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Effect of Gibberellin and Light on Germination of Seeds in *Codonopsis lanceolata* Benth

Bimal Kumar Ghimire*[1], Chul Min Shin*, Cheng Hao Li, Ill Min Chung****[1], Dong wook Lee*, Hee Young Kim*, Na Young Kim***, Jung Dae Lim*, Jae Kwang Kim*, Myong Jo Kim*, Dong Ha Cho*, Sun Joo Lee****, and Chang Yeon Yu*[†]**

*Bioherb Research Institute, Kangwon National University, Chunchon 200-701,Korea.
**Key Laboratory of Forest Genetics and Tree Breeding, Northeast Forestry University, Harbin 150040, China.
***Department of Food & Nutrition, Kyunghee University, Seoul 130-701, Korea.
****Department of Applied Life Science, Konkuk University, Seoul 143-701, Korea.

ABSTRACT : Seed of *Codonopsis lanceolata* exhibits low germination due to impermeable seed coat. Prolonged seed dormancy can be overcome by the application of gibberellins, as it promotes growth of the embryo and weakens the structures surrounding of embryo. The effects of photoperiod, sugar and gibberellin concentration were investigated at constant temperature for 12 days and 22 days *in vitro* and *invivo* conditions respectively. The rate of germination of seeds of *Codonopsis lanceolata* in wet filter paper in both complete dark and light treatments was significantly lower than that of seed treated with GA_3. It clearly indicates that there is significant coat imposed dormancy in the seed of *Codonopsis lanceolata*. The rate of germination *in vivo* condition was lower than that of the *invitro* condition supplemented with GA_3. Thus, the physical dormancy due to impermeable seed coat and low level of endogenous gibberellins in the seed was the cause of poor germination rate in *Codonopsis lanceolata*.

Key words : germination, gibberellins, *Codonopsis lanceolata*.

INTRODUCTION

Codonopsis lanceolata (common name : Bounet bell flower, deoduck in Korea) is a perennial climber and belongs to family Companulacea, grows naturally in moist places in woods, low mountains and hills (Hong *et al.*, 1984). Its root is widely used as herbal medicine for the remedy of depurative, emmenagogue, galactogogue, dyspepsia, poor appetite, fatigue and psychoneurosis (Zhang, 1982), anticancer as well as a wild vegetable in Korea, China and Japan. Its natural habitats are fast depleting with the increase of its demand in market. Its natural population is very low due to poor germination rate.

The germination of seed of plant widely depends on the light and temperature condition of its environment (Kambizia, 2006). Since the regeneration of *Codonopsis lanceolata* takes place mostly by seeds with low rate of germination (Li *et al.*, 2003), therefore the study of germination behavior to improve the rate of seed germination assumes to be of great importance. Dormant seeds which require chilling, dry storage after ripening and light as a germination stimulator, are often treated with GA_3 to overcome their dormancy (Gupta, 2003). Gibberellic acid (GA_3) is one of the hormone which can control primary dormancy by inducing germination (Iglesias & Babiano,1997). Hence, the objective of present study is to develop an efficient system, which would improve the rate of germination of seed of *Codonopsis lanceolata*.

MATERIALS AND METHODS

Seeds of *Codonopsis lanceolata* were collected from bioherb research center, Kangwon National University, Chuncheon. Seeds were hand-separated from the pods and stored in plastic pot at room temperature under the laboratory condition at 18~25 ℃. Seeds were sterilized in 70% ethyl alcohol for 1 min followed by rinsing twice with sterile deionized water and subsequently soaked in 5% NaOCl supplemented with 2 drops of tween 20 and agitated on mechanical shaker for 30 min followed by 6 times rinsing with sterilized water.

Germination test in filter paper

To determine the effect of light and GA_3 on the rate of seed germination of *Codonopsis lanceolata*, surface sterilized seeds were planted on the single layer of Whitman No. 1 filter paper

† Corresponding author: (Phone) +82-33-250-6411 (E-mail) cyyu@kangwon.ac.kr
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[1] These two authors are equally contributed to this work.

moistened with 3 mℓ of sterile deionized water in petridishes with dimension of 90 × 15 mm. The dishes were covered and sealed with parafilm to prevent evaporation during germination period. Seeds were incubated at 25~27℃ in light and dark conditions. 1 mℓ of sterile deionized water was replenished after every alternative day to prevent drying. All experiments were replicated at least thrice with five replica of 15 seeds per treatment. Seeds were considered as germinated when radical protrusion was visible under the microscope. The percentage of germination was evaluated after every 3 days. Germination test at constant temperature were concluded after 12 days.

