近畿大農紀要 28：45～50 (1995)
Mem. Fac. Agr. Kinki Univ. 28：45～50 (1995)

Studies on Cultivation of *Panax ginseng* in Thailand II. Effect of Stratification and GA_3 on Seed Germination

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Synopsis

To promote the germination of ginseng seeds under highland conditions in northern Thailand, some experiments were conducted for the treatment of GA_3 and stratification under natural low temperature of northern Thailand or under controlled cold temperature of 5°C. The results suggested that ginseng seeds should be firstly soaked in GA_3 100 ppm solution then stratificated at 5°C in a refrigerator for 4 ～ 5 months. Appearance of radicles from the seeds is the main criterium for appropriating time to bring out the seeds and sowing in the growing bed.

1. Introduction

Ginseng is usually propagated by seed in Japan. In general, full ripening fruits are harvested and the red exocarps are pulped out. Shade dried seeds are then firstly treated to break dormancy by stratification in moistened river sand and under ambient low temperature or by soaking in GA_3 solution before stratification. The relatively low air temperature of September to October in Japan will trigger the embryo development and the embryo swells within 3-4 months. Seeds with opened seed coat will be sown in November and achieve activegermination under warm climate in March to April (Miyazawa, 1990 and Sruamsiri et. al. 1991)[1,2].

To develop the technology for securing the seed germination in Thailand the same procedure of seed treatment as practiced in Japan was firstly followed up under Thailand conditions to find out the possibility and some basic constraints. The follow up experimentswere then conducted based on the limiting factors found in previous trials.

2. Experiment 1: Effect of GA_3 and Ambient Temperature on Seed Germination

In Japan ginseng seeds are stratificated in moistened sand under ambient low temperature to break dormancy. This same basic procedure will be followed in this experiment to find out the possibility of using GA_3 and / or stratification under normal ambient temperature in northern highland area of Thailand for seed germination.

Materials and Methods:

Two groups of ginseng seeds of each 1,000 seeds were soaked for 24 hours in deionized water of GA_3 100 ppm solution before mixing with moistened river sand and kept in double layer of clay in pots under shade room with relatively constant moisture conditions. Seed germination were inspected every month until 5 months of stratification.

The experiment was conducted at Nong Hoi Experimental Station (1,000 m. above sea level), where the ambient temperature during the trial were recorded as shown in Table 1.

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Table 1. Monthly maximum and minimum air temperature at Nong Hoi Experimental Station, Chiang Mai Univ., Thailand in the year 1990-1991

	1990			1991	
Month	Oct.	Nov.	Dec.	Jan.	Feb.
Max. Temp. (°C)	25.1	24.8	21.9	29.5	31.0
Min. Temp. (°C)	20.6	19.3	13.5	10.5	12.1

Table 2. Effect of GA_3 and stratification under ambient temperature at Nong Hoi Station. Chiang Mai on the development of ginseng seeds.

	Water		GA_3-treatment		
Month after stratification	A	B	A	B	Seed condition
1 (October)	0	0	0	0	good
2 (November)	0	0	240	760	good
3 (December)	0	0	368 (36.8)	632	good
4 (January)	40 (4.0)	460	544 (54.4)	476	good, some seed coat turned brown
5 (February)	146 (14.6)	854	500 (50.0)	394	some seeds dried & rot in both treatments

A: Number of seeds with opened seed coat.
B: Number of seeds with closed seed coat (no germination).
The figures in brackets represent the percentage.

Results and Discussion:

As shown in Table 2, GA_3 could clearly promoted the seed germination more than water application. Seeds soaked in the GA_3 solution prior to stratification had a good embryo development ane swelling. The percentage of seeds with opened seed coat had already reached to 24.0% at 2 months and increased to be 36.8% and 56.4% within 3 and 4 months, respeectively. whereas only 4% of the seed soaked in water opened the seed coat after 4 months of stratification.

These results suggested that GA_3 treatment was effective for breaking dormancy and triggering the embryo development of ginseng seed. However, seed germination seems to be achieve only through GA_3 application. The relativly low percentage of seed with opening seed coat (56.4% within 4 months) and the problem of seed rot observed during the 4 and 5 months of stratification suggested the necessity of additive cold treatment for the seed germination (Table 2). This additional factor would be similar effect of low temperature in winter as procticed in conventional ginseng cultivation in Japan.

In Japan, the seeds with opened seed coat will be sown in November to let the seeds expose to cold winter temperature and germinate at warm temperature of the next spring. Soil temperature in Nagano Prefecture, Japan were around 2°C from December to February and 9°C in April (Kitamimaki Reserch Report, 1990)[3]. The low temperature treatment is therefore necessary for seed germination and it should be proved experimentally.

