Germination and Emergence of *Eclipta prostrata*(L.) L.

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한련초의 發芽 및 出現

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ABSTRACT

Several experiments were conducted to investigate the effects of external factors on germination and emergence of *Eclipta prostrata*(L.)L. The weight of viable achenes doubled as a result of 90 minutes soaking in water. The germination of *E. prostrata* was significantly improved by alternating temperatures. At a constant temperature of 35℃, only 78% of the achenes germination, whereas at alternating temperatures of 35/20℃, 96.5% of the achenes germinated. *E. prostrata* was more sensitive than rice to moisture stress. No germination of *E. prostrata* achenes occurred in the absence of oxygen. No germination of *E. prostrata* achenes occurred in the dark or when they were exposed to green, blue, and far-red light. Germination of *E. prostrata* achenes was influenced by the duration of illumination after absorption of water. Ten hours of illumination was needed for maximum germination and 2 hours for 50% germination. No significant changes in germination of *E. prostrata* achenes were observed between pH 3 and 10. A high tolerance of *E. prostrata* achenes to salt was observed. Emergence of *E. prostrata* achenes was greatly affected by planting depth. In the upland soil, 74.0% of the achenes planted on the soil surface germinated, and no emergence was at planting depths of 0.5 cm or greater.

Key words ; *Eclipta prostrata*, germination, emergence.

INTRODUCTION

Eclipta prostrata (L.) L. is an important weed which is distributed throughout the warmer areas of the world and into temperate regions. It occurs under both lowland and upland condition. Thus, it has a high dispersibility and a high adaptability to changing environmental conditions. In spite of this, few physiological and ecological studies have been conducted for this species.

For a seed to germination, it must be placed in favorable environmental conditions such as an adequate supply of water, a suitable temperature and oxygen supply as well as light for certain seeds. The requirements vary according to the species as well as variety and ecotype. Although research on seeds of different weeds has contributed to the current knowledge of seed germination and emergence, the results are not directly interchangeable.

Of the many environmental factors which affect germination, temperature is probably the most important, assuming adequate moisture is available (19), because most of the germination processes are enzymatic and highly temperature

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dependent (7). Over the range of temperature in which a seed germinates, there is an optimum germination temperature. There are also minimum and maximum temperatures at which germination is delayed but not prevented. The optimum temperature for germination of non-dormant seeds of various weeds is generally quite specific. Although non-dormant seeds of most weed species have a single temperature optimum for germination, in many species biomodal temperature optima have been reported.

Germination is a process related to living cells and requires an expenditure of energy by the cells. The energy requirement of living cells is usually sustained by processes of oxidation. Although metabolic processes involved in plant growth have high oxygen demands, anaerobiosis or reduced oxygen levels may induce germination of some weed seeds (1, 9, 34).

Light is required for the germination of seeds of many weed species including *E. prostrata* (26). In photoblastic seeds, sensitivity to light changes with time after the beginning of imbibition of dry seeds in the dark (7, 17). In all cases, sensitivity to light increases relatively rapidly, peaks, and then declines slowly, relative to the initial increase.

The mechanism by which phytochrome induces germination is still unknown (8), but its primary action is thought to be through direct interaction with cellular membranes (21). The time required for phytochrome to induce germination after convension to the far-red light absorbing form of phytochrome (P_{fr}) varies. However, the average time is generally several hours. Gorski et al. (15) found that light filtered through leaf canopies inhibited or retarded germination of seeds of many species that readly germinated under diffuse white light.

The light requirement of weed seeds is the principal means by which seed germination is restricted to the area close to the soil surface. Most soils attenuate light very effectively so that sufficient light for inducing germination is present only in the first few millimeters from the soil surface (43). Therefore, some people question whether light is the major controlling factor, because light cannot penetrate to a depth of 2.5 cm in soil (33). Thus, in an agricultural environment, many weed seeds are buried by cultivation and can germinate only when they are reexposed to light by subsequent cultivation. Increased emergence of weed seedlings caused by soil disturbance is a well-known phenomenon (29, 40, 41).

Imbibition is a prerequisite for germination. The swelling of seeds reflects the storage materials present in the seeds (22). Without sufficient initial water uptake, germination cannot occur. If the water content of the embryo does not reach the level required to support biochemical events that lead to cell expansion, germination cannot occur.

