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Effects of Plant Growth Regulators on Physiology of Germinating Panax ginseng Seed.

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植物 生長調節劑가 人參 Punax ginseng) 種子의 発芽生理에 미치는 影響

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ABSTRACT

The undehiscent ginseng seed did not germinate, even if the seeds were treated with GA_3, kinetin or IAA. Only GA^3 stimulated germination of dehiscent ginseng seed. The physiological roles of gibberellic acid on stimulation of the seed germination were enhancing production of soluble carbohydrate and sucrose. Then gibberellic acid stimulated biosynthesis of insoluble cellural materials and amino acids from sugars and incorporation of amino acids into protein. The fruit coat of ginseng seed did not impede water imbibition, but did function as water absorbor and reservoir.

INTRODUCTION

The root of ginseng (Panax ginseng) has been an object of intensive studies, particularly with respect to the pharmacological properties[6,8] and clinical effects. Little is known about the physiology of ginseng seed germination.

Some plant hormones have been reported to stimulate certain seed germination. Gibberellic acid [4,11,16], and kinetine [4,7,10] have stimulatory effects on various seed germination. Mode of action of gibberellic acid on some plants is influencing the permeability of membrance systems[17], the hydrolysis of reserve materials [13], and biosynthesis of protein and RNA [1,3].

It was reported that gibberellic acid stimulated ginseng seed germination [5,9,12] However, the physiolo-ical effects of gibberellic acid in ginseng seed germination has not been clear.

On this present research, three hypotheses are proposed to explain the stimulatory effects of plant hormones on ginseng seed germination. The first hypothesis is that the hormones enhance water uptake. The second is that the hormones stimulate the hydr-olysis of reserve material and the third is that the hormones stimulate biosynthesis of new products.

MATERIALS AND METHODS

Seed : Ginseng (Panax ginseng) seeds, dehiscent and undehiscent, were given by the Ginseng Reseach Experimental Station, Gwachun. The seed surface was sterilized in 1% NaOCI soultion for 5 minutes and washed three times with sterilized water and sown on filter paper moistened with 10^{-6}M solution of GA^3 kinetin, IAA and water respectively. It was placed in plastic box and the boxes were kept in 18℃ incubator for seed germination test and water imbibition test. The days for initiation of seed germination and germination percent were monitored every day.

Analysis of soluble carbonydrate : Two ginseng

seeds from each treatment were grounuded with 5 ml of distilled water with a mortar and pestle. The homogenate was centrifuged at 2,800 rpm for 5 minutes. The supernatant was diluted 50 times with distilled water. A 2 ml sample was added to 0.2 ml of 80 % phenol in test tube. This was mixed with 5 ml of conc. H_2SO_4 and shaked at room temperature for 10 minutes, then placed in 28℃ water bath for 20 minutes. The O. D. of the reaction mixture was measured in a Spectronic 20 at 490 nm. Total soluble carbohydrate was calculated from a standard curve for glucose.

Chromatography :

Paper chromatography : Sugars in 80% ethanol of the seeds were separated on paper chromatograms using a descending solvent system of ethyl acetate : pyridine : water (8 : 2 : 1, v/v) and developed for 24 hours. Raffinose, sucrose, glucose and fructose standards were applied to the margin of all chromatograms. Sugars were located on the paper by first drawing the marker strips through a saturated solution of $AgNO_3$ in acetone and allowing to dry before drawing through 0.5 N KOH in ethanol. When the deposition of silver was completed (judged visually) the paper was dipped into a 5 % solution of $Na_2S_2O_3$ for 30 minutes, then washed in running tap water for a further 60 minutes, and finally dried. This procedure produced an intense black zone where sugars were pre present, on a light grey back ground. Elution of sugar zones from the paper was achieved by cutting and washing with 2 ml of 80 % ethanol. The amounts of sugars were analyzed by O. D. as previously described

