韓藥作誌(Korean J. Medicinal Crop Sci.) 32(5) : 295 – 304 (2024)
ISSN(Print) 1225-9306
ISSN(Online) 2288-0186
http://www.medicinalcrop.org
http://dx.doi.org/10.7783/KJMCS.2024.32.5.295

식물생장조절제를 이용한 인삼 개갑률 향상 및 이후 발아와 식물체 형성에 미치는 영향

김소정[1] · 김장욱[2] · 이정우[3†]

Improvement of Seed Dehiscence using Plant Growth Regulators and Its Effect on Subsequent Germination and Growth of *Panax ginseng* C. A. Meyer

So-Jeong Kim[1], Jang-Uk Kim[2], and Jung-Woo Lee[3†]

Received: 2024 August 12
1st Revised: 2024 August 28
2nd Revised: 2024 September 18
3rd Revised: 2024 September 22
Accepted: 2024 September 22

ABSTRACT

Background: Plant growth regulators are frequently used to improve the dehiscence rate of seeds in *Panax ginseng* C. A. Meyer. However, research on the appropriate type and concentration of treatment is lacking. In addition, there is no information on the effects of plant growth regulator treatments on germination and plant formation before dehiscence.
Methods and Results: Indehiscent seeds were soaked in gibberellic acid (GA_3) and kinetin at concentrations of 10 ㎎/ℓ – 300 ㎎/ℓ for 24 hours. The highest dehiscence rate was observed with the 10 ㎎/ℓ GA_3 treatment. The dehiscence rate was significantly lower than that of the control, and the seed contamination rate significantly increased in the treatments containing 100 ㎎/ℓ or more GA_3. GA_3 treatment also affected the types of dehiscent seeds and embryo development. However, no differences were observed in the dehiscence rate or embryo development between the kinetin-treated and control groups. GA_3 treatment before warm stratification affected seed germination but did not significantly affect plant development.
Conclusions: In this study, we determined the type and concentration of plant growth regulators suitable for improving dehiscence and forming stable *P. ginseng* C. A. Meyer plants were presented. These results are expected to contribute to stable ginseng seed production.

Key Words: *Panax ginseng* C. A. Meyer, Dehiscence, Embryo, Germination, Gibberellic Acid, Kinentin

INTRODUCTION

Korean ginseng (*Panax ginseng* C. A. Meyer), a perennial herbaceous plant, is a representative medicinal plant in Korea that has been proven effective in treating various diseases due to its excellent pharmacological properties. Although new propagation methods based on plant tissue culture have been developed (Lee *et al*., 2023a, b, c), the primary propagation method for ginseng remains seed propagation.

Ginseng requires at least 3 years to produce seeds from the mother plant due to its juvenility (Kim *et al*., 2016). Ginseng seeds have a morphologically dormant characteristic, in which the seed coat is very hard and closed (Min *et al*., 2022). Additionally, the zygotic embryo of the seed is too small to germinate. Therefore, after-ripening is essential for embryo development (Kim *et al*., 2014a). The development of the undifferentiated embryo in ginseng is highly influenced by the flesh and endocarp (Lim *et al*., 2008).

Ginseng seed ripening takes approximately 18 months under natural conditions. To shorten this period, seeds are artificially subjected to stratification through a process known as dehiscence (Lee *et al*., 2018). Even after dehiscence, the zygotic embryo remains immature and requires additional cold treatment for more than 3 months to break its physiological dormancy. This

†Corresponding author: (Phone) +82-43-871-5612 (E-mail) enzymer@korea.kr
[1]농촌진흥청 인삼특작부 연구사 / Researcher, Department of Herbal Crop Research, NIHHS, RDA, Eumseong 27709, Korea.
[2]농촌진흥청 인삼특작부 연구사 / Researcher, Department of Herbal Crop Research, NIHHS, RDA, Eumseong 27709, Korea.
[3]농촌진흥청 인삼특작부 연구사 / Researcher, Department of Herbal Crop Research, NIHHS, RDA, Eumseong 27709, Korea.

cold treatment is known as cold stratification (Suh *et al*., 2022). Various factors affect the dehiscence rate of ginseng seeds, including variety, seed condition, and seed harvest time (Kim *et al*., 2014a).

Seeds are an important stage in the plant life cycle (Soppe and Bentsink, 2020). Seed dormancy and germination are complex physiological processes controlled by both endogenous and environmental factors (Yang *et al*., 2020).

Plant growth regulators, such as abscisic acid (ABA) and gibberellin (GA), have a direct effect on the regulation of seed dormancy and germination, and their balance is an essential factor (Tuan *et al*., 2021). Exogenous plant growth regulators can improve the dehiscence and germination of ginseng seeds.

For example, GA and cytokinins directly affect the dormancy and germination of ginseng seeds (Kwon and Lee, 1997). In particular, GA has a significant impact on the development of early embryos (Hu *et al*., 2018), and treatment with GA_3 is known to improve the seed dehiscence rate and promote the breaking of dormancy in ginseng (Kim *et al*., 2014a; Lee *et al*., 2016).

In addition, the treatment of ginseng seeds with exogenous GA_3 promotes embryo development (Lee *et al*. 2018). Improvements in germination rates due to GA_3 treatment have been reported in other medicinal plants (Han *et al*., 2022).

Cytokinins are essential plant growth regulators involved in cell differentiation, the movement of inorganic and organic nutrients, and senescence (Skoog and Armstrong, 1970). Also, cytokinins can affect germination and break dormancy (Prasad *et al*., 1983; Kabar, 1998).

