when the seeds were treated with GA_3 tswice; first before stratification and second before germination, while no statistical significant differences among the treatment levels were observed(Table 2). A little higher percentages were obtained when the seeds were treated once before germination or once before stratification (Table 2 and 3). The seeds treated with GA_3 twice showed the average germination percentage of 46%, while those which were treated only once before stratification or before germination showed the average germination of 16% or 22% respectively. The average germination percentage of the seeds treated with GA_3 twice was 8 times as much as that of the control. Further the germination of the seeds treated with GA_3 twice started 20—22 days after seeding but the seeds in all other treatments started 27 days after seeding No clear differences were obtained among the treatments with Ethrel, H_2O_2 and control (water). It was the same in the treatments with GA_3 once before stratification or once before germination. It was believed that not only the dehiscent ratio and germination ratio could be increased but also the period of germination of ginseng seeds could be shortened, if the GA_3 is applied either in the dehisence or germination of the seeds. It was also suggested that increase of yield would be possible, because the period of growth will be extended if the period of germination is shortened. Further studies would be needed for this line. In the test with control seeds

Table 1. Effects of chemicals at different concentrations on embryo ratio and Keakapp ratio of ginseng seeds. (unit in %)

Chemicals	Ratio	Treat. / Obse.	0	100	200	400 ppm	L.S.D.
GA_3	Embryo Ratio	9/20 date	18	20	33	34	
		11/ 8	53	69	80	76	0.05:7.24
	Kaekapp Ratio	9/20	0	0	16	47	
		11/ 8	80	90	95	94	0.01:10.98
Ethrel	Embryo Ratio	9/20	20	16	18	18	
		11/ 8	52	68	68	78	0.05:6.10
	Kaekapp Ratio	9/20	0	0	0	0	
		11/ 8	79	89	89	94	0.01:9.25
H_2O_2	Embryo Ratio	9/20	19	20	22	20	
		11/ 8	53	66	73	73	0.05:11.46
	Kaekapp Ratio	9/20	0	0	0	0	
		11/ 8	80	84	93	92	0.01:17.39

Embryo ratio=(Embryo length/Endosperm length)×100
Kaekapp ratio=(No. of dehisced seeds/Total seeds)×100

Table 2. Effects of chemicals at different concentrations on germination of ginseng seeds treated twice before stratification and germination. (units in %)

Chem. / Treat.	GA_3		GA_3*		Ethrel		H_2O_2	
	Germ. seed	Rot seed	Germ.	Rot	Germ.	Rot	Germ.	Rot
0 ppm	2	3	4	3	2	3	0	4
100	48	12	16	9	2	11	0	7
200	42	21	15	9	1	12	0	7
400	46	24	17	11	1	17	0	6
L.S.D.	0.05: 7.51 0.01:11.39		0.05: 2.97 0.01:4.51					

Germination of seeds were checks for more than 50 days.
* treated once before stratification treatment.

Table 3. Effects of chemicals at different concentrations on germination of ginseng seeds treated once before germination. (unit in %)

Chem. / Treat.	GA_3		Ethrel		H_2O_2	
	Germ.	Rot	Grem.	Rot	Germ.	Rot
0ppm	0	5	0	4	0	4
200	18	10	0	9	0	6
400	25	14	0	12	0	7
800	24	18	0	12	0	9
L.S.D. 0.05 0.01	 9.52 15.82					

(Germination of seeds were checked for more than 50 days.)

of A part which were treated with 0, 200ppm, 400ppm and 800ppm solution of GA_3, Ethrel and H_2O_2 at once only before germination, the seeds treated with GA_3 showed 18—25% of germination ratio 22 days after seeding and no germination was found even after 50 days in all other treatments.

Since GA_3 has been found effective in hastening the breakage of dormancy and accelerating the germination in several plant seeds[4)6)19)14)], it is reasonable to assume that low temperature after ripening results in an increased synthesis of endogenous gibberellins in the seeds[8)21)21)] and so increased germination ratio on ginseng seed might be obtained. However no data on chilling test were obtained because the tested seeds showed no germination at all until 50 days after placing on the petri dishes. Hence further studies on this line will be carried out later.

Figure 3 shows the respiration rates (ppm/100 seeds at 20°C) of ginseng seeds at the germinating stage. In general, low respiration rates were observed but the endosperm obtained from the stratified seeds by removing the seed coat, showed the highest respiration rate of 3.8ppm/100seed at 20°C in comparison with the stratified seeds and the endosperms obtained from the non-stratified seeds by removing the seed coat. The stratified seeds showed a slightly lower respiration rate than that of the endosperms of non-stratified seeds. Those results indicated that the germination of ginseng seeds might be inhibited by seed coat in some way or other.

The embryo germinated in vitro about 18—20 days after placing in test tube, but only two of them which were excised from the endosperm of stratified seeds developed into normal seedlings (Fig. 4a). Rest of them including the embryos excised from stratified and non-stratified seeds showed only the expansion of its cotyledon and radicle (Fig. 4b). A less development of embryo excised from the

