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Improved Germination of American Ginseng Seeds Under Controlled Environments

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SUMMARY. Traditionally, American ginseng (*Panax quinquefolium* L.) seeds are stratified for 18 to 22 months, before seeding, in a sandbox buried outdoors in late August or early September. Uncontrolled fluctuating temperature and moisture levels and the presence of pathogenic organisms in the seed box can cause seeds to sprout prematurely, rot, dry out and die. A study was initiated to shorten the lengthy stratification period, and to increase seed viability and percentage of germination by stratifying seeds indoors under a controlled environment. Seeds were subjected to various periods of warm [15 or 20 °C (59 or 68 °F)] and cold [2 °C (35.6 °F)] temperature stratification regimes in growth chambers. Embryo growth and viability, and

seed moisture content were tested periodically during stratification. The best warm regime for embryo development, seed viability and germination after subsequent cold treatment was 15 °C (59 °F). The first "split" seeds, indicating incipient germination, were observed after 3 months of warm [15 °C (59 °F)] and 4 months of cold [2 °C (35.6 °F)] treatment, when average embryo length reached 6 mm (0.24 inch). Greenhouse germination of stratified seeds was as high as 80%. The results from this study indicate that good germination is possible when ginseng seeds are stratified indoors under a controlled environment and seeds can be made to germinate at any time of the year.

American ginseng cultivation in Canada has expanded rapidly in the last few years, making ginseng one of the major cash crops in the provinces of British Columbia and Ontario, and making Canada one of the major ginseng production regions of the world. With a suitable climate and environment, British Columbia produces high yields of ginseng roots with excellent quality.

Ginseng is propagated from seed. The embryo in the newly harvested seed is not fully developed (Baranov, 1966), and needs a stratification period of 18 to 22 months before germination (Xiao et al., 1987). During the stratification period, seeds complete anatomical development and physiological after-ripening stages (Li, 1995). Under suitable temperatures of 15 to 20 °C (59 to 68 °F) (Jo et al., 1988; Stoltz and Garland, 1980), the embryos begin to develop further and cotyledons, hypocotyls, radicles and epicotyls become visible as the embryos continue to develop and reach a length of 3 to 3.5 mm (0.12 to 0.14

inch) (Yu and Kim, 1992). Equally important is physiological after-ripening. Seeds with fully developed embryos need low temperature stratification before germination (Lee et al., 1983; Proctor and Bailey, 1987). Anatomical development and physiological after-ripening stages are very vulnerable developmental stages for ginseng seeds and without proper or suitable environmental conditions, seeds will either rot, fail or be slow to germinate.

Traditionally, in North America, seeds are harvested in late August or early September and stratified in a sand box buried outdoors (Polczynski, 1982; Proctor and Louttit, 1995). Uncontrolled fluctuating temperature and moisture levels, and the presence of pathogenic organisms in the seed box, cause seeds to sprout prematurely, rot, dry out and die, or be delayed in germination by up to 2 years after seeding, with severely reduced germination rates (Li, 1995). Stratifying seeds in a controlled environment is a potentially reliable method of reducing or eliminating these serious problems. In an indoor controlled environment, ginseng growers should be able to maintain a disease-free environment and provide ideal temperatures and moisture levels for seeds to stratify as well as to monitor seed conditions.

The purpose of this project was to study the feasibility of stratifying ginseng seeds in a controlled environment indoors and to investigate the effect on seed germination of various stratification regimes using combinations of warm [15 or 20 °C (59 or 68 °F)] and cold [2 °C (35.6 °F)] temperatures, for varying lengths of the stratification period.

Materials and methods

EXPERIMENT 1. In Expt. 1, ginseng seeds were harvested randomly from 4-year-old plants in a commercial ginseng field in September, 1996. Seeds were depulped, cleaned and soaked in 1% formalin for 10 min. Seeds were planted 1 cm (0.4 inch) deep in plastic pots [15 cm (5.9 inch diameter)] containing pasteurized sandy loam soil. There were 20 seeds per pot and 10 pots per treatment. Seeds were kept moist at all times and were placed in growth chambers (Convion, Winnipeg, Man.) in the dark under controlled temperature regimes, a combination of warm [20 °C (68 °F)]

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Table 1. Effect of seed stratification temperatures and the length of stratification period on American ginseng seed germination.