GA_3 could trigger embryo development but do not induce a complete germination of ginseng seeds. Cold temperature treatment seems to be the necessary procedure to secure seed germination after GA_3 application.

3. Experiment 2: Effect of GA_3 and Cold treatment on Seed Germination

GA_3 treatment was found to promote embryo development, swelling and seed coat opening but

Table 3. Gemination of ginseng seeds after soaking in water or GA_3 solution and kept at 5°C.

Month of stratification at 5°C	Number of germinating seeds*1		State of the seed
	Water	GA_3	
1	0 (0)	0 (0)	seed coat stay closing
2	143 (57.0)	148 (59.4)	seed coat opened
3	160 (64.0)	194 (77.8)	seed coat opened, the radicle protrusion occurred in some seeds

*1 Total number of seeds for each treatment was 250. The figures in brackets are percentage

not complete germination of ginseng seeds. The low temperature in winter in Nagano might be necessary to induce the protrusion of radicle and hypocotyl. In this experiment the necessity of cold temperature after GA_3 treatment for the seed germination was examined.

Materials and Methods:

The healthy seeds without opened seed coat were selected from the previous experiment and used in this experiment. The total of 500 seeds were divided into two groups of each 250 seeds and soaked for 24 hours in GA_3 100 ppm solution or in water. After mixing with moistened river sand in beakers, all the seeds were then kept at 5°C in a refrigerator. The experiment was started in March 1991 and germination of seeds were inspected once a month.

Results and Discussion:

Both GA_3 and water soaking induced germination to a similar extent, and seed coat opening was already observed in the second month of stratification in a refrigerator. The number of opening seed coat increased to 64-77.8% in the third month, a half of which had started the radicle protrusion (Table 3). Another half of seeds were therefore planted out in a ginseng house at the highland station and emergence of overground part occured within two weeks after transplanting.

The results confirmed the necessity of cold treatment for the induction of seedling emergence, regardless of previous treatment of GA_3. It can be concluded that keeping the seeds at very low temperature in refrigerator could replace the cold temperature exposure in winter (December to February) at Nagano. Moreover, it should be emphasized that the stratification temperature used in this study was appropiate not only for embryo development but also for seeding emergence. Most of the seeds germinated in the refrigerator (5 C and in darkness).

Comparing the effect of GA_3 treatment with the stratification (cold treatment), the latter is seemed to be more effective than GA_3-treatment. In this experiment, cold treatment could promote the germination even of the seeds soaked in water and at a similar rate as those treated with GA_3 (Table 3). This result is similar to the normal procedure used by the farmers in Nagano Prefecture, Japan (Sruamsiri et al. 1991)[2], where most of the farmers perfer to stratify the ginseng seed without GA_3 application. The method of soaking in GA_3 prior to stratification will be carried out only for the late harvested seeds to accellerate embryo development before exposing to the normal cold winter temperature.

Together with the achieved results, it may be concluded that cold treatment is necessary for seed germination. The effective temperature for cold treatment must be low of about 5°C.

A Practical Implication:

For promotion of ginseng seed germination in Thailand a cold treatment in a refrigerator is necessary.

4. Experiment 3: Effect of Duration of Cold Treatment on Seed Germination

Cold temperature is necessary for seed germination, but the appropriate duration of cold treatment is unknown. It was shown in the second experiment that seeds kept at 5°C in refrigerator already started the germination within 90 days of stratification. These emerging seedlings are however very fragile and inappropiate for transportation to mountainous area and difficult to planting.

In practice, the most appropiate time for seeding should theoretically be at the shortest time before radicle protrusion. In this exoeriment the duration of the cold treatment was varied to find out the shortest duration of cold treatment for promotion of seed germination.

Materials and Methods:

Ginseng seeds received from the farmers in Nagano Prefecture were used in this study. The seeds were previously stratificated with conventional procedure from August to November in Nagano and some seeds had already showed a slightly seed coat opening. To ensure a regular seed performance all the seeds were again soaked in GA_3 100 ppm solution before mixing with moistened river sand and kept at 5°C in the refrigerator. The stratification times were 30, 60 and 90 days, respectively.