Ion exchange chromatography : Dowex 50 and Dowex 1 ion exchange resins were packed in 2 ml syrinages to a bed volume of 1.5 ml respectively. The alcohol soluble fraction from the seeds was evaporated to dryness and dissolved in 2 ml of water. One tenth of the solution (0.2 ml) was loaded on the Dowex 50 column. The column was washed with 5 ml of water. Amino acids were retained on the column. The eluate from this column was placed on a Deowes 1 column. This column was washed with 20 ml of water. Organic acids were retained on the Dowex 1 resin. The neutral fraction (mainly sugars) which was not retained on either column was collected in a scintillation vial. Amino acids were eluted from Dowex 50 column with 10 ml of 1 N NH_4OH, and organic acids from Dowex 1 column with 10 ml of 4 N formic acid. Each fractionwas collected in a small vial and evaporated to dryness in a hot air oven.

Metabolism of ^{14}C-glucose : The seeds were on filter papers moistened with water and 10^{-6} M of GA_3 solution. 2 days after sowing, thee seeds were covered with ^{14}C– glucose (10 uC/ml) for 3 hrs. After 3 hours exposure, the seeds were quickly washed in cold water, ground and extracted with 80 % ethanol (v/v). The alcohol-soluble fraction and insoluble fraction were separated by centrifugation at 2,800 rpm for 5 minutes. The soluble fraction was chromatographed on paper, and on ion exchange chromato - graphy as previously described. The radioactivity in each fraction was determined by Deckman Liquid Scintillation system2.

Protein synthesis in germinating seed : The dehiscent ginseng seeds were sown on filter papers which were moistened with solution of 10^{-6} M of GA_3, kinetin, IAA and water, respectively. These were incubated at 10℃ for 2 weeks. Lots of 6 seeds from each treatment were exposed to ^{14}C-valine (5uC/ml) in 10^{-3} M of unradioactive valine solution as a carrier system, for 3 hours. The seeds were washed sucessively in ice-cold water, nonradioactive valine solution (10^{-4} M), and ice-cold water. The seeds were homogenized in 5 ml of 0.2 M NaCl + 1mM valine solution with a mortar and pestle. The homogenate was cent - rifuged at 12,000 x g for 15 minutes One tenth of the supernatant fraction was precipitated with an equal volume of 15 % trichloroacetic acid. The precipitate was collected on a millipore filter by filtration. The membrane was dried at 70 ℃ over night and counted by a Beckman liquid scintillation system.

RESULTS

Effects of Plant Growth Regulators on Ginseng Seed Germination : Undehiscent seeds did not germinate, even the seeds were treated with plant hormones, such as GA_3, kinetin and IAA. (Table 1). The dehiscent seeds sown in water, initiated germination

in 6 days after sowing. Their germination percent was 38% in 11 days and 61.4% in 21 days. Only GA^3

TABLE 1. Effect of plant hormones on ginseng seed germination.

Treatment	Initiation	11 days	21 days
	(day)	(%)	(%)
Water	6	38	61.4
GA_3	4	60	67.0
Kinetine	7	27	59.9
IAA	5	35	65.8

stimulated the seed germination. The seeds in GA^3 initiated germination in 4 days. The germination percent was 60% in 11 days, and 67% in 21 days. Neither kinetin, nor IAA could stimulate the seed germination (Table 1).

wing, the fresh weight of kinetin treated seeds increased to 177% of original dry weight, GA^3 treated seeds increased to 191%, IAA treated seeds increased in 197%. The seeds in water (control) increased to 189%. The seeds without fruit coats absorbed water slowly. The seeds gradually increased their fresh weight until 9 days after sowing (Fig. 2). The fresh weight of seeds treated with GA^3, IAA and water increased up to 200% of original weight. But the seeds treated with kinetin increased to 225%. (Fig. 2).