Treatment	Time at warm [20 °C (68 °F)] (month)	Time at cold [2 °C (35.6 °F)] (month)	Germination (%)
1	1	3	2 c ^z
2	2	3	39 b
3	3	3	55 a
4	1	2	7 c
5	2	2	2 c
6	3	2	12 c
7	1	1	7 c
8	2	1	24 b
9	3	1	5 c

^zValues with a common letter are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.

temperature for 1, 2, or 3 months and a subsequent cool [2 °C (35.6 °F)] period for 1, 2, or 3 months for a total of nine treatments. Pots were moved to a greenhouse [20 to 22 °C (68 to 71.6 °F)] day and night with 16-h photoperiod) for germination tests immediately after treatments. The number of seedlings were counted once a week for 6 weeks and percentage of germination were calculated. Analysis of variance was conducted using the SAS GLM procedure (SAS Institute, 1990) and Duncan's New Multiple Range Test was applied to separate treatment means.

EXPERIMENT 2. In September 1997, ginseng seeds were randomly harvested from 4-year-old plants in a commercial ginseng field. Newly picked seeds were depulped, cleaned, and soaked in a 1% formalin for 10 min, then mixed with pasteurized sand in a 1:2 (v/v) ratio. The sand was moistened with 500 to 600 mL (16.9 to 20.3 oz) of 1% formalin, and additional sterile distilled water was added to provide a 10% (w/w) moisture level. The sand and seed [2 kg (4.5 lb)] mixtures were placed in 11.3-L (3-gal) plastic containers (Rubber-maid Inc., Wooster, Ohio) with removable covers. Several holes had been drilled for drainage and a 2-cm (0.8-inch) layer of gravel had been placed at the bottom.

The containers were placed in controlled temperature chambers in the dark. Two containers each were subjected to

the following treatments. 1) Warm temperature regimes [15 or 20 °C (59 or 68 °F)] for 3.5 months followed by a cold temperature regime [2 °C (35.6 °F)] for 9 months. Seeds were sampled 5, 7, and 9 months after placement in the cold for germination tests. 2) Seeds were first stored in cold storage [1 to 2 °C (33.8 to 35.6 °F)] for 3 months followed by warm temperature [15 or 20 °C (59 or 68 °F)] for 3.5 or 4.5 months, then stratified at cold [2 °C (35.6 °F)] for 5.5 months. Seeds were sampled 3.5 and 5.5 months after placement in cold [2 °C (35.6 °F)] for germination tests. 3) After warm and cold stratification [3.5 months at 15 or 20 °C (59 or 68 °F) followed by 5.5 months at 2 °C (35.6 °F)], seeds were separated based on development stage, split or not split, for germination tests. Statistical analysis was as described in Expt. 1.

Thirty seeds were sampled monthly from each container to deter-

mine viability and to measure embryo length. Seeds were bisected diagonally using a single edged razor blade, and soaked in 0.1% tetrazolium chloride in water at 35 °C (95 °F) for 2 h. The embryo and surrounding endosperm tissue of seeds which stained pink with the tetrazolium test were considered viable. Ten embryos were excised from the bisected seeds, placed in water on a glass slide to prevent desiccation and embryo lengths were measured with a calibrated ocular micrometer on a binocular microscope, or, for larger embryos, a dissecting microscope with a stage micrometer or ruler.

Results

EXPERIMENT 1. Percentage of germination varied among treatments. Treatment 3, in which seed was at 20 °C (68 °F) for 3 months followed by 3 months at 2 °C (35.6 °F) produced a significantly higher germination rate (55%), than any of the other treatments (Table 1). Seeds at 20 °C (68 °F) for 2 months followed by 1 and 3 months at 2 °C (35.6 °F) (Treatments 8 and 2) had the second best percentage of germination at 24 and 39%, respectively.

EXPERIMENT 2. Seed viability and moisture content. Initially, all of the seeds sampled were viable; the embryo and surrounding endosperm tissue stained pink with the tetrazolium test. Under cold stratification [2 °C (35.6

Fig. 1. American ginseng embryo development during warm [15 °C (59 °F)] and cold [2 °C (35.6 °F)] seed stratification treatments; 25.4 mm = 1.0 inch; 9/5 (°C) + 32 = °F.

Fig. 2. American ginseng embryo development during warm [20 °C (68 °F)] and cold [2 °C (35.6 °F)] seed stratification treatments; 25.4 mm =1.0 inch; 9/5 (°C) + 32 = °F.