Germination test with GA_3

To determine the effects of plant growth regulator on seed germination, surface sterilized seeds were planted in MS (Murashige & Skoog, 1962) medium supplemented with 0.8% agar at different concentration of GA_3. Seeds were incubated at 25~27 ℃ in light and dark conditions. All experiments were replicated at least thrice with five replicas of 15 seeds per treatment. Petri dishes with seeds for treatments in darkness were wrapped with a double layer of aluminum foil. 16 hrs in light and 8 hrs in dark photoperiod at 30 $\mu E/m^2/s$ from cool white fluorescent tubes were used for light condition. The percentage of germination was monitored in every three day.

Germination test in green house

To determine the effect of light on rate of seed germination *in vivo* condition, seeds were washed with tap water and single seed was sown in each 3 × 3 cm cylindrical chamber of 54 × 28 cm size plug tray supplemented with bed soil (Hangnong Chongmyo) and perlite at the ratio of 3 : 1. Plug tray with seeds for treatment in darkness was wrapped by double layer black polyethylene sheet with tiny pores to permit free air exchange. Each (light + dark treatment) combination had 64 seeds and watering was done in every 2 days. Data were collected in every 4 days.

RESULTS

Germination of seed of *Codonopsis lanceolata* was stimulated by the treatment of GA_3. Percentage of germination was higher in case of seed exposed to 16/8 hr photoperiod than that of seed germinated under complete darkness. Similarly, percentage of germination of seed without GA_3 treatment was significantly lower than that of seed germination treated with GA_3. Maximum percentage of germination of seeds was obtained in MS medium supplemented with GA_3 3 mg/ℓ in both 16/8 hrs photoperiod and complete dark condition (Table 1 and 2). Thus, it strongly suggests that gibberellin concentration in some way plays significant role in the seed germination of *Codonopsis lanceolata.* Increasing in the GA_3 concentration increased both germination rate and percentage (Nadjafia, 2006).

The rate of germination was markedly higher in the medium treated with GA_3 than that of control seeds. It suggests that exogenous treatment of GA_3 be responsible for early seed germination.

The percentage of germination of *Codonopsis lanceolata*

Table 1. Germination of seed of *Codonopsis lanceolata* under 16/8 hrs photoperiod.

Treatments	No. of seed	3rd day		6th day		9th day		12th day	
		Germination	(%)	Germination	(%)	Germination	(%)	Germination	(%)
½ MS	50	4 ± 0.9	8.0	11.3 ± 0.3	22.7	12.0 ± 3.3	24.0	18.7 ± 0.2	37.3
½ MS + GA_3 1 mg/ℓ	50	6 ± 1.3	12.0	10.3 ± 0.3	20.7	15.7 ± 1.8	31.3	26.0 ± 0.5	52.0
½ MS + GA_3 2 mg/ℓ	50	3 ± 0.5	6.0	9.3 ± 0.3	18.7	13.7 ± 1.4	27.3	33.7 ± 0.7	67.3
½ MS + GA_3 3 mg/ℓ	50	5 ± 1.3	10.0	13.7 ± 1.9	27.3	19.7 ± 1.5	39.3	38.0 ± 0.8	76.0

Table 2. Rate of germination of seed of *Codonopsis lanceolata* under complete darkness.

Treatments	No. of seed	3rd day		6th day		9th day		12th day	
		Germination	(%)	Germination	(%)	Germination	(%)	Germination	(%)
½ MS	50	4.3 ± 0.9	8.7	11.0 ± 0.5	22.0	14.6 ± 0.5	29.3	17.7 ± 0.9	35.3
½ MS + GA_3 1 mg/ℓ	50	5.0 ± 0.5	10.0	10.6 ± 0.3	21.3	16.6 ± 1..5	33.3	21.3 ± 2.2	42.7
½ MS + GA_3 2 mg/ℓ	50	7.3 ± 0.9	14.7	15.6 ± 3.3	31.3	24.0 ± 2.6	48.0	29.7 ± 0.9	59.3
½ MS + GA_3 3 mg/ℓ	50	4.7 ± 1.2	8.9	10.3 ± 1.4	20.7	20.3 ± 3.1	40.7	37.6 ± 1.1	75.3

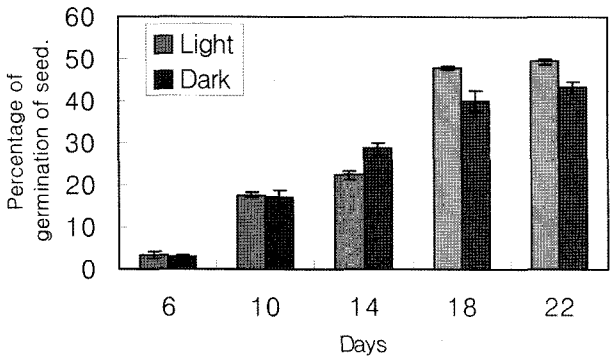

Fig. 1. Rate of germination of seed of *Codonopsis lanceolata* in green house.