Total soluble carbohydrate and sugars : The seeds were dissected into endosperm and embryo in 1 and 2 weeks after sowing on moistened filter paper. Each portion was ground and extracted with 80% ethanol. One tenth of the extract was spotted on paper chromatogram and the separated sugars were quantitatively analyzed by O. D. Sucrose was the main composition

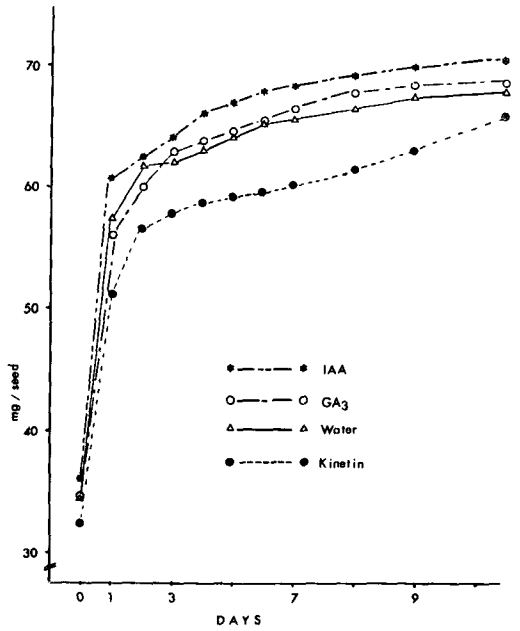

Fig. 1. Water absoption by ginseng seed.

Fig 1. Breaking strength of 3rd internode under different mixing ratios of several barley varieties.

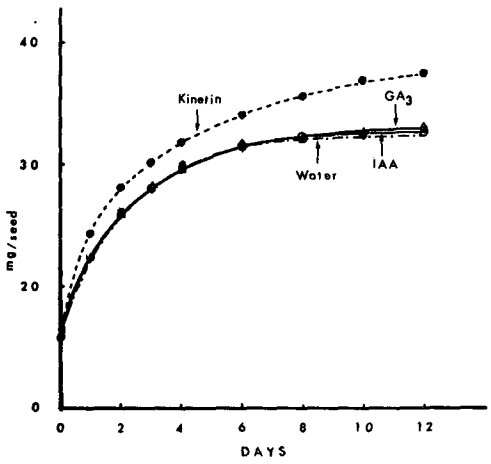

Fig. 2. Water absorption by ginseng seed without seed coat.

Fig. 2 Yield components of Kangbori +Bunong mixture

Water imbibition : The seeds with fruit coats in all treatments absorbed water rapidly in 2 days after sowing (Fig.1). After 3 days, the seeds absorbed water very little. The seed treated with kinetin absorbed water less than any other treatment. 9 days after so-

of soluble carbohydrates in both endosperm and embryo. In the dry seed, 0.55 mg and 0.5 mg of sucrose was detected from embryo and endosperm, respectively. There were 0.005 mg of glucose and trace amounts of fructose in embryo, and 0.02 mg

TABLE 2. Sugar analysis in eneosperm and embryo of germinating dehiscent ginseng seed (mg /seed)

Sugars	Embryo			Endosperm		
	0	1	2	0	1	2
Sucrose	0.55	0.22	0.07	0.50	0.54	0.56
Glucose	0.005	t	t	0.02	t[1]	t
Fructose	t	t	t	t	t	t
Total Sol. Carbohydrate	0.78	0 67	0.24	0.52	1.48	1.70

1 Trace amount.

of glucose and trace amount of fructose in endosperm (Table 2). The amount of sucrose in embryo decreased from 0.55 mg to 0.22 mg in one week and to 0.07 mg in 2 weeks. But the sucrose concentration in endosperm increased from 0.5 mg to 0.54 mg in one week and to 0.56 mg in 2 weeks. The amonut of total soluble carbohydrate in embryo decreased from 0.78 mg to 0.77 mg in one week, and to 0.24 mg in 2 weeks. However, it increased in endosperm from 0.52 mg to 1.48 mg in 1 week and to 1.7 mg in 2 weeks (Table 2).

