Effect of Various Factors on Dormancy-Breaking of *Digitaria sanguinalis* Seeds

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바랭이 種子의 休眠打破에 관한 硏究

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ABSTRACT

The effects of various factors on germination of dormant *Digitaria sanguinalis* seeds were studied to obtain the basic informations on establishing an effective control method in upland crops. Germination of dormant seeds was most significantly promoted by light treatment, about 88-89%, and followed by the treatment of alternating temperatures (15 or 20°C to 35°C), showing the similar effects like light. Removal of seed coats also increased germination of dormant seeds by 72%, next to the treatments of light and altering temperature. Potassium nitrate (KNO_3) at 10^{-2}M and concentrated sulfuric acid (H_2SO_4) treatment at the duration of 8 minutes resulted in 20% and 37.8% of germination of dormant seeds, respectively, which were significantly higher than that of the untreated control. Under the conditions of altering temperature, gibberellic acid (GA) at 10^{-3}M to 10^{-6}M, benzylandenin (BA) at 10^{-4}M to 10^{-7}M and their combination had no effect on breaking of dormant weed seeds, and instead inhibited the promotive effects induced by the alternating temperature. However, under the constant temperature at 35°C in dark, all these treatments increased germination as much as 20% over the untreated control.

Key words: dormancy-breaking, Digitaria sanguinalis.

INTRODUCTION

Digitaria sanguinalis, an annual grass is an important weed that poses a serious problems in both temperate and tropical crops because of its ability of adaptation to wide ranges of environmental conditions[4]. In Korea, this weed was reported as a major weed and one signle dominant species occurring in orchard and upland crops[6]

Pladeck[9] reported that germination of freshly matured *Digitaria sanguinalis* seeds varied from 2% to 5% and this increased up to 49% to 75% when the seeds were allowed for after-ripening at room temperature for several months. Toole and Toole[12] found that 196 days were required for complete germination of freshly picked crab grass seeds.

Maguire and Steen[7] reported that KNO_3 does not overcome dormancy per se but may act in conjunction with dormancy-breaking treatments such as light and alternating temperature to increase germination rate.

Steinbauer and Grigsby[10] reported that field bindweed and hedge bineseed tend to produce

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seeds having coats that are impermeable to water and this impermeability can be effectively broken by immersion of the seeds in concentrated sulfuric acid for 1 hour followed by rinsing with ditilled water.

Ikuma and Thimann[5] observed that light triggered the synthesis of pectinase and cellulase which probably destroyed the integrity of the restrictive integument thereby allowing the emerging embryo to penetrate it.

Holm and Miller[3] suggested that the germination of lightrequiring seeds was promoted by red light, GA, acetylcholine (ACh), and adenosine 3',5' -monophosphate (cyclic AMP). The phytochrome in response to the red light may trigger the synthesis of ACh which causes the release of GA from a bound form and/or GA synthesis that enhances the production of cyclic AMP, which in turn could alter the metabolism of the seed in a way as to cause its germination.

There are no much informations available on germination or dormancy-breaking of *Digitaria sanguinalis*. These informations are directly needed to establish an effective control measure in upland crops. This study was conducted to investigate the effects of various factors on dormancy-breaking of *Digitaria sanguinalis* seeds.

METERIALS AND METHODS

Seeds of *Digitaria sanguinalis* harvested from the experimental farms belonging to the Kyungpook National University in the month of October, 1981 and air dried for 2 weeks prior to storage at 5°C refrigerator. In order to determine effect of various factors on germination of *Digiatria sanguinalis*, seeds previously stored for about 120 days at 5°C refrigerator were placed on two sheets of Whatman No. 1 filter paper in 9-cm petri dishes with 30 seeds and allowed to germinate on an incubator under dark or green house conditions (10 hr light and 14 hr darkness). The seeds used for this experiment were less than 165 days of storage unless otherwise noted. Germination ratios were determined when radicle or coleoptile attained a length of 2 mm or more. The following factors were used to investigate their effects on dormancy-breaking of *Digitaria sanguinalis* seeds,

Potassium nitrate. To investigate the effect of KNO_3 on dormancy-breaking of *Digitaria sanguinalis* seeds, various concentrations of KNO_3 such as 1 M, 10^{-1} M, 10^{-2} M. 10^{-3} M, 10^{-4} M, 10^{-5} M, and 10^{-6} M were treated. Seeds were placed on filter paper and moistened with 8ml of these concentrations and kept at 35°C of dark condition. Germination ratios were detected at 10 days after treatment.