In previous studies, kinetin, a cytokinin, was found to have a positive effect on dormancy breaking and sprouting in one-year-old ginseng (Park *et al*., 1979), and improved the dehiscence rate of ginseng (Lee *et al*., 2018). However, there has been no research thus far on the effects of treatment with plant growth regulators before dehiscence on subsequent seed germination and plant growth, and further research on this is needed.

In this study, the effects of GA_3 and kinetin treatment on the dehiscence of ginseng seeds were confirmed. Additionally, the germination of seeds treated with GA_3 for dehiscence and their subsequent growth characteristics were investigated to evaluate the stability of treatment with growth regulators before dehiscence.

MATERIAL AND METHOD

1. Plant materials

'Gumpoong' berries were harvested from Eumseong, Chungcheongbuk-do, Korea (latitude 36°94, longitude 127°75) at the end of July 2023. The berries were washed and rubbed by hand to completely remove the sarcocarp. Indehiscent seeds were soaked in running water for 24 hours.

2. Plant growth regulators treatment

GA_3 (Duchefa Biochemie, Haarlem, Netherlands) was used for GA treatment, and kinetin (Duchefa Biochemie, Haarlem, Netherlands) was used for cytokinin treatment. Each plant growth regulator was dissolved in a solvent and diluted in dH_2O to concentrations of 10, 50, 100, and 500 ㎎/ℓ. Indehiscent seeds (15 g) were soaked in each plant growth regulator for 24 hours, or in dH_2O as a control.

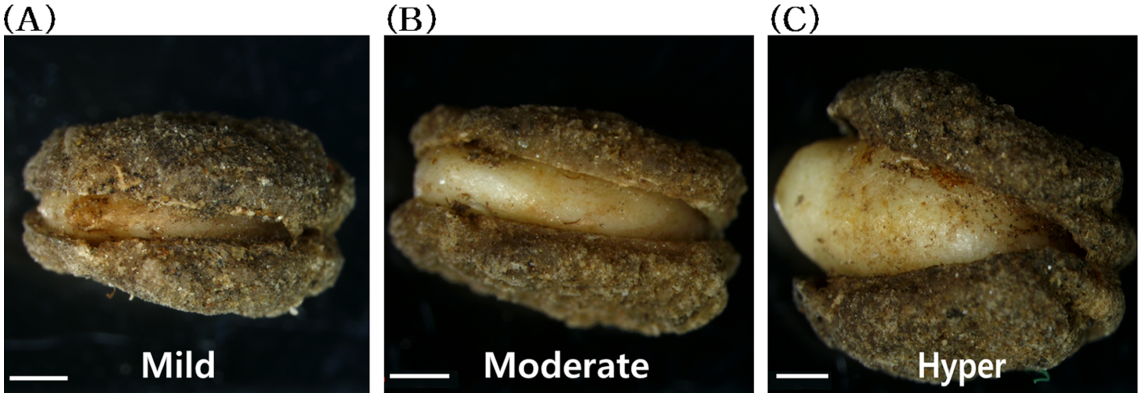

Fig. 1. Types of dehiscent seed according to the size of the seed coat width of *P. ginseng* C. A. Meyer. (A) mild type, seed coat width: < 0.9 ㎝, (B) moderate type, seed coat width: 0.9 ㎝ - 1.5 ㎝, (C) hyper type, seed coat width: > 1.5 ㎝. Scale bar; 1 ㎝

3. Seed dehiscence

The treated indehiscent seeds were stratified according to a previously described method (Lee *et al*., 2018). Irrigation was performed twice a day for 45 days after the start of stratification, once a day from days 46 to 80, and once every two days thereafter. After 90 days of stratification, the seeds were considered dehiscent once the seed coats had split. In addition, the contamination rate of the seeds by fungi and bacteria was assessed. Dehiscent seeds were classified into three groups according to the length of the seed coat (Fig. 1): seeds with a width less than 0.9 ㎝ were classified as mild type, between 0.9 ㎝ - 1.5 ㎝ as moderate type, and greater than 1.5 ㎝ as a hyper-type.

4. Seed development

Dehiscent seeds were randomly sampled (30 seeds from each treatment). Seed coats were removed for seed development analysis, and the embryo and endosperm were observed after cutting half of the seeds.

The lengths of the embryo and endosperm were measured using a microscope (S8AP0, Leica, Wetzlar, Germany), and the ratio of embryo to endosperm length was calculated using previously described methods (Lee *et al*., 2018).

5. Germination

The dehiscent seeds, excluding kinetin-treated seeds whose dehiscence rate was not different from that of the control, were stored in cold storage at 2℃ for 90 days to break dormancy. For each treatment, 50 dehiscent seeds were sown in a 100 ㎝ × 20 ㎝ petri dish containing artificial soil (Nonggyung, Jincheon, Korea) consisting of peat and perlite in a 7 : 3 ratio.

This process was repeated three times. The sown seeds were placed in an incubator (VS-1203PFC-L, Vision Scientific Co. Ltd., Daejeon, Korea) maintained at 15℃ and kept away from light. Seed germination was monitored daily for 30 days. A radicle longer than 1 ㎜ was considered germinated, and the germination rate, mean germination time, and T_{50} were calculated using methods described in a previous study (Lee *et al*., 2018).

6. Growth of aerial and underground parts

Germinated seeds were transplanted into plastic boxes containing artificial soil (Nonggyung, Jincheon, Korea). Sixty days after sowing, the characteristics of the aerial parts, including length, stem diameter, leaf length, and leaf width, were measured for 20 plants in each treatment group.

Then, 150 days after sowing, the characteristics of underground parts, including root length, root diameter, and root weight, were measured (n = 20 for each treatment).