The hydrogen ion has been found to have no effect on the germination of seeds of several species within the pH range normally found in soil (3, 10, 27, 31). In general pH has little influence on germination or dormancy. Weed seeds usually remain in contact with soil much longer than crop seeds, therefore more attention should be paid to the effect of pH on germination.

The high salt content of soils, especially sodium chloride, can inhibit germination, primarily due to osmotic effects. In saline environments, the development of the seedling is extremely poor, However, a number of plants, which have resistance to salt, can develop.

Kobayashi (18) noted that in the same families showed a similar degree of salt resistance. He found that weeds belonging to the Asteraceae family were highly tolerant to high salt contents compared to the other nine families which occurred in reclaimed tidal land. The adaptability of *E. prostrata* to high salt contents was reported by Choudhuri and Varshney (6) and Varshney and Sharma (37).

MATERIALS AND METHODS

Matured achenes collected from the experimental fields at IRRI were planted in the greenhouse in order to collect uniform achenes for the tests.

Unless otherwise stated, 50 achenes were placed in a 9 cm diameter plastic petri dish containing Whatman No. 1 filter paper moistened with 7 ml of distilled water for each replication. The petri dishes were randomly arranged in an incubator and their positions were changed both horizontally and vertically daily. Germination counts were made until the end of germinaion. The protrusion of the radicle through the seed wall was used as the criterion for germination. For all experiments, a completely randomized design with four replications was used.

Imbibition. Five hundred achenes were placed in each petri dish containing water. After 10 and 20 minutes, 0.5, 1, 1.5, 2, 3, 48, 72, and 96 hours, the achenes were removed from the moistened paper, dried by blotting, and weighed. The number of germinated achenes was also counted. The imbibition of heat-killed achenes was compared to that of viable achenes. Achenes were subjected to a constant temperature of 35℃.

Temperature. Achenes were subjected to constant light, seven constant temperatures (15, 20, 25, 30, 35, 40, and 45℃), and three alternating temperatures (35/15, 35/20, and 35/25℃) for 12 hr each.

Simulated soil moisture content. Aqueous solutions of polyethylene glycol (PEG) having an average molecular weight of 6000, were prepared by dissolving 0, 41, 63, 93, 117, and 200 g of PEG in 1 L of distilled water to obtain osmotic potentials of 0, −0.25, −0.5, −1.0, −1.5, −2.0, −3.0, and −4.0 bars, respectively. The concentration of each solution was determined from the incubation temperature and the desired osmotic potential by using the following equation (24),

$$\psi s = -(1.18\times10^{-2})C - (1.18\times10^{-4})C^2 + (2.67\times10^{-4})CT + (8.39\times10^{-7})C^2T$$

where

C=concentration of PEG-6000 in g/kg H_2O

T=temperature in ℃

Achenes were moistened with 7 ml the appropriate PEG solution and were placed in an incubator at 35℃ and constant light. Fifty rice seeds (UPLRi]-5) were also treated in the same manner.

Oxygen. The oxygen content was adjusted by using a continuous flow of oxygen for the 100% level, air for the 20% level, and nitrogen for the 0% level.

Fifty achenes were placed on a disk of 9 cm diameter filter paper in a 500ml flask. The filter paper was supported by glass beads to prevent excess water from submerging the achenes and yet maintaining maximum moisture conditions by keeping the filter paper wet. The flasks containing the achenes were placed in an incubator at alternating temperatures of 35/20℃ for 12 hr each with constant light.

Light quality. Six levels of light quality (white, red, yellow green, blue, and far-red) and dark condition were used. These were achieved as follows:

White light : The petri dish was exposed to light.

Red light : The petri dish was wrapped in red cellophane.

Yellow light : The petri dish was wrapped in yellow cellophane.

Green light : The petri dish was wrapped in green cellophane.

Blue Light : The petri dish was wrapped in blue cellophane.

Far-red light : The petri dish was wrapped in one layer of blue cellophane between two layers of red cellophane.

Dark : The petri dish was wrapped with aluminum foil.

Achenes were placed in an incubator with alternating temperatures of 35/20℃ for 12 hr each and constant light.

Illumination length. The achenes fully imbibed for 5 days in darkness were subjected to nine different illumination lengths 0, 0.5, 1, 2, 4, 6, 8, 10, and 12 hours. A treatment consisting of

constant illumination was also included for comparison purposes. The achenes were placed in an incubator having a constant temperature of 35℃. Each petri dish was wrapped in aluminum foil before and after desired illumination length.

pH. Eight different buffer solutions of pH 3, 4, 5, 6, 7, 8, 9, and 10, were used for the germination media.