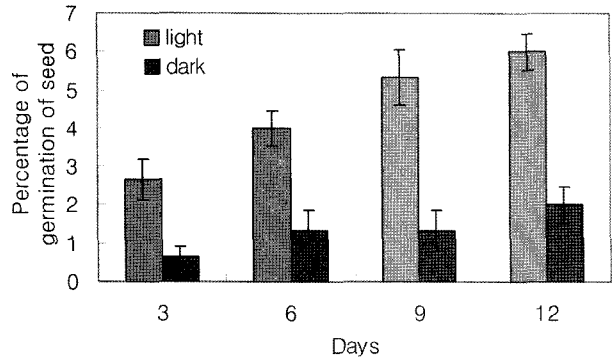

Fig. 2. Rate of seed germination of *Codonopsis lanceolata* in filter paper.

seeds *in vivo* condition (Green House) showed significant differences among seeds treated with 16/8 hrs photoperiod and complete darkness conditions. Germination rate was higher in case of seed exposed under 16/8 hrs photoperiod (49.5%) than seed germinated under complete dark condition (43.2%). A marked increase in the percentage of seed germination in light treatment indicates that the light treatment can change the endogenous GA_3 level of seeds (Fig. 1).

Similarly, in the filter paper germination test the seed started germinating after 3 days and 9 days of inoculation in light and dark condition respectively. The majority of seeds did not germinate (Fig. 2). The percentage of germination in dark treatment under constant temperature was much lower (2%) than the seed treated in 16/8 hrs photoperiod (6%).

DISCUSSIONS

The overall germination performance of seeds incubated in 16/8 hrs photoperiod was higher than that of seed incubated in complete darkness. According to Baskin and Baskin (1998) light is one of the most important environmental factors that interact with temperature to regulate seed germination in many plant species. Light requirement for germination may vary with temperature (El-Keblawy & Al- Rawai, 2005).

Application of GA_3 stimulated the germination and this response was dependent on the concentration of applied GA_3. Gibberellic acid (GA_3) promoted seed germination in species exhibiting physiological dormancy (Bewley & Black, 1994; Baskin & Baskin, 1998). Germination rate (early germination) was higher in GA_3 treatment seed and it was positively correlated with germination percentage. Therefore, fast germination was related with high germination percentage. As suggested by the De Greef & Fredericq (1983) gibberellin (GA_3) was known to mimic the effect of red light on the germination of seed. Several biochemical processes in germinating seed seem to be mediated directly by gibberellins, such as food reserve mobilization and cell wall softening. Evidences has been provided that gibberellins decrease the permeability of the plasma membrane to Ca^{2+}, with consequences for the effect of GA_3 on wall hydrolases (Thomas, 1992). The rate of germination of seed was much lower in the filter paper both in light and dark treatment. It suggests that *Codonopsis lanceolata* seeds are light responsive. According to Yamaguchi and Kamiya (2000), light controls the seed germination or dormancy by promoting gibberellin biosynthesis or increases seed sensitivity to gibberellins by phytochrome. The role that gibberellic acid (GA) plays in the germination of seeds, in which the embryo as a source of GA, supplying the aleurone layer which stimulates the production of a-amylase, a hydrolytic enzyme responsible for the digestion of food reserves within the seed, required by the growing embryo and developing seedling during seed germination (Hamman, 2003).

CONCLUSIONS

The poor rate of germination of seed of *Codonopsis lanceolata* was due to impermeable seed coat, which induced considerable amount of seed dormancy. As suggested by the Perez-Garcia (2005), the mechanism of dormancy lies in the seed coat and physical dormancy caused by impermeable seed coat is the main reason of poor germination. The present work established an efficient strategy for breaking the seed dormancy of *Codonopsis lanceolata* with appropriate amount of exogenous GA_3 and suitable photoperiod.

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