7. Statistical analysis

Statistical analyses were conducted using R (R version 4.1.2, R Foundation for Statistical Computing, Vienna, Austria). Significance was tested using analysis of variance (ANOVA). When significance was confirmed, post hoc analysis was performed using Duncan's Multiple Range Test (DMRT) at the 5% level ($p < 0.05$).

RESULTS

1. Seed dehiscence

The dehiscence rate of the seeds was investigated after 90 days of stratification. The highest dehiscence rate of 97.2% was observed in the group treated with 10 ㎎/ℓ of GA_3 (Fig. 2A).

In the 50 ㎎/ℓ GA_3 treatment group, the dehiscence rate was 89.4%, but there was no significant difference compared to the control group (88.9%). The 100 ㎎/ℓ GA_3 treatment group exhibited a dehiscence rate of 81.8%, which was significantly lower than that of the control group. In the 300 ㎎/ℓ GA_3 treatment group, the dehiscence rate was 42.8%.

No seed contamination was observed in the control group. However, in the GA_3 treatment group, the contamination rate increased with the concentration. In particular, in the GA_3 300 ㎎/ℓ treatment group, 42.7% of the seeds were contaminated (Fig. 2A), and as the seeds grew excessively, the seed coat fell off naturally; in severe cases, early germination was also observed (Fig. 2B).

There was no significant difference compared to the control group in kinetin concentration from 10 to 100 ㎎/ℓ. However, the 300 ㎎/ℓ kinetin treatment group had a significantly lower dehiscence rate than the control group. Seed contamination was not observed in the kinetin-treated groups.

The effect of the concentration of treated plant growth regulators on the type of dehiscent seeds was investigated. In the control group, the moderate type was most frequently identified, followed by the mild type; the hyper-type was not observed (Fig. 2C).

As the GA_3 concentration increased, the proportion of hyper-types increased. In the 100 ㎎/ℓ GA_3 treatment group, the proportion of hypertype was exceeded 50%, and in the 300 ㎎/ℓ

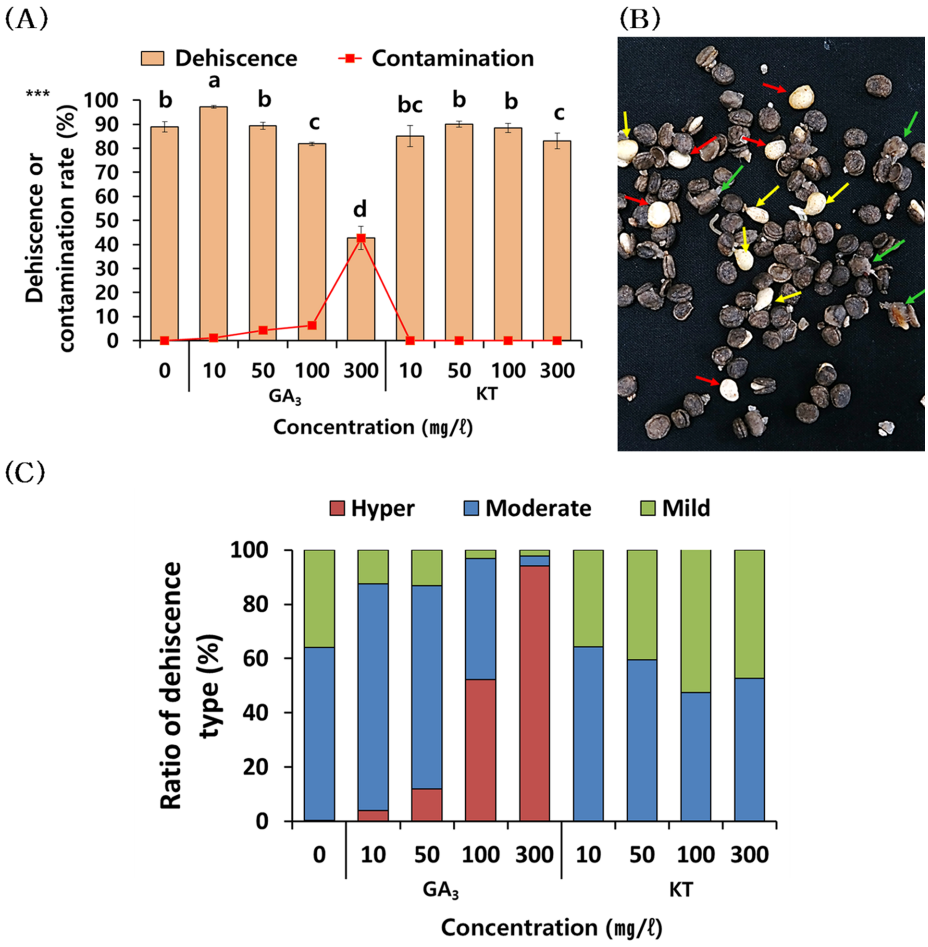

Fig. 2. Effects of plant growth regulators on dehiscence after 90 days of warm stratification in *P. ginseng* C. A. Meyer. (A) dehiscence and contamination rates according to plant growth regulators treatment, (B) characteristics of dehiscence by GA_3 300 ㎎/ℓ treatment before warm stratification. Red arrow; seeds with detached seed coat due to excessive dehiscence, yellow arrow; early germinated seeds, green arrow; contaminated seeds. (C) ratio of dehiscence types by concentration of plant growth regulators.

GA_3 treatment group, most seeds were observed to be hypertype. The moderate type was maximized at 83.5% in the 10 ㎎/ℓ GA_3 treatment group, followed by the 50 ㎎/ℓ GA_3 treatment group and the control group. In contrast, in the kinetin-treated groups, the hyper-type was not observed, and the proportion of the moderate type was lower in the kinetin-treated groups than in the control group.