The buffered solutions were prepared using 0.1 M potassium hydrogen phthalate in combination with either 0.1 M HCl or 0.1 M NaOH to obtain pH levels of 3, 4, 5, 6, and 7. A 0.025 M borax solution in combination with 0.1 M HCl was used for pH levels of 8, 9, and 10. Achenes were exposed to continuous light at 35/20℃ for 12 hr each. Fifty rice seeds (UPLRi-5) were also treated in the same manner.

Salinity. Achenes were subjected to nine levels of NaCl --0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6%. Achenes were placed in petri dishes containing filter paper and 7 ml of the appropriate NaCl solution. The dishes were placed in an incubator at alternating temperatures of 35/20℃ for 12 hr each with constant light. Fifty rice seeds (UPLRi-5) were treated in the same manner.

Planting depth. One hundered achenes were planted in a plastic tray measuring 32 x 24 x 11 cm. The tray was filled with either upland soil or lowland soil.

The treatments were as follows:

field condition	planting depth (cm)	water regime
Upland	0	Saturated
	0.2	
	0.5	
	1.0	
Lowland	0	Saturated
	0.2	Flooded 2 cm deep
	0.5	Flooded 5 cm deep
	1.0	

After planting, the trays were sub-irrigated to minimize soil compaction and disturbance.

RESULTS AND DISCUSSION

Imbibition

The weight viable achenes was doubled as a result of 90 minutes soaking in water (Fig. 1). Heat-killed achenes absorbed water more rapidly compared to viable achenes. After 10 minutes soaking, the weight of heatkilled and viable achenes increased to 142% and 127% of their initial weight, respectively. The weight of viable achenes after full imbibition increased as germination increased. When there was 63% and 91% germination, their weights increased to 278% and 302% of their initial weight, respectively.

The high absorption of the heat-killed achenes might be because fruit wall permeability was improved by heat treatment. This indicates that achene imbibition of *E. prostrata* is not related to viability but to physical properties. The thick fruit wall is probably a morphological character for rapid absorption of greater amounts of water. Vidaver and Hsiao (38) noted that the germination response in some light sensitive seeds was influenced by seed water content during earlier exposure to light.

Temperature

The germination of *E. prostrata* was significant-

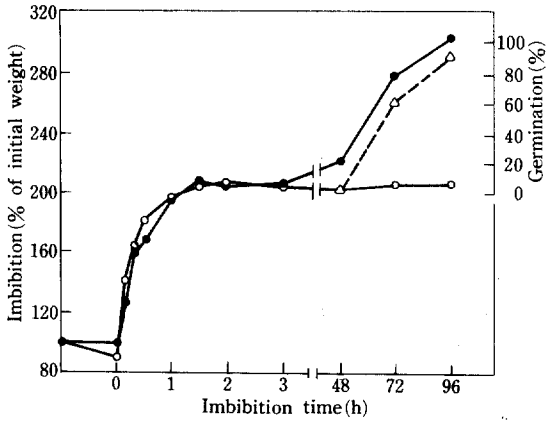

Fig. 1. Comparison of imbibition between viable and heat-killed achenes of *Eclipta prostrata*.

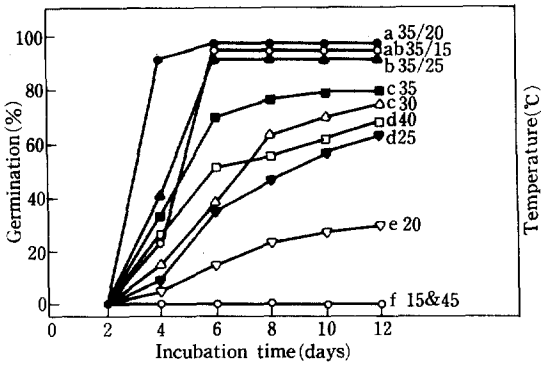

Fig. 2. Percent germination of *Eclipta prostrata* achenes incubated under different temperatures; alternating temperatures were for 12 hours each. (Means followed by the same letter are not significantly different at the 5% level by DMTR.)

ly improved by alternating temperatures. Maximum germination occurred at the 35/20℃ temperature regime with a 12-hr thermoperiod and a constant photoperiod (Fig. 2). The final germination percentages of achenes incubated at 35/25℃, 35/20℃ and 35/15℃ did not differ markedly. At a constant temperature of 35℃, only 78% of the achenes germinated, whereas at alternating temperatures of 35/20℃, 96.5% of the achenes germinated. No germination occurred at constant temperature of 15℃ and 45℃. The minimum and maximum temperatures for germination were 20℃ and 40℃, respectively.