Fig. 3. American ginseng embryo development during warm [15 °C (59 °F)] and cold [2 °C (35.6 °F)] seed stratification treatments after stored in cold (2 °C) storage for 3 months; 25.4 mm =1.0 inch; 9/5 (°C) + 32 = °F.

well. Under warm stratification [15 or 20 °C (59 or 68 °F)], embryos continued to stain well but later on, the endosperm tissue stained progressively pinker. This trend was most apparent in the containers stratified at 20 °C (68 °F). After a prolonged period of time, uneven staining of some of the embryos was observed. Specifically, white patches appeared on the cotyledons indicating nonviable tissue. The moisture content of seeds in all temperature regimes was consistently at ≈50%.

Embryo growth. The initial mean embryo length of ginseng seeds before being placed in the various experimental containers was ≈0.5 mm (0.02 inch). Embryo growth in the seeds began immediately at 20 °C (68 °F) while at 15 °C (59 °F), embryo growth was slower to start (Figs. 1 and 2). After 72 d of stratification, mean embryo length in the two containers was the same (2.0 mm, 0.08 inch), and after 89 d, mean embryo length was greater for the seeds from 15 °C (59 °F), 3.0 mm

(0.12 inch), than the seeds from 20 °C (68 °F), 2.2 mm (0.09 inch). Embryo growth in the seeds stratified at 20 °C (68 °F) essentially stopped after 72 d and did not resume until after the seeds had been transferred to 2 °C (35.6 °F) for over a month (day 141) (Fig. 1). Embryo growth continued when seeds in the 15 °C (59 °F) treatment were placed at 2 °C (35.6 °F) (Fig. 2).

A similar pattern of embryo growth was observed in other stratification regimes. No embryo growth occurred in seeds held in cold storage [1 to 2 °C (35.6 °F)] for 3 months (Figs. 3 and 4). Embryo development

Fig. 4. American ginseng embryo development during warm [20 °C (68 °F)] and cold [2 °C (35.6 °F)] seed stratification treatments after stored in cold (2 °C) storage for 3 months; 25.4 mm = 1.0 inch; 9/5 (°C) + 32 = °F.

began when seeds were placed at warm regimes [15 or 20 °C (59 or 68 °F)] and continued when they were returned to a cold temperature [2 °C (35.6 °F)] (Fig. 3 and 4). A lag period of 2 and 4 weeks, respectively for embryo growth was observed when seeds were placed at 15 or 20 °C (59 or 68 °F) after being stored at 2 °C (35.6 °F) for 3 months. Mean embryo length after 5 months (day 241) was 4.6 and 2.5 mm (0.18 and 0.1 inch), respectively, for seeds stratified at 15 or 20 °C (59 or 68 °F). After a subsequent cold stratification period of 3.5 months, the respective mean embryo lengths were 5.9 and 6.1 mm (0.23 and 0.24 inch).

GERMINATION TESTS. Germination was assessed in the greenhouse for each of the experimental temperature regimes after 3.5 months of warm [15 or 20 °C (59 or 68 °F)] followed by 5, 7, and 9 months of cold [2 °C (35.6 °F)] stratification. Seeds were chosen at random for the tests. The percentage of germination shown are means of germination tests conducted on 10 lots of 100 seeds at 2 months intervals. The germination improved significantly as length of cold stratification increased (Table 2). No interaction effect of stratification regime and time was detected. germination percentage for the seeds in warm regime at 15 °C (59 °F) were significantly higher than those in regimes at 20 °C (68 °F). Germination tests were also conducted on seeds stored at 2 °C (35.6 °F) for 3

months before starting stratification. As shown in Table 3, a longer cold stratification period significantly improved germination percentage regard-

less of the length of warm stratification. Increasing the length of the warm period from 3.5 to 4.5 months also increased germination. Percentage of germination was higher for seeds stratified at 15 °C (59 °F) than 20 °C (68 °F) as was found in the preliminary experiment.

The first split seeds were observed 6 months after the beginning of stratification.

The appearance of split seeds, or split seeds with radicles, was not uniform. A large number of seeds (20% to 30%) did not split despite staining pink with tetrazolium and having well-developed embryos. Test conducted to compare the germination rates of split and nonsplit seeds showed that split seeds

Table 2. Effects of the length of cold stratification period on the percentage of germination after 3.5 months of warm stratification treatments on American ginseng seeds.