2. Embryo growth

The lengths of the endosperm and embryo were measured for each treatment on seeds for which the dehiscence investigation was completed. GA_3 treatment before dehiscence had a significant effect on both endosperm and embryo length. The length of the endosperm was 4.7 ㎝ in the control group and increased with GA_3 concentration, reaching 5.8 ㎝ in the 300 ㎎/ℓ GA_3 treatment group (Fig. 3A). On the other hand, kinetin treatment slightly increased the size of the endosperm at 50 ㎎/ℓ and 100 ㎎/ℓ compared to the control, but no clear difference was observed.

Embryo length increased significantly with an increasing GA_3 concentration (Fig. 3B). The embryo length was 2.2 ㎝ in the control group, but it reached 4.3 ㎝ in the 300 ㎎/ℓ GA_3 treatment group. However, no significant differences were observed between the kinetin-treated and control groups.

In the control group, the embryo-to-endosperm length ratio was 0.46 (Fig. 3B). In the GA_3 treatment groups, the ratio was significantly higher than in the control and increased with increasing GA_3 concentration. The ratio was maximized at 0.74

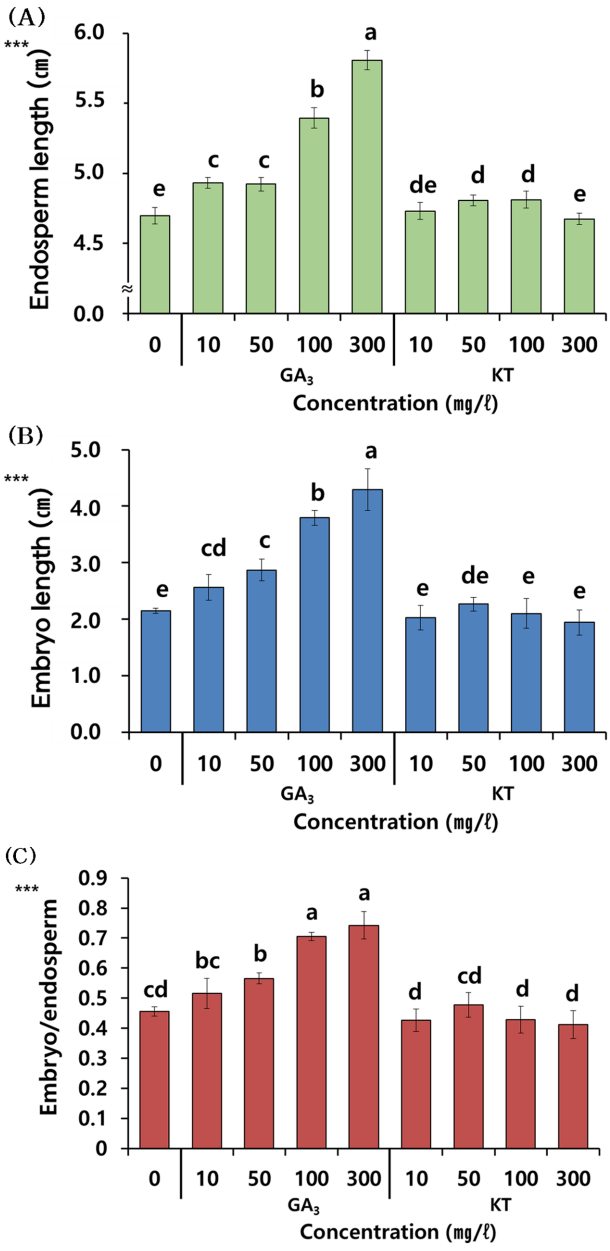

Fig. 3. Effects of plant growth regulators on endosperm and embryo length after 90 days of warm stratification in *P. ginseng* C. A. Meyer. (A) endosperm length, (B) embryo length, (C) ratio of embryo to endosperm length.

in the 300 ㎎/ℓ GA_3 treatment group. However, no significant differences were observed between kinetin-treated and control groups.

3. Germination

Dehiscent seeds were sown in the soil 90 days after cold treatment, and germination was observed for 30 days. The first germination was observed 3 days after sowing in the 100 ㎎/ℓ and 300 ㎎/ℓ GA_3 treatment groups (Fig. 4A).

In the 10 ㎎/ℓ and 50 ㎎/ℓ GA_3 treatment groups, the first germination was observed 7 days after sowing, and the control group germinated 8 days after sowing. The germination rate in the GA_3 treatment group was significantly higher than that in the control group (65.3%). The highest rate was 92.0% in the 100 ㎎/ℓ GA_3 treatment group, and no significant difference was observed depending on the GA_3 concentration (Fig. 4B).

The mean germination time was shortest at 14.3 days and 14.9 days in the 100 ㎎/ℓ and 300 ㎎/ℓ GA_3 treatment groups, respectively. As the GA_3 concentration decreased, the mean germination time gradually increased, and the longest germination time of 19.1 days was observed in the control group (Fig. 4C).

The T_{50} was also lowest in the 100 ㎎/ℓ and 300 ㎎/ℓ GA_3 treatment groups at 13.2 days and 13.3 days, respectively. As the GA_3 treatment concentration decreased, the T_{50} increased, reaching the longest time in the control group at 18.3 days (Fig. 4D).

4. Growth of aerial and underground parts

Germinated seeds were examined for the growth of aerial parts 60 days after transplantation into the greenhouse. Stem length was significantly increased in the 50 ㎎/ℓ and 100 ㎎/ℓ GA_3 treatment groups compared to the control group, and the other GA_3 treatment groups were similar to the control group (Table 1).