Germination is a complex autocatalytic process composed of many interdependendent steps, most of which are enzymatic and, thus, are highly temperature dependent (8). Although most weed species have a single temperature optimum for germination, alternating temperatures stimulate it (28, 42). This may be due to different temperature optima for different metabolic steps required for germination. Toole (35) suggested that stimulation of *A. smithii* seed by alternating temperatures is due to facilitation of the proper balance and interaction of hormones, enzymes and substrates.

Simulated Soil Moisture Content

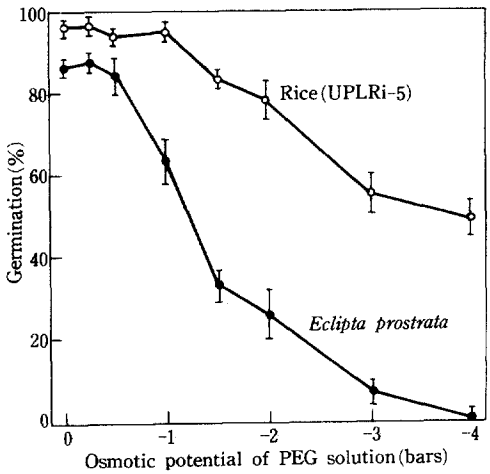

Fig. 3. Percent germination of *Eclipta prostrata* achenes and rice seeds (cv. UPLRi-5) at different osmotic potentials of polyethylene glycol solution. (Vertical bars indicate standard deviation of mean.)

High sensitivity of *E. prostrata* achenes to different osmotic stresses was observed (Fig.3). The lower the osmotic potential of aqueous solutions of PEG, the lower the germination percent-- 84.5% at -0.5 bars, 63.0% at -1.0 bars, and 33.0% at -1.5 bars. *E. prostrata* was more sensitive than rice to moisture stress.

Without sufficient initial water uptake, germination cannot occur. When the water content of the embryo reaches the level required to support biochemical events that lead to cell expansion then germination occurs. Imbibition is determined by the properties of the seed and the soil and the degree of contact between them (2). Therefore, although seed water potential is determined by soil water potential, the critical soil water potential permitting germination may change because of poor seed-soil contact and may result in more severe water stress during germination even at soil water potentials suitable for germination (16, 44). Young *et al* (44). reported that the relation between *Sisymbrium altissimum* L. germination under osmotic stress and soil moisture stress was dependent on the soil substrate.

Table 1. Percent germination of *Eclipta prostrata* achenes as affected by oxygen content.[a]

Oxygen content (%)	germination (%)
100	95.5a
20	96.5a
0	0.0b

[a] In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Oxygen

No germination of *E. prostrata* achenes occurred in the absence of oxygen. An increase in oxygen content above atmospheric level did not stimulate germination (Table 1).

Germination processes require energy expenditure by cells, and the energy is sustained by processes of oxidation through respiration. Hence, most seeds show lower germination when oxygen tension is decreased below that normally present in the atomosphere. But there are many cases in which germination of some species like *Cynodon dactylon* L., *Typha latifolia* L. (25), and *Zizania aquatica* L. (30) are enhenced as the oxygen content of the air is decreased below 20%. No germination of *E. prostrata* achenes in the absence of oxygen implies that germination may be inhibited by flooding.

In some seeds like *Rumex crispus* L. (13) and *Sicyos angulata* L. (20), germination increases with increase in oxygen content above atmospheric levels. Frequently in these cases, seed coats are impermeable to oxygen. However, based on the results obtained in this experiment, the fruit wall and seed coat of *E. prostrata* achenes are not a barrier to oxygen diffusion.

Light quality

No germination of *E. prostrata* achenes occurred in the dark or when they were exposed to green, blue, and far-red light, but germination under red and yellow light was as good as that under white light (Table 2). This means that light having wavelengths between 580 and 700 nm promotes the germination of *E. prostrata* achenes.