Sulfuric acid. To determine the effect of concentrated sulfuric acid on dormancy-breaking of *Digitaria sanguinalis*, intact seeds were soaked in concentrated sulfuric acid for varying duration of 0, 2, 4, 6, 8, 10, 12, 14, 16, and 18 minutes followed by rinsing with distilled water repeatedly. These seeds were allowed to germinate on an incubator at 35°C under dark condition, and then germination ratios were determined at 10 days after soaking.

Alternating temperature. To investigate the role of alternating temperature on dormancy-breaking, germination tests were conducted on seeds treated with following sets of alternating temperature; 15°C to 35°C and 20°C to 35°C. The periods of the low and high temperature treatments at daily basis were 10 hr and 14 hr, respectively. The lengths of alternating temperature treatment were for 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 days, and evaluations of germination ratio were made at 11 days after each treatment.

Removal of seed coats. To determine the role of seed coat on germination of *Digitaria sanguinalis*, seeds were dehulled before sowing and allowed to germinate under light (10 hr light and 14 hr darkness) and dark condition at 35°C. The temperature during the light treatment was 35°C. Light intensity directly above the petri dishes was about 20,000 lux. Germination ratios were determined at 3, 4, and 5 days after soaking.

Growth regulators. To determine effects of

growth regulators on dormancy-breaking of *Digitaria sanguinalis*, gibberellic acid (GA), bezyladenine (BA), and their combination were used. Concentrations of growth regulators tested were as follows: 1) GA at 10^{-3}M, 10^{-4}M, 10^{-5}M, and 10^{-6}M. 2) BA at 10^{-3}M, 10^{-4}M, 10^{-5}M, and 10^{-6}M. 3) GA 10^{-3}M plus BA 10^{-4}M, GA 10^{-4}M plus BA 10^{-5}M, GA 10^{-5}M plus BA 10^{-6}M, and GA 10^{-6}M plus BA 10^{-7}M. Both petri dishes teated with growth regulators and the untreated control were placed under dark condition, and kept at 35°C constant temperature and alternating temperature from 20°C (10 hr) to 35°C (14 hr). Germination ratio was taken at 10 days after each treatment.

RESULTS AND DISCUSSION

Effect of potassium nitrate. Germination of *Digitaria sanguinalis* seeds was promoted about 20.0% compared with the untreated control when treated with KNO_3 at 10^{-2}M (Figure 1). Concentrations of KNO_3 at 10^{-1}M and 10^{-3}M also significantly increased germination ratio of *Digitaria sanguinalis* seeds, showing 18.9 and 13.3%, respectively. These data indicate that KNO_3 is partly effective on dormancy-breaking of *Digitaria sanguinalis* seeds. The physiological role of KNO_3 is known to stimulate the germination of a number of seeds in dark (8) However, Maguire and Steen (7) reported that probably KNO_3 treatment makes the seeds more susceptible to the promotive effects of light and alternating temperature to which the seeds may be exposured during germination.

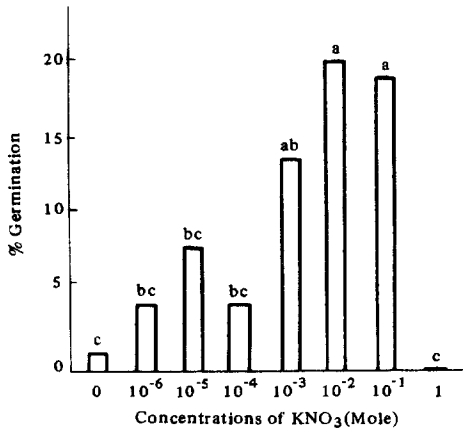

Fig. 1. Effect of KNO_3 on dormancy-breaking of *D. sanguinalis*.*
*Each value is average of 30 seeds.
Means within a figure with the same letter are not significantly different at 0.05 level (Duncan's multiple range test).

Effect of sulfuric acid *Digitaria Sanguinalis* seeds were immersed in concentrated sulfuric acid for 2 to 18 minutes to break restriction of its seed coats if coat of *Digitaria sanguinalis* seeds restricts to penetrate water or gas. Sulfuric acid treatment resulted in promotive effect on the germination of the seeds in dark condition, and with 8 minutes treatment, 37.8% of them were germinated and this duration gave the highest germination percentage although the range of 6 to 18 minutes treatments were also effective on dormancy-breaking of this weed seeds, as compared with the untreated control which showed no germination (Figure 2). Steinbauer and Gribsby (10) reported that germination of field bindweed and hedge bindweed which are also serious weeds in upland was greatly enhanced by soaking of the seeds in concentrated sulfuric acid for 1 hour.

With the results obtained from the effects of potassium nitrate and sulfuric acid treatments on dormancy-breaking, it can be concluded that the seed coat of *Digitaria sanguinalis* seed may play some role as a mechanical barrier to the germination of *Digitaria sanguinalis* seeds, showing more promotive effect of sulfuric acid on breaking dormancy.