No significant difference was observed in the stem diameter. In contrast, leaf length was significantly increased in the 50 ㎎/ℓ and 100 ㎎/ℓ GA_3 treatment groups compared with that in the control group. The leaf length of the 10 ㎎/ℓ GA_3 treatment group was similar to that of the control group, whereas in the 300 ㎎/ℓ GA_3 treatment group, the leaf length was significantly reduced compared to the control group. Leaf widths were similar in all treatments except in the 300 ㎎/ℓ GA_3 treatment group.

The characteristics of the underground parts were also investigated. The longest root length was observed in the 50 ㎎/ℓ GA_3 treatment group, although no significant difference was observed compared to the other treatment groups, including the control (Table 2).

The root diameter was largest in the 10 ㎎/ℓ GA_3 treatment group, but there was no significant difference compared to the

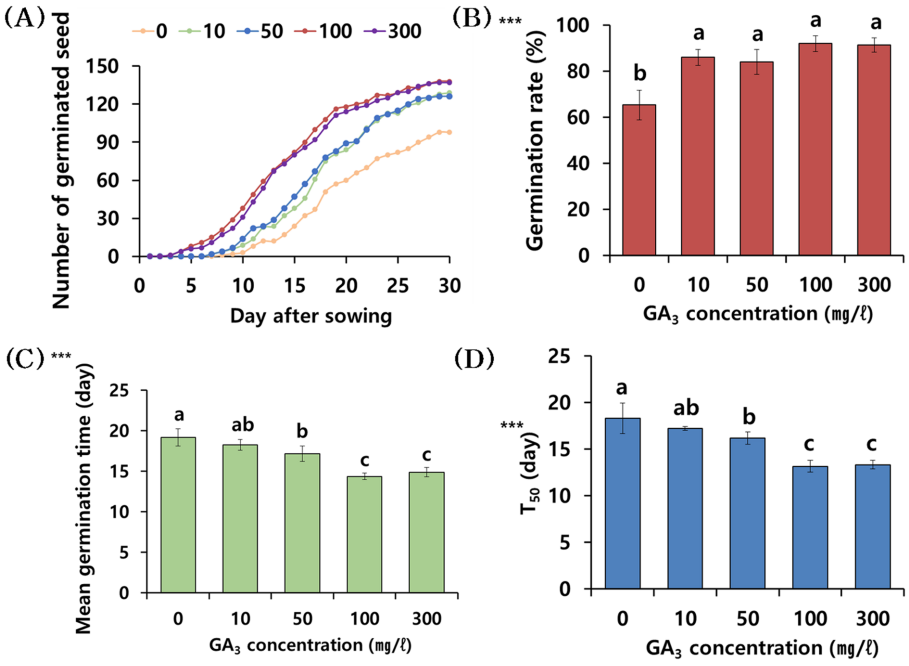

Fig. 4. Characteristics of germination by GA_3 treatment before warm stratification in *P. ginseng* C. A. Meyer. (A) cumulative germination, (B) germination rate, (C) mean germination time, (D) T_{50}.

Table 1. Characteristics of aerial parts in *P. ginseng* C. A. Meyer by GA_3 treatment before warm stratification.

GA_3 (mg/ℓ)	Stem length (cm)	Stem diameter (mm)	Leaf length (cm)	Leaf width (cm)
0	$4.9\pm0.8^{b***}$	1.13 ± 0.13^{ns}	$4.0\pm0.4^{b***}$	$2.1\pm0.2^{a***}$
10	4.9 ± 0.7^{b}	1.11 ± 0.12	4.1 ± 0.4^{b}	2.1 ± 0.2^{a}
50	6.0 ± 0.8^{a}	1.21 ± 0.09	4.5 ± 0.4^{a}	2.1 ± 0.2^{a}
100	5.8 ± 0.7^{a}	1.19 ± 0.12	4.3 ± 0.4^{a}	2.1 ± 0.2^{a}
300	4.9 ± 0.9^{b}	1.13 ± 0.21	3.7 ± 0.6^{c}	1.9 ± 0.3^{b}

Data were collected 60 days after seed sowing. Values represent the mean of three independent experiments. ^{NS}; not significant. Significant differences ($^{***}p < 0.001$) were determined using one-way ANOVA. Different letters indicate significant differences according to Duncan's Multiple Range Test (DMRT, $p < 0.05$).

Table 2. Characteristics of underground parts in *P. ginseng* C. A. Meyer by GA_3 treatment before warm stratification.

GA_3 (mg/ℓ)	Root length (cm)	Root diameter (mm)	Root weight (g)
0	12.2 ± 3.8^{ns}	3.36 ± 0.56^{ns}	0.27 ± 0.07^{ns}
10	13.1 ± 3.1	3.40 ± 0.68	0.27 ± 0.05
50	13.8 ± 3.5	3.25 ± 0.49	0.30 ± 0.06
100	12.2 ± 2.4	3.35 ± 0.49	0.29 ± 0.07
300	12.9 ± 2.2	3.31 ± 0.71	0.27 ± 0.07

Data were collected 150 days after seed sowing. Values represent the mean of three independent experiments. ^{NS}; not significant.

other treatments. Similarly, the root weight was highest in the 50 mg/ℓ GA_3 treatment group, although no significant difference was observed between the treatments.

DISCUSSION

Dehiscence, the starting point of seed production, is an important process in ginseng cultivation. Ginseng seeds are hard seeds that can take up to 21 months to germinate without artificial treatment (Lee *et al.*, 2018). Therefore, the after-ripening process of seeds is essential, and various attempts have been made to improve their dehiscence rate. The dehiscence rate has been improved by endophytic microbes (Kim *et al.*, 2017), as well as by treatment with plant growth regulators (Kim *et al.*, 2014a; Lee *et al.*, 2018). Some farmers treat seeds with GA_3 for stable dehiscence. However, research on the appropriate concentrations of plant growth regulators for seed dehiscence and their effects on plant growth remains scarce. In

addition, in South Korea, the use of plant growth regulators for the dehiscence of ginseng seeds requires an official safety evaluation and registration of the substances. However, there is a lack of information regarding this process.