Table 2. Percent germination of *Eclipta prostrata* achenes under different light qualities.[a]

Light quality[b]	Germination (%)
White light	94.5a
Red light	93.0a
Yellow light	94.0a
Green light	0.0b
Blue light	0.0b
Far-red light	0.0b
Dark	0.0b

[a] In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

[b] Achenes were exposed to prolonged illumination of different light qualities during incubation.

In addition, the P_r and P_{fr} forms of phytochrome are associated with the germination process.

Sunlight has a ratio of red/far-red light of about 10/9. This is the ratio needed to induce germination in photoblastic seeds (36). However, the leaf canopy transmits very little radiation in the 400-700 nm wavelengths and many times as much radiation in the longer wavelengths (14, 32, 36). Gorski (14) reported that a single rhubarb (*Rheum rhaponticum* L.) leaf transmited 2.5, 3, 8 and 28% in the blue, red, green and far-red light ranges. The ratio of far- red to red was about 9. Therefore, poor germination of *E. prostrata* achenes may occur a crop canopy even though other facters are favorable for germination (11, 12, 14, 39). Van der Veen (36) noted that not only photoblastic seeds but also seeds requiring darkness for germination can be inhibited by light under vegetation.

Illumination Length

Germination of *E. prostrata* achenes was influenced by the duration of illumination after absorption water (Fig. 4). Ten hours of illumination was needed for maximum germination, and 2 hours for 50% germination. Exposure to unfiltered light for 30 minutes or less did not result in germination.

Mayer and Poljakoff-Mayber (22) noted that the time at which seeds reach maximum sensitiv-

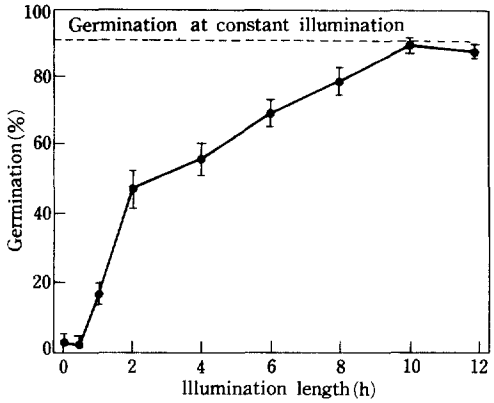

Fig. 4. Percent germination of *Eclipta prostrata* achenes as affected by illumination length after imbibition for 5 days in darkness. (Vertical bars indicate standard deviation of mean.)

ity for germination is related to the light intensity or the amount of radiation. Duke et al. (7) reported that the sensitivity of Portulaca *oleracea* L. seed to light increased and then declined slowly with time after the beginning of imbibition of dry seeds.

Thus, the response to illumination length may be changed by light intensity and the time in darkness before illumination. These results imply that the germination of achenes under field conditions may vary, although the achenes are exposed to light for the same length of time.

pH

No significant changes in germination of *E. prostrata* achenes were observed pH 3 and 10 (Table 3).

Soil pH influences plant growth. However, growth limitation is not due to the pH effect but is due to one or more secondary factors which are pH dependent (23). Burdett (5) reported that low pH enhanced the effectiveness of gibberellic acid in stimulating germiation of lettuce (*Lactuca sativa* L.) seeds but the effect was not substantial. Many researchers have reported that pH does not have an effect on seed germination of many species within the pH range normally found in soil

Table 3. Percent germination of *Eclipta prostrata* achenes and rice seeds (cv. UPLRi-5) as affected by pH.[a]

pH	Germination (%)	
	Rice seed	*Eclipta prostrata*
3	95.5a	93.0a
4	95.0a	91.5a
5	94.5a	93.0a
6	97.0a	92.5a
7	96.0a	93.0a
8	95.5a	91.5a
9	92.5a	91.0a
10	95.5a	90.0a

[a] In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

(10, 27, 31). Thus, pH is not a major limiting factor to metabolic activity during germination of *E. prostrata* achenes.