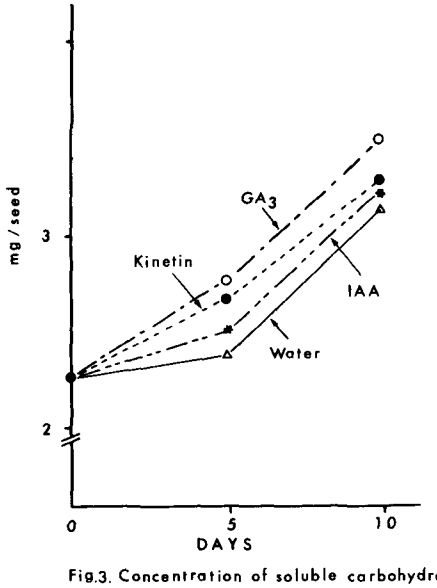

Fig.3. Concentration of soluble carbohydrate

Fig. 3 Yield components of Kangbori +Suweon 177 mixture.

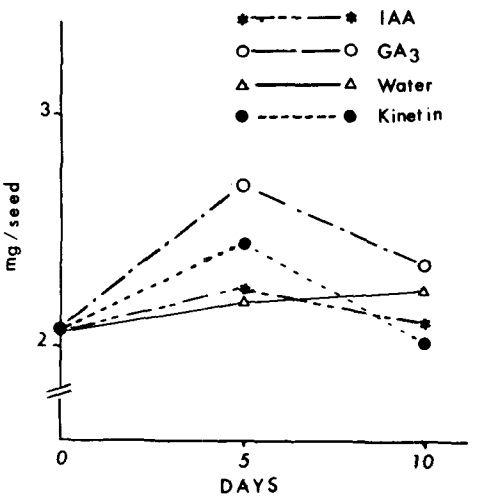

Fig. 4. Amout of soluble carbohydrate in ginseng seed without seed coat.

Fig. 4 Yield components of Kangbori +Olbori mixture.

The amount of soluble carbohydrate in the seed with fruit coat increased even in 10 days after sowing (Fig 3). But the amount in seed without fruit coat increased in 5 days, but decreased in 10 days (Fig. 4). GA_3 stimulated the production of soluble carbohydrate in both experiments (Fig. 3 & 4). It indicated that the solubilization of fruit coat and transportation of the soluble carbohydrate from fruit coat to endosperm occurred on the process of seed germination.

^{14}C-Glucose metabolism : The GA_3 treated seeds had higher radioactivity in sucrose and glucose than those of untreated seeds. The radioactivity of sucrose in GA_3 treated seeds and untreated seeds were 190 cpm (34%) and 64 cpm (14.2%) respectively. The radioactivity of glucose in GA_3 treated seeds and untreated seeds were 71 cpm (12.6%) and 33 cpm (7.3%) respectively. The untreated seeds had higher counts at unmoving materials and raffinose that GA_3 treated seeds (Table 3 & 4). GA_3 treated seeds converted ^{14}C-glucose into insoluble materials more rapidly than untreated seeds (Table 5). One week after sowing, the GA_3 treated seeds converted 76.3% of ^{14}C- glucose into insoluble cellular materials, while the untreated seeds converted only 43.8%. In two weeks, both GA_3 treated and untreated seeds converted almost same

TABLE 3. Distribution of ^{14}C—glucose in ginseng seed (2 days after soaking)

Sugars	cpm	%
Origine	228	50.5
Raffinose	122	27.0
Sucrose	64	14.2
Glucose	33	7.3
Fructose	4	1.0
TOTAL	451	100.0

TABLE 4. Distribution of ^{14}C—glucose in GA_3 treated seed (2 days after soaking)

Sugars	cpm	%
Origine	204	36.4
Raffinose	78	14.0
Sucrose	190	34.0
Glucoes	71	12.6
Fructose	16	3.0
TOTAL	559	100.0

TABLE 5. Rate of ^{14}C—glucose converted into insoluble cellular material in germinating ginseng seed.