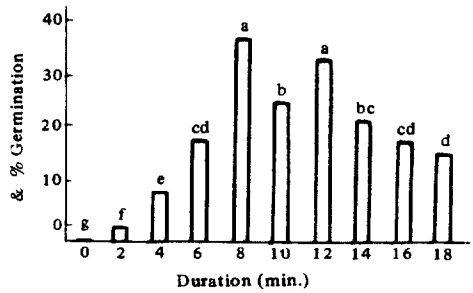

Fig. 2. Effect of H_2SO_4(Concentrated) treatment on dormancy-breaking of *D. sanguinalis*.*
*Each value is average of 30 seeds.
Means within a figure with the same letter are not significantly different at 0.05 level (Duncan's multiple range test).

Effect of alternating temperature. Daily alternating temperature treatments in both 15°C to 35°C and 20°C to 35°C significantly increased germination percentage in the similar trend as the duration of the alternating temperature treatments increased. More than 80% of *Digitaria sanguinalis* seeds were germinated after the continuous 10 days alternating temperature treatments in both cases. In case of 15°C to 35°C treatment, 139 days stored seeds were tested, and percent germination increased as incubation period increased, and 80% of germination was obtained about 6 days incubation and about 89% at 10 days incubation. But, no germination was observed in the constant 35°C treatment like the untreated control. However, in 20°C to 35°C treatment 160 days stored seeds were used and the similar trend like 15°C to 35°C treatment was observed. Approximately 10% was germinated in the constant 35°C treatment used as the untreated control (Figure 3). Different percentage of germination in both the untreated controls indicates that the dormancy of *Digitaria sanguinalis* seeds can be broken as the storage duration become longer. This facts verified that all the *Digitaria sanguinalis* seed can be naturally germinated under the natural conditions because weed seeds are naturally exposed to various ranges of alternating temperature. In another weed such as Florida pusley seeds, Biswas et al. (2) reported that the alternating temperature accelerated germination. Taylorson and Hendricks (11) reported that an alternating temperature interacts strongly with the phytochrome action induced by light during germination of seeds. It seems that the altering temperature may be one of the effective means to break the dormant *Digitaria sanguinalis* seeds and this response may be related with phytochrome action.

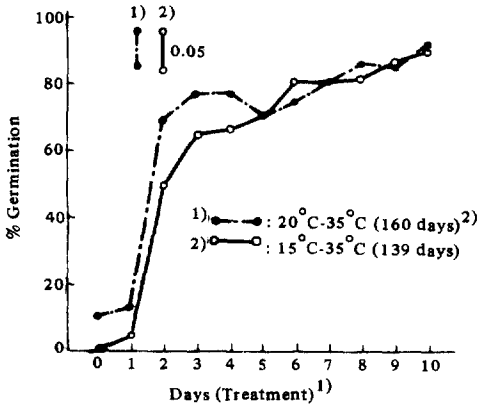

Fig. 3. Effect of alternating temperatures on dormancy-breaking of *D. sanguinalis*. (10hrs low temp. and 14 hrs high temp. treatment per day)
1) Alternating temperature treatment: the period of alternating temperature, 0, 1, 2, 3,4,5,6,7,8,9 and 10 days. (0:no treatment)
2) Storage period of seeds: 139 days for 15-35°C treatment. 160 days for 20-35°C treatment.

Effect of light and removal of seed coat. Germination of dormant *Digitaria sanguinalis* seeds was promoted by the removal of its seed coat itself, about 72% under dark condition, as compared with the unhulled seeds which showed no germination at all (Table 1). The results indicate that seed coat may play an important barrier on germination of dorment seeds. This fact can be further supported by the effects on dormancy-breaking of these seeds rendered by potassium nitrate and concentrated sulfuric acid treatments. In one way, synthesis of pectinase and cellulase which presumably destroyed the integrity of the impermeable integument was triggered by light on germination of seeds and these enzymes render the emerging embryo to penetrate its seed coats. Furthermore, light is the most important factor to induce its germination. Regardless of the presence or the absence of the seed coats, light alone can result in approximately 88-89% of germination

Tab. 1. Germination of *D. sanguinalis* affected by removal of its seed coat.