In this study, the effects of treatment with GA_3 and kinetin on dehiscence in *P. ginseng* C. A. Meyer were evaluated. The dehiscence rate in the 10 ㎎/ℓ GA_3 treatment group improved by 9.3% compared to the control group (Fig. 2A). On the other hand, the 50 ㎎/ℓ GA_3 treatment group showed no significant difference compared to the control. When treated with more than 100 ㎎/ℓ GA_3, the dehiscence rate significantly decreased compared to the control. In particular, when 300 ㎎/ℓ of GA_3 was applied, the dehiscence rate sharply decreased as the contamination rate increased. Our results contradict a previous study, which found that the dehiscence rate increased as the GA_3 concentration increased up to 300 ㎎/ℓ. These varying results may be due to differences in cultivar and harvested seed conditions, as well as differences in the environmental conditions under which the seed-mother plants grew during the test year, resulting in differences in the maturity state of the seeds. Additionally, in this study, contaminated seeds were considered indehiscent, which is thought to have influenced the difference in dehiscence rates between the present study and those reported in previous studies. A similar study was reported in *Eleutherococcus senticosus*, a close relative of ginseng, where the dehiscence rate increased with the GA_3 concentration up to 500 ㎎/ℓ, but at 700 ㎎/ℓ, the dehiscence rate decreased as the contamination rate increased (Lim *et al.*, 2008). The increase in contamination is believed to be due to excessively high GA_3 concentrations, which overly stimulated the cell development of the zygotic embryo, hindering its normal differentiation and maturation. Further research is required to elucidate this issue.

In contrast, kinetin treatment did not cause a significant difference in seed dehiscence or contamination rates compared to the control group (Fig. 2A). Contrast to the results of the present study, Lee *et al*. (2018) found that treatment with 50 ㎎/ℓ of GA_3 or 100 ㎎/ℓ of kinetin significantly increased the dehiscence rate compared to the control. Kim *et al*. (2014b) reported that the treatment of indehiscent seeds with more than 100 ㎎/ℓ of GA_3 significantly increased the dehiscence rate, while treatment with 10 ㎎/ℓ of GA_3 showed no difference from the control.

An evaluation of the effect of plant growth regulators on the dehiscence type revealed clear differences between the GA_3 treatment groups (Fig. 2C). In the control group, most of the dehiscent seeds were of the moderate or mild type, whereas very few were hyper-types. In contrast, in the GA_3 treatment groups, as the concentration increased, the proportion of hyper-types increased, and in the 100 ㎎/ℓ GA_3 treatment group, more than half of the seeds were hyper-types.

This study is the first to report on the effect of GA_3 treatment on the type of dehiscent seeds, demonstrating that GA_3 treatment has a significant effect not only on the dehiscence rate but also on the type of dehiscent seeds. In contrast, no significant difference was observed between the kinetin treatment groups and the control group before dehiscence.

The size of the endosperm in ginseng seeds is known to grow rapidly until 30 days after fertilization, and does not change significantly thereafter. Before seed dehiscence, the length of the embryo is about 500 ㎛, increasing rapidly after 1 month (Kim *et al.*, 2014a). However, there have been no previous studies on changes in embryo and endosperm size caused by treatment with plant growth regulators before dehiscence.

In this study, the effects of GA_3 and kinetin treatments on endosperm and embryo growth before dehiscence were evaluated. GA_3 treatment before dehiscence increased the endosperm size, and as the concentration of GA_3 increased, the endosperm size significantly increased (Fig. 3A).

For example, the endosperm size in the 300 ㎎/ℓ GA_3 treatment group increased by over 20% compared to the control group. Embryo size also increased after GA_3 treatment before dehiscence, increasing with the concentration (Fig. 3B). The embryo size of the 300 ㎎/ℓ GA_3 treatment group increased by approximately two-fold compared to the control group. The ratio of embryo length to endosperm length also increased as the GA_3 concentration increased, reaching 0.74 in the group treated with 300 ㎎/ℓ GA_3 (Fig. 3C).

After dehiscence, in seeds exposed to low temperatures, the abscisic acid (ABA) concentration decreases as the GA_3 concentration rapidly increases (Kim *et al*., 2014b). External GA_3 treatment increased GA and decreased ABA in the seeds, thereby promoting embryo development in the GA_3 treatment group. Similarly, Lee *et al*. (2018) found that GA_3 treatment had a positive effect on seed maturation. However, kinetin treatment showed no differences, except for some parts of the endosperm length, compared to the control group (Fig. 3A, B, C).

Germination is an important process for plant development and is affected by endogenous GA and ABA (Chen *et al*., 2021). As the amount of GA in the seed increases, ABA

activity decreases, thereby inducing germination (Chen *et al*., 2008). In ginseng seeds, the GA content increases rapidly starting 30 days after dehiscence is completed, after which the ABA content tends to decrease rapidly (Kim *et al*., 2014b).

In this study, the germination characteristics of the control and GA_3 treatment groups were evaluated, excluding the kinetin treatment group, which showed little improvement in dehiscence. In the GA_3 treatment group, as the treatment concentration increased, the initial germination period became shorter than that of the control, and GA_3 treatment before dehiscence was considered to have a positive effect on breaking dormancy (Fig. 4A). The germination rate was low in the control group (65.3%) (Fig. 4B), and cold treatment for 90 days was thought to be insufficient to break dormancy (Lee *et al*., 2018).