Warm temp [°C (°F)]	Length of warm stratification (month)	Length of cold stratification (month)	Germination (%)
15, 59	3.5	5	20 a ^z
		7	47 b
		9	80 c
20, 68	3.5	5	2 a
		7	18 b
		9	60 c

^zValues in each column and row with a common letter are not significantly different ($P=0.05$) according to Duncan's new multiple range test.

Table 3. Effects of the length of warm and cold stratification periods on American ginseng seed germination after freshly seeds were stored in cold storage [1 to 2 °C (33.8 to 35.6 °F)] for 3 months.

Warm temp [°C (°F)]	Length of warm stratification (month)	Length of cold stratification (month)	Germination (%)
15, 59	3.5	3.5	2 a ^z
		5.5	45 b
		4.5	26 a
20, 68	3.5	3.5	71 b
		5.5	1 a
		5.5	25 b
	4.5	3.5	1 a
		5.5	53 b
		5.5	

^zValues in each column and row with a common letter are not significantly different ($P=0.05$) according to Duncan's new multiple range test.

had higher percentage of germination at either 15 or 20 °C (59 or 68 °F), however, split seeds from 15 °C (59 °F) had higher germination rate than from 20 °C (68 °F), 64% and 18%, respectively.

Discussion

Many factors affect ginseng seed germination, including stratification period (Li, 1995), seeding time and depth (Liu, 1988), temperature (Bae, 1978), and spacing (Park, 1987). In Expt. 1 (Table 1), 55% of seeds germinated after 6 months of stratification (3 months warm period followed by 3 months cold period) in a controlled environment indoors compared to the 18 to 22 months outdoor stratification period required by the conventional method (Baranov, 1966; Xiao et al., 1987). The results demonstrated that the seed stratification can be shortened substantially with appropriate temperature treatments. Percentage of germination was reduced significantly when each of the warm and cold periods was shorter than 3 months, indicating that the lack of full embryo development decreased the germination (Baranov, 1966; Li, 1995).

The embryo in the newly harvested ginseng seed is not fully developed and its average length was 0.5 mm (0.02 inch), as previously reported by Hovius (1996). Stoltz and Snyder (1985) indicated that ginseng seed germination is a two-stage process. In the first (warm) stage, the embryo begins to grow and in the second (cold) stage, the embryo completes its development and the endogenous dormancy of the seed is overcome. In this experiment, embryo growth started almost immediately after seeds were placed in warm regimes [15 or 20 °C (59 or 68 °F)]; however, embryo development was better at 15 °C (59 °F) than 20 °C (68 °F) (Fig. 1) at the end of 3 months (Figs. 1 and 2), in agreement with Jo et al. (1988) and Hovius (1996). Similar patterns were observed when the seeds were stored at 2 °C (35.6 °F) (cold treatment) for 3 months before exposing them to a warm and a cold stratification treatment (Figs. 3 and 4). Embryo growth continued after seeds were moved from a warm to

a cold environment, and subsequently the average embryo length reached 6.0 mm (0.24 inch). Similar results were reported by Hovius (1966), who measured embryo growth at the end of the conventional seed stratification outdoors. These results suggest that prematured ginseng seeds can be stored for a period of time to delay embryo development for later germination if required. Seeds can be handled differently to prolong the availability of seedlings beyond the single annual spring period provided by conventional stratification.

Stratification of ginseng seeds under a controlled environment indoors is an relatively new experimental procedure not yet fully evaluated or accepted for American ginseng. Results from Expt. 1 (Table 1) suggested that it was possible to induce germination by manipulating seeds through 3 months each of warm [20 °C (68 °F)] and cold [2 °C (35.6 °F)] temperatures. Results from subsequent experiments revealed that by lowering the temperature of the warm period to 15 °C (59 °F) and extending the length of the cold period, percentage of germination could be increased to 80% (Table 2). This is a significant improvement for ginseng stratification, as it shortens the time required for stratification and increases percentage of germination.

We conclude that the length of warm and cold periods during stratification is an important factor for ginseng seed germination. More importantly, seed stratification carried out in a controlled environment indoors can shorten the length of seed stratification, increase percentage of germination and avoid hazards experienced outdoors such as pathogens and fluctuation of temperature and moisture levels.

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