Days after soaking	Hulled		Unhulled	
	Light	Dark	Light	Dark
	%		%	
3	85.5	46.7	58.9	0.0
4	88.9	70.0	86.7	0.0
5	88.9	72.2	87.8	0.0

[a]Each value is average of 3 repli (30 seeds per repli.).
[b]8 hrs illumination per day.
[c]Complete dark condition per day

under conditions of optimum moisture and a temperature of 35°C. The fact that light is a single dominant factor to induce germination of *Digitaria sanguinalis* suggests that phytochrome system may be involved in the breaking-dormancy of this weed seeds. Many works on the involvement of phytochrome system in relation with breaking of dormancy were reported. Bewley et al. (1) also indicated similarly that the active form of phytochrome (Pfr) induced by light may increase the permeability of cell membranes. Further, Holm and Miller (3) also found out that the phytochrome in response to the red light stimulates the synthesis of ACh that brings out the release of GA from a bound form and/or GA synthesized promotes the production of cyclic AMP, which initiated the germination response.

With the effect of seed coat removal and light on dormancy-breaking of *Digitaria sanguinalis* seeds, it can be assumed that dormant characteristics of its seeds may be closely related to phytochrome system. It has been generally known that the effectivity of alternating temperature and light on the breaking of dormancy may be closely related to the phytochrome system, which may in turn induce the action of promoter or promote the synthesis of promoting hormone.

Combining effect of GA with BA. Combination of GA and BA under the constant temperature, 35°C, significantly increased germination, approximately 20% as compared with the untreated control, having germination percentage about 4%. Under the constant temperature at 35°C, the combination of GA and BA had more promotive effects on the breaking-dormancy than individually applied one.

A single application of GA and BA, and their combination under the alternating temperature showed inhibitory effects on germination than that of the untreated control (Figure 4). This indicates that hormonal application rather inhibits the promotive effects induced by the altering temperature treatment which significantly accerelated germination of dormant *Digitaria sanguinalis* seeds. The

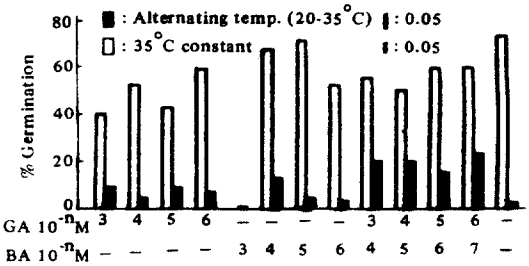

Fig. 4. Combining effect of GA with BA on dormancy-breaking of *D. sanguinalis*.
: Untreated control

higher percentage germinations when applied with growth regulators were obtained under the alternating temperature treatment rather than those of the constant temperature treatments. It was known that the exogenous GA treatment may nullify its inhibitory effect of ABA presented in seed extracts(13). With these results, it is rather difficult to generalize the effect of growth regulators on the dormancy-breaking of this weed because they had the promotive effect under the constant temperature at 35°C, but inhibitory effects under the alternating temperature. The promotive effect under the constant temperature indicates that dormancy of *Digitaria sanguinalis* may be partly caused by inhibitory substance. It needs further studies along this line to clarify their effect in relation with with dormancy-breaking.

Based on the results and observations, the following suggestions on the germination of dormant *Digitaria sanguinalis* seeds can be made from this study. Firstly, the seed coats of *Digitaria sanguinalis* may act as a mechanical barrier to the germination of this seeds. Secondly, light and alternating temperature conditions are the most effective treatments to break dormancy of *Digitaria sanguinalis* seeds. On this basis it is assumed that the breaking of its dormancy may be closely related to phytochrome system which can influence seed membrane properties, and which can control the level of promoting hormones for germination, and other physiological changes during germination.

Further studies on the role of inhibitory substances that might be existed in the seed coats may

shed more light on understanding of the dormancy-breaking of *Digitaria sanguinalis* seeds.

摘 要

休眠狀態에 있는 바랭이 種子의 發芽에 影響미치는 여러 가지 要因을 處理하여 얻어진 結果를 要約하면 다음과 같다.

1. 窒酸處理는 10^{-2}M 濃度에서 約 20%, 濃黃酸 連續 8分間 處理에서 37.8%의 發芽力을 보여 이들 두 處理는 無處理에 比하여 有意한 休眠打破效果를 보였다.

2. 變溫(15°−35°C 및 20°−35°C) 處理는 處理後 10일째 約 89%의 높은 發芽力을 보여서 빛과 더불어 바랭이 種子의 休眠打破에 가장 效果的인 方法으로 간주되었다.

3. 種皮除去로 72%의 發芽力을, 種皮의 有無에 關係없이 빛만의 照射로 約 88−89%의 극히 높은 發芽力을 보여서 變溫處理와 더불어 가장 效果的인 休眠打破方法으로 究明되었다.

4. 變温下에서 GA, BA 및 이들의 混合處理 等은 變混 그 자체에 依한 休眠打破 效果를 增加시키지 못하고 抑制시키는 현상을 보였다. 定温下에서의 平均發芽力은 變温處理時보다 훨씬 낮았으나 生長調節劑 相互處理間에는 有意한 發芽力을 보였다.

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