A previous study reported that the low-temperature period needed for breaking dormancy in ginseng seeds was more than 90 days, and that treatment at alternate temperatures was effective (Lee *et al*., 2016). The germination rate of the GA_3 treatment group was significantly higher than that of the control group (Fig. 4B).

Similarly, Lee *et al*. (2016, 2018) reported that treatment with 50–100 ㎎/ℓ GA_3 promoted breaking dormancy in ginseng seeds. Additionally, the mean germination time and T_{50} of the GA_3 treatment group at 50 ㎎/ℓ or higher were significantly lower than those of the control group (Fig. 4C and Fig. 4D).

Lee *et al*. (2018) reported that GA_3 treatment in dehiscent seeds improved the germination rate and decreased the mean germination time and T_{50} values. For seed germination, a certain concentration of gibberellins must be present in the seeds (Groot and Karssen, 1987; Karssen *et al*., 1989), and as the low-temperature treatment period passes, the concentration of synthesized in the seed increases, enabling seed germination (Ross and Bradbeer, 1971). Shortening the post-ripening period and promoting seed germination by GA_3 treatment are also known to occur in *P. notoginseng*, which is closely related to ginseng (Ge *et al*., 2023).

GA is a plant growth regulator that affects seed dormancy and germination, as well as growth and development. Although studies have been conducted to improve the dehiscence rate of ginseng seeds by GA_3 treatment before dehiscence (Kim *et al*., 2014a; Lee *et al*., 2018), research on its effects on subsequent growth is scarce.

The purpose of this study was to evaluate the effects of GA_3 treatment before dehiscence on the growth of germinated plants. In the treatment groups above 50 ㎎/ℓ GA_3, the lengths of stems and leaves were significantly increased compared to those in the control group, but no significant differences were observed in terms of stem diameter or leaf width (Table 1).

The effect of improving the growth of the aerial parts by GA_3 treatment on plants has been previously reported (Hong *et al*., 2021). When zygote embryos of *P. quinquefolius* were placed on a medium containing GA_3, stem length increased compared to that of the control (Hovius *et al*., 2007).

On the other hand, both the leaf length and width of the 300 ㎎/ℓ GA_3 treatment group decreased compared to the control, and excessively high GA_3 treatment before dehiscence was considered to have a negative effect on the growth of the aerial plant parts of ginseng.

GA_3 was previously found to promote the secondary growth of roots in 1-year-old ginseng (Hong *et al*., 2021). However, in the present study, the promotion of growth in the underground parts by treatment with gibberellin before dehiscence was not observed (Table 2). Based on these results, GA_3 treatment before dehiscence was considered to decompose as germination progressed, having no effect on the subsequent growth of the underground parts in *P. ginseng* C. A. Meyer.

In this study, the effects of plant growth regulators on the development and germination of ginseng seeds before dehiscence were evaluated. In addition, subsequent growth characteristics were evaluated, confirming that the plants were able to develop into normal plants. In conclusion, our research findings suggest that the use of plant growth regulators at appropriate concentrations during the dehiscence of ginseng seeds can be applied in agricultural practices. Subsequent processes are expected to be carried out to officially allow the use of plant growth regulators for ginseng seed stratification.

ACKNOWLEDGEMENTS

This work was carried out with the support of "Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ01476002)" Rural Development Administration, Republic of Korea.

This study was supported by 2024 the RDA Fellowship Program of National Institute of Horticultural & Herbal Science, Rural Development Administration, Republic of Korea.

REFERENCES

Chen L, Lu B, Liu L, Duan W, Jiang D, Li J, Zhang K, Sun

H, Zhang Y, Li C and Bai Z. (2021). Melatonin promotes seed germination under salt stress by regulating ABA and GA_3 in cotton(*Gossypium hirsutum* L.). Plant Physiology and Biochemistry. 162:506-516.

Chen S, Kuo SR and Chien CT. (2008). Roles of gibberellins and abscisic acid in dormancy and germination of red bayberry(*Myrica rubra*) seeds. Tree Physiology. 28:1431-1439.

Ge N, Jia JS, Yang L, Huang RM, Wang QY, Chen C, Meng ZG, Li LG and Chen JW. (2023). Exogenous gibberellic acid shortening after-ripening process and promoting seed germination in a medicinal plant *Panax notoginseng*. BMC Plant Biology. 23:67. https://link.springer.com/article/10.1186/s12870-023-04084-3 (cited by 2024 May 22).

Groot SPC and Karssen CM. (1987). Gibberellins regulate seed germination in tomato by endosperm weakening: A study with gibberellin-deficient mutants. Planta. 171:525-531.

Han JW, Ki, EH, Oh MW, Lee JH, Ma KH, Yoon TH and Jeong JT. (2022). Seed morphology and germination characteristics of *Rehmannia glutinosa.* Korean Journal of Medicinal Crop Science. 30:187-194.

Hong CP, Kim J, Lee J, Yoo SI, Bae W, Geem KR, Yu J, Jang IB, Jo IH, Cho H, Shim D and Ryu H. (2021). Gibberellin signaling promotes the secondary growth of storage roots in *Panax ginseng*. International Journal of Molecular Sciences. 22:8694. https://www.mdpi.com/1422-0067/22/16/8694 (cited by 2024 May 22).

Hovius MHY, Saxena PK and Proctor JTA. (2007). Effects of date and growth regulators on the culture of immature zygotic embryos of north american ginseng. Journal of Ginseng Research. 31:14-22.

Hu Y, Zhou L, Huang M, He X, Yang Y, Liu X, Li Y and Hou X. (2018). Gibberellins play an essential role in late embryogenesis of Arabidopsis. Nature Plants. 4:289-298.