Treatment		0 wk	1 wk	2 wks
Water	Total (cpm)	174	202	389.3
	% of sol. material	85.7	57.2	27.8
	% of insol. material	16.3	428	72.2
GA_3	Total (cpm)	—	240.0	370.0
	% of insol. material	—	23.7	27.0
	% of insol. material	—	76.3	73.0

amount (73%) of ^{14}C-glucose into insoluble materials (Table 5). Alcohol soluble portion of the extract was further separated by ion exchange chromatography by using Dowex 50 and Dowex 1 resines. Redioactivities in amino acids were 152 cpm (45.8%), 92 cpm (32.7%) and 121 cpm (40.0%) from the seeds treated with GA_3, kinetin and water, respectively. It indicated that GA_3 treated seeds produced more amino acids than any other treated seeds (Table 6).

Protein synthesis : The seeds treated kinetin (2185.4 cpm) and GA_3 (2008 cpm) absorbed more ^{14}C-valine than water (1638.6 cpm) or IAA 1781.4 cpm) treated seeds (Table 7). ^{14}C-valine incorporation into protein was the highest in the seeds treated with GA_3 among all treatments (Table 7).

TABLE 6. Effect of plant hormones on converion of ^{14}C—glucose in ginseng seed1

	GA_3		Kinetine		Water	
	cpm	%	cpm	%	cpm	%
Sugar	180	54.2	189	67.5	188	60.0
Amino acid	152	45.8	92	32.7	121	40.0
Organic acid	0	0	0	0	0	0

TABLE 7. ^{14}C—valine incorporation into protein in dehiscent ginseng seed (2 weeks after soaking)

Treatment	Total (cpm)	Incorporation (cpm)	%
Water	1638.6	116	7.0
GA_3	2008.0	161	8.0
Kinetine	2185.4	144	6.6
IAA	1781.4	94	5.2

DISCUSSION

The undehiscent seed did not germinate, even the seeds were treated with GA_3, kinetin or IAA (Table 1) It indicated that the three plant hormones could not replace the dehiscence process and the inability of germination of undehiscent seed might not due to the lack of the above hormones. It took 21 days for dehiscent seeds to reach 60% germination percent in wa-

ter. Almost the same germination percent obtained in kinetin and IAA treated seeds. While the seeds treated with GA_3 were stimulated germination. They germinated 60% in 11 days (Table 1). The same results were reported by others [9,12]. The question was was how GA_3 stimulated the seed germination. To solve this problem, the three hypotheses were proposed. The first hypothesis was that GA_3 might inhance water uptake, this resulted acceleration of the seed germination. Wood and Paleg (1972) reported that GA_3 influenced the permeability of model membrane system. To determine water uptake, the fresh weight of the ginseng seeds was weighed every day. The fresh weight of the seed with fruit coat rapidly increased in 2 days after sowing (Fig. 1) and the weight of the seed without fruit coat slowly increased until 9 days (Fig. 2). It indicated that fruit coat of ginseng seed did not impede water imbibition, but worked as a water absorbor and reservoir. The fresh weight of GA_3 treated seed equally increased as those of other treatments, such as kinetin, IAA and water. This result did not support the first hypothesis. GA_3 could not stimulate water imbibition.

The second hypothesis was that GA_3 stimulated breakdown of reserve materials and transport the material into newly building parts. The amount of soluble carbohydrate and composition of sugars in endosperm and embryo of germinating ginseng seed were monitored. The interesting thing is the main composition of soluble carbohydrate in the seed (Table 2). The amount of sucrose and soluble carbohydrate in embryo decreased by time. Possibly they were used up for respiration and transformed into insoluble cellular materials for process of germination, such as cell wall. The amount of sucrose and soluble carbohydrate in endosperm increased (Table 2). Since the demand of soluble carbohydrate in embryo became high, the production of the soluble carbohydrate in endosperm should be high to catch up with the demand. The amount of soluble carbohydrate in the seed with fruit coat increased, while the amount in the seed without fruit coat decreased in 10 days after sowing. It showed the possibility that fruit coat was hybrolyzed into soluble carbohydrate and this was absoraed by the seed. The hydrolyzation of the fruit coat occurred late. The results (Fig. 3 & 4) showed the amount of total soluble carbohydrate in seed with fruit coat and without fruit coat were almost same in 5 days, but there were big difference in the amout between them in 10 days after sowing. GA_3 treated seeds contained higher level of soluble carbohydrates than any other treatment (Fig. 3 & 4). It indicated that GA_3 stimulated hydrolyzatition of reserve material in endosperm, which was ready to move to the growing tissues. This results suppoport the second hypothesis.