To study the stimulative effect of GA_3 on seed germination a half amount of the seeds were soaked again in GA_3 for 30 minutes after cold treatment and before sowing. Hence, the experiment was conducted according to factorial design in which factor A means stratificating duration of 30, 60, 90 days and factor B means GA_3-application : once prior to stratification and twice pre- and post-stratification. All the seeds were soaked for 24 hour in GA_3 100 ppm solution on 25 December 1993 and kept in moist sand under 5°C in a refrigerator. The 30, 60 and 90 days stratificated seeds were sown in the field on 26 January 26 February and 26 March 1994, respectively. Number of emerged seedling of each replication (25 seeds for 1 replication) were firstly counted at 30 days after sowing and then at every 15 days until 50% germination was observed. The result was shown in Table 4.

Table 4. Percentage germination of ginseng seeds after cold treatment (5°C) for 30 days

Treatment of GA_3 soaking	Percentages of Germinating seed at the days after the end of cold-treatment		
	30	45	60
Once ; prior to stratification	0	24	42
Twice ; pre- and post-stratification	14	41	68

Result and Discussion:

Both stratification duration and the GA_3 application showed a similar strong stimulation on seed germination. Whereas a good embryo swelling and the seed coat opening were observed at 30 days of cold treatment in some seeds, most of the seeds were however attained their complete germination after 50 days of stratification and protrusion of the radicle and hypocotyl were alredy observed in 267 seeds out of total 600 seeds. Practically seed should therefore be sown after 50 days cold treatment.

As shown in Table 4, at 30 days after sowing only the seeds treated twice with GA_3 showed 14% germination. Seeds with one GA_3 application germinated firstly at 45 days after sowing. More than 400 seeds of the total 800 seeds kept in a refrigerator for 60 days were sown in the growing bed at Nong Hoi Experimental Station. The seedlings developed very well.

It must be noticed that seeds used in this study were alredy treated with conventional stratifica-

tion in Nagano for 4 months. Some seeds had already open the seed coat. This might explain why ginseng seeds respond to the GA_3 and cold treatment with a very high sensibility and germinated in the refrigerator within only 50 days. For the non-treated seed, more longer duration of cold treatment might be necessary to ensure germination.

Soaking the seed in GA_3 solution twice pre- and post-stratification seems to promote a faster germination without any negative effect on seedling development, when compared to those with only one application before stratification. This might be another technology to promote a better seed germination.

Practical Implication :

With previous and present results, it might be suggested that ginseng seed should be firstly soaked in 100 ppm GA_3 for 24 hour and then mixed with moistened sand before keeping at 5°C in a refrigerator. This procedure will be useful for breaking seed dormancy and promotion of germination. Seeds must be kept under cold temperature until the tip of radicle protrudes out of the seed coat. Then the stratification should be stopped and all the seeds must be planted out. Germination should be achieved within 30 to 45 days thereafter.

5. Conclusion

Ginseng seed needs cold temperature for germination. Soaking in 100 ppm. GA_3 solution will shorten the duration of cold treatment but stratification is still necessary for their complete germination. Both GA_3 and coldness can break dormancy of ginseng seed, triggering the embryo development, seed coat opening and embryo swelling. However only the coldness around 5°C is needed to promote the protrusion of radicle and hypocotyl from opened seed coat in order to be completed the germination procedure.

For cultivation of ginseng in Thailand, the seeds should be soaked in GA_3 100 ppm solution and mixed with moistened sand before keeping at 5°C in a refrigerator until protrusion of the embryo. Through this procedure embryo swelling and seed coat opening should be observed within 2 or 3 months of stratification and protrusion of radicle would occured within another 2 months.

Acknowledement

The studies on "Cultivation of Panax ginseng in Thailand" are part of the cooperative program for scientific exchange and works between Kinki University, Japan and Chiang Mai University, Thailand. The program has been started since 1990.

All the sincere thanks would be given to Kinki University for the financial support and staff training.

6. References

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タイ国における薬用ニンジンの栽培学的研究（第2報）種子の発芽に及ぼす層積（低温）処理とGA処理の影響について

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要 約

タイ北部山地域で薬用人参，*Panax ginseng*，を栽培するに当たって，まず休眠種子の発芽促進が前提となる。本植物の自生地では冬季の適当な低温と水分供給により休眠打破と発芽促進が可能である。そこで，長野県の主産地で行われている栽培技術に倣って，タイ北部山地域にあるノンホイ試験場において薬用人参種子の層積処理とジベレリン処理による一連の発芽促進実験を行った。その結果薬用人参種子は，まずGA_3 100 ppm溶液に24時間浸漬した後，冷蔵庫内で約5℃，4～5か月おいて低温（層積）処理をすることにより十分な催芽種子が得られた。そしてさらにそれらの種子をノンホイ試験場の圃場に播種すると，すみやかに健全な芽生え個体が得られることが明らかになった。