Kabar K. (1998). Comparative effects of kinetin, benzyladenine, and gibberellic acid on abscisic acid inhibited seed germination and seedling growth of red pine and arbor vitae. Turkish Journal of Botany. 22:1-6.

Karssen CM, Zagorski S, Kepczynski J and Groot SPC. (1989). Key role for endogenous in the control of seed germination. Annals of Botany. 63:71-80.

Kim DH, Kim YC, Bang KH, Kim JU, Lee JW, Cho IH, Kim YB, Lim JY and Kim KH. (2016). Flowering and fruits formation characteristics in major varieties of *Panax ginseng*. Korean Journal of Medicinal Crop Science. 24:207-213.

Kim MJ, Shim CK, Kim YK, Hong SJ, Park JH, Han EJ and Kim SC. (2017). Enhancement of seed dehiscence by seed treatment with *Talaromyces flavus* GG01 and GG04 in ginseng (*Panax ginseng*). Plant Pathology Journal. 33:1-8.

Kim YC, Kim YB, Park HW, Bang KH, Kim JU, Jo IH, Kim KH, Song BH and Kim DH. (2014a). Optimal harvesting time of ginseng seeds and effect of gibberellic acid(GA_3) treatment for improving stratification rate of ginseng(*Panax ginseng* C. A. Meyer) seeds. Korean Journal of Medicinal Crop Science. 22: 423-428.

Kim YH, Ahn IO, Khan AL, Kamran M, Waqas M, Lee JS, Kim DH, Jang SW and Lee IJ. (2014b). Regulation of endogenous and abscisic acid levels during different seed collection periods in *Panax ginseng*. Horticulture, Environment, and Biotechnology. 55:166-174.

Kwon WS and Lee JM. (1997). Change of cytokinins and gibberellin contents during low temperature storage of dehisced ginseng seeds. Journal of the Korean Society for Horticultural Science. 38:111-115.

Lee JW, Jo IH, Kim JU, Hong CE, Kim YC, Kim DH and Park YD. (2018). Improvement of seed dehiscence and germination in ginseng by stratification, gibberellin, and/or kinetin treatments. Horticulture, Environment, and Biotechnology. 59:293-301.

Lee JW, Kim JU, Bang KH, Kim DH, Jo IH and Park YD. (2023a). Efficient micropropagation of genetically stable *Panax ginseng* Meyer by somatic embryogenesis. Agronomy. 13:1139. https://www.mdpi.com/2073-4395/13/4/1139 (cited by 2024 May 29).

Lee JW, Kim JU, Bang KH, Kwon N, Kim YC, Jo IH and Park YD. (2023b). Efficient somatic embryogenesis, regeneration and acclimatization of *Panax ginseng* Meyer: True-to-type conformity of plantlets as confirmed by ISSR analysis. Plants. 12:1270. https://www.mdpi.com/2223-7747/12/6/1270 (cited by 2024 May 29).

Lee JW, Kim YC, Kim JA, Jo IH, Kim KH and Kim DH. (2016). Effects of gibberellic acid and alternating temperature on breaking seed dormancy of *Panax ginseng* C. A. Meyer. Korean Journal of Medicinal Crop Science. 24:284-293.

Lee JW, Kwon N, Kim JU, Bang KH, Jung SM, Lee SW, Kim DH, Kim YC, Jo IH and Park YD. (2023c). *In vitro* micro-propagation of commercial ginseng cultivars(*Panax ginseng* Meyer) *via* somatic embryogenesis compared to traditional seed production. Horticulturae. 9:435. https://www.mdpi.com/2311-7524/9/4/435 (cited by 2024 May 29).

Lim SH, Jeong HN, Kang AS and Joen MS. (2008). Influence of GA_3 soak and seed dressing with Toros(tolclofos methyl) wp. on the dehiscence of *Eleutherococcus senticosus* Maxim seeds. Korean Journal of Medicinal Crop Science. 16:106-111.

Min JE, Long NP, Hong JY, Kim SJ, Anh NH, Wang D, Park JH, Kwon SW and Lee SJ. (2022). The dehiscence process in *Panax ginseng* seeds and the stigmasterol biosynthesis pathway in terms of metabolomics. Journal of Ginseng Research. 46: 225-234.

Park H, Kim SK and Bae HW. (1979). Effect of gibberellin and kinetin on bud dormancy breaking and growth of Korean ginseng root(*Panax ginseng* C. A. meyer.). Journal of Ginseng Research. 3:105-112.

Prasad VN, Gupta VNP and Bajracharya D. (1983). Alleviation by gibberellic acid and kinetin of the inhibition of seed germination in Maize(*Zea mays* L.) under submerged conditions. Annals of Botany. 52:649-652.

Ross JD and Bradbeer JW. (1971). Studies in seed dormancy. VI. The effects of growth retardants on the gibberellin content and germination of chilled seeds of *Corylus avellana* L. Planta. 100:303-308.

Skoog F and Armstrong DJ. (1970). Cytokinins. Annual Review of Plant Biology. 21:359-384.

Soppe WJJ and Bentsink L. (2020). Seed dormancy back on track; its definition and regulation by DOG1. The New Phytologist. 228:816-819.

Suh SJ, Yu J, Jang IB and Kim YC. (2022). Effect of seed moisture content on seed storage of dehisced ginseng seeds. Korean Journal of Plant Resources. 32:457-462.

Tuan PA, Nguyen TN, Jordan MC and Ayele BT. (2021). A shift in abscisic acid/gibberellin balance underlies retention of dormancy induced by seed development temperature. Plant Cell and Environment. 44:2230-2244.

Yang L, Liu S and Lin R. (2020). The role of light in regulating seed dormancy and germination. Journal of Integrative Plant Biology. 62:1310-1326.