The third hypothesis was that GA_3 stimulated biosynthesis of new products. ^{14}C-glucose was given to the seeds treated with GA_3 and nontreated seeds, in order to find if GA_3 affected synthesis of sucrose which is the main sugar in ginseng seed and movable from in plant tissue. GA_3 treated seeds converted more ^{14}C-glucose to sucrose (Table 3 & 4) and produced more insoluble cellular materials than untreated seeds (Table 5). In order to grow, the tissue of germinating seed synthesize amino acids from sugars. Because amino acids are the building blocks for enzymes and structural proteins. This process was investigated by admitting ^{14}C-glucose to the seeds, and the radioactivity in amino acid fractions were counted. The activity in amino acids from GA_3 treated seeds was twice as much as that from untreated seeds (Table 6). Finally, a test was made to see if GA_3 could enhance biosynthesis of protein. ^{14}C-valine incorporation was investigated. The GA_3 treated seeds synthesized more protein than anyother treatment (Table 7).

For the conclusion, it is possible clue that the stimulatory effects of GA_3 on germination of ginseng seed were enhancing production of soluble carbohydrate and sucrose which is movable form of sugar. Then GA_3 stimulated production of insoluble cellural materials and amino acids from sugars. Also GA_3 enhanced production of protein from amino acids, as Chen and Park [1] reported.

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적 요

본 연구는 식물 생장조절제가 인삼 종자의 발아 생리에 미치는 영향을 구명코져 실시 하였으며, 그 결과를 아래와 같이 요약할 수 있었다.

1. 미개갑종자는 전연 발아하지 못하였고, 지베레린, Kinetin 및 IAA를 처리하였어도 발아하지 못하였다. 즉, 미개갑종자가 발아치 못하는 것은 上記 식물생장 조절제와는 관계가 없는 것 같았다.

2. 개갑종자는 파종후 21일이면 60%의 발아율을 보였고, kinetin과 IAA처리구에서도 비슷한 결과를 보였으므로, 위의 두 생장조절제는발아촉진 효과를 보이지 않았으나, 지베레린 만은 발아를 촉진시켜 처리후 11일에 60%이상 발아 되었다.

3. 지베레린은 수분흡수에 영향을 끼치지 않는

것 같고, 수분흡수 속도와 발아율과는 관계가 없는 것 같음. 종피는 수분흡수를 조장 및 수분저장 역할을 하였다.

4. 인삼종자에 내포된 당류는 주로 Sucrose 이였다. 발아가 진전됨에 따라 胚에서는 sucrose와 가용성 탄수화물의 양이 줄어드는 반면, 배유에서는 그들의 함량이 늘어났다. 지베레린 처리종자는 가용성 탄수화물의 생성이 많았다. 발아과정 후기에서는 종피에서도 탄수화물의 공급 가능성을 보여 주었다.

5. 방사선 동위원소를 포함한 glucose를 사용한 결과, 지베레린 처리종자는 sucrose와 불용성 물질의 합성이 속하였다.

6. 지베레린처리 종자는 당류를 아미노산으로의 전환이 속하고, 또한 아미노산을 단백질로 합성능력이 높았다.

7. 즉, 지베레린의 인삼종자 발아촉진 효과에 대한 생리학적 역할은 저장양분의 가수분해 촉진, 가수분해된 물질의 신진대사 촉진, 즉 아미노산 및 단백질 합성을 조장하는 것 같았다.