RESEARCH ARTICLE
J. Crop Sci. Biotech. 2008 (September) 11 (3) : 199 ~ 204

# Role of Red Light, Temperature, Stratification and Nitrogen in Breaking Seed Dormancy of *Chenopodium album* L.

**Dong-Sheng Tang[1], Muhammad Hamayun[1], Young-Moon Ko[1], Yi-Ping Zhang[2], Sang-Mo Kang[1], In-Jung Lee[1*]**

*[1]Division of Plant Biosciences, College of Agriculture and Life Sciences, Kyunpook National University, Republic of Korea*
*[2]Flower Research Institute, Yunnan Academy of Agricultural Sciences, P.R. China*

<Received August 15, 2008 / Accepted September 10, 2008>

## Abstract

Seed dormancy behavior of weed seeds is a critical determinant of their survival rates in a given cropping system as it helps the weeds to evade herbicides and other weeding practices. We investigated the effects of red light, alternating temperature, stratification duration and different doses of nitrogen containing compounds alone or in combination with red light on breaking seed dormancy of *Chenopodium album* L. The application of red light (80 $\mu mols^{-1}m^{-2}$) significantly increased seed germination of *C. album* in all treatments. Germination rates of 12 h incubated seeds were highest under 20 min of red light irradiation than 1 min, 5 min and 10 min treated seeds. Germination rate was significantly higher at alternating temperatures of 25°C and 5°C for 12 h each with an irradiation of red light (80 $\mu mols^{-1}$ $m^{-2}$) for 10 min than other treatments. Stratification period of 15 days significantly stimulated germination percentage of seeds incubated in dark, although 5 days of stratification along with red light application for 10 minutes exhibit similar effects on seeds. Seed germination was also enhanced