Salinity

A high tolerance of *E. prostrata* achenes to salt was observed (Fig.5). There was 81.5% germination in a 0.8% NaCl solution and 13.5%

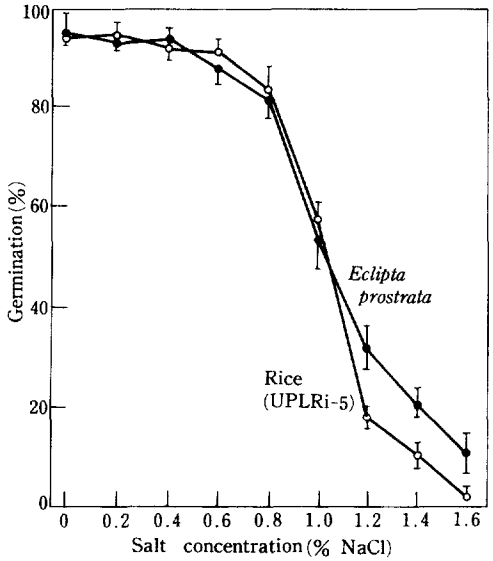

Fig. 5. Percent germination of *Eclipta prostrata* achenes and rice seeds (cv. UPLRi-5) at different salt concentrations. (Vertical bars indicate standard deviation of mean.)

germination in a 1.6% NaCl solution. This tolerace to salinity was similar to that of rice 'UPLRi-5'.

There may be a discrepancy in germination percentages between laboratory and field conditions, because soil moisture in saline soil is frequently depleted due to evaporation which leads to higher salt contents. Varshney and Sharma (37) reported that *E. prostrata* had a high salt adaptive capacity at the germination phase and subsequent stages of growth.

In general low seed germination at high NaCl concentrations is attributed to low osmotic potential. The response of *E. prostrata* achenes to salt seemed to be due to the Na concentration not the osmotic potential because the responses to salt concentration and osmotic potential were different between *E. prostrata* achenes and rice seeds (Fig. 3 and 5). Boucard and Uager (4) reported that cytokinin levels were reduced when seeds of *Suaeda* spp. were imbibed in NaCl solutions.

Planting Depth

Emergence of *E. prostrata* achenes was greatly affected by planting depth (Table 4). In the upland soil, 74.0% of the achenes planted on the soil surface germinated, 18.5% emerged when planted 0.2 cm deep, and no emergence was observed at planting depths of 0.5 cm or greater. Under lowland conditions, emergence was more sensitive to planting depth --less than 10% of the achenes germinated when planted on the soil surface, and no emergence was observed when the planting depth was 0.2 cm or greater.

Table 4. Percent emergence of *Eclipta prostrata* achenes as affected by planting depths under different field conditions.[a]

Planting depth (cm)	Upland	Lowland		
	saturated	saturated	flooded	
			2 cm deep	5 cm deep
	emergence (%)			
0	74.0a	76.5a	8.5c	9.0c
0.2	18.5b	10.5c	0.0d	0.0d
0.5	0.0d	0.0d	0.0d	0.0d
1.0	0.0d	0.0d	0.0d	0.0d

[a] Means followed by a common letter are not significantly different at the 5% level by DMRT.

E. prostrata achenes needed light for germination (see Table 2). The light requirement obviously played a major role in the poor emergence observed when seeds were sown below the soil surface. Much lower germination and emergence percentages were observed in flooded conditions probably because of the low oxygen content in water (see Table 1). Because of the light and oxgen requirements for germination, there was no emergence at the 0.2 cm planting depth in lowland conditions. More compacted soil might be another factor responsible for the low emergence in lowland conditions. Therefore, the occurrence of *E. prostrata* in lowland fields may be determined entirely by water management.

摘 要

한련초의 發芽와 出現에 미치는 外的 要因의 影響을 究明하기 爲하여 數個의 試驗이 遂行되었다. 한련초의 種子는 90分 浸種에 依해 2倍 무게로 增加되었다. 發芽率은 常溫보다 變溫에서 더 높아 35℃에서 78%, 35/20℃에서 96.5%였다. 種子의 發芽는 水分Stress에 極히 敏感하였다. 酸素 缺乏條件에서는 發芽하지 않았으나 大氣水準 以上의 O_2含量에서도 發芽는 促進되지 않았다. 暗條件, 綠色, 靑色, far-red光條件에서는 發芽하지 않았고 赤色, 黃色光條件에서는 正常 發芽하였다. pH3-10 範圍에서는 發芽率에 有意變化가 없었다. 한련초 種子는 salt에 높은 耐性을 보여, NaCl 0.8%에서 81.5%의 種子가 發芽하였다. 土壤內 種子 깊이에 따라 出現이 極히 敏感하여, 밭條件에서는 0.5cm 깊이에서도 出現하지 않았고 논湛水條件에서는 더 얕은 깊이에서도 出現하지 않았다.

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