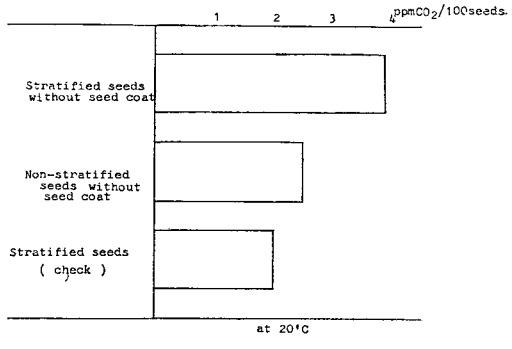

Fig. 3. Respiration rate of treated *Panax ginseng* seeds

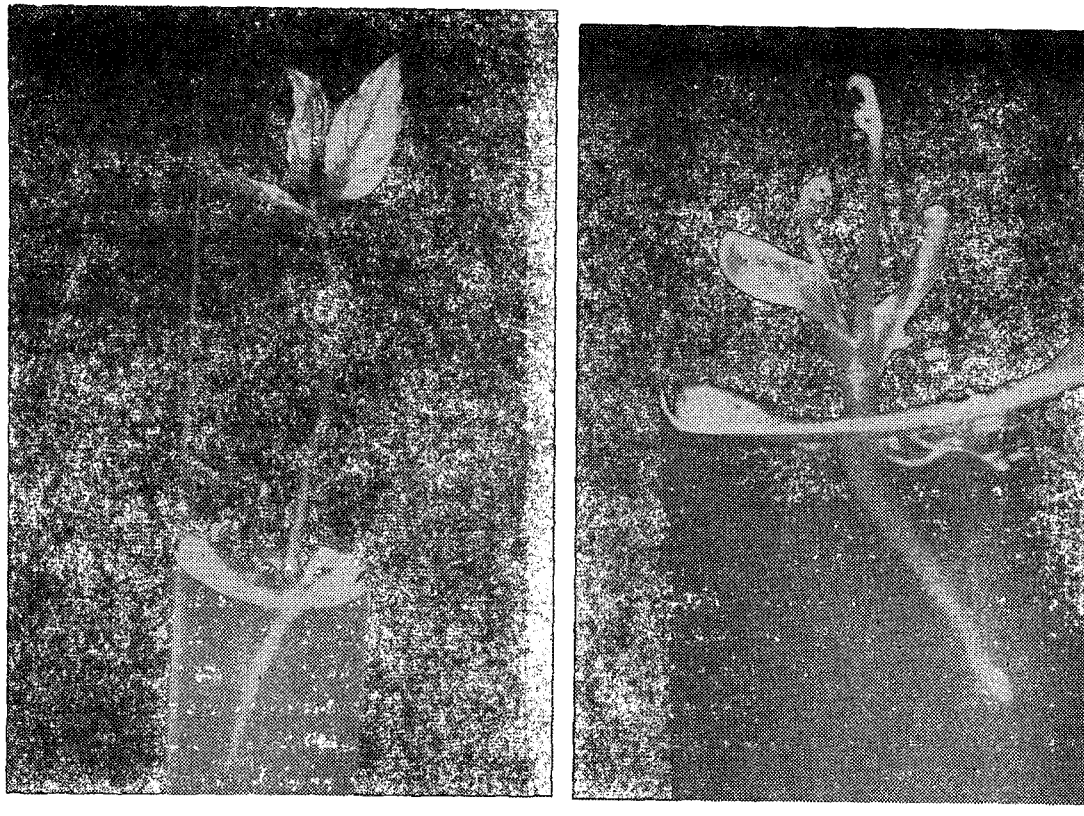

Fig. 4. Ginseng seedling developing from exiced embryo cultured in vitro.
a: Normal developing b: Abnormal developing.

stratified seeds (with seed coat) and no development of embryo excised from the endosperm which obtained from the non-stratified seeds by removing the seed coat were observed. This results also indicated that the slow germination of ginseng seeds is mainly due to the dormancy of endosperm or seed coat rather than of embryo in the stratified seeds.

SUMMARY

In order to know the effects of GA_3, Ethrel and H_2O_2 on breaking dormancy and germination in ginseng seeds, studies were carried out on the stratification and germination at Braunschweig FAL, The Institute for Botany, Geisenheim and Korea Univerity, Seoul. The results were summarized as follows:

1. GA_3 stimulated the embryo growth and increased the dehiscent percentage of ginseng seeds far more than Ethrel and H_2O_2 and the seeds started dehiscing when the embryo ratio reached around 30%. While there were no statistical differences among the treatment levels.
2. The germination ratio not only was increased but also the period of germination was shortened by soaking with GA_3 solution either before stratification or before germination than at once before stratification or at once before germination.
3. The endosperms obtained from the stratified seeds showed the highest respiration ratio in comparison with the stratified seeds and the endosperms obtained from the non-stratified seeds. The seed coat appeared to inhibit the germination of ginseng seeds in someway or other.
4. Only two embryos excised from stratified seeds developed in vitro to normal seedlings; the rest however developed uncompletely. Cotyledon and radicle growth was observed in cultured embryos as well from stratified seeds as nonstratified seeds and whole seeds after removing the seed coat. The slow germination of ginseng seeds seemed to be

due to the dormancy of endosperm or seed coat than of the embryo in the stratified phase.

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摘　　要

本 硏究는 人蔘種子의 休眠打破 및 發芽에 미치는 GA_3, Ethrel 및 H_2O_2의 影響을 알기 爲하여 實施하였는 바 GA_3는 胚의 生長과 種子의 개갑比率을 높이는데 效果的이었다. GA_3 處理는 人蔘種子의 發芽率을 높이고 發芽期間을 短縮시키는데도 效果가 있었다. 그러나 Ethrel과 H_2O_2處理는 發芽에 影響을 미치지 못했으며 GA_3의 處理濃度間에도 差異가 없었다.

本 實驗結果에 依하면 人蔘種子의 發芽速度가 늦은 것은 胚의 影響보다는 胚乳 또는 種皮의 影響을 더 크게 받는 것으로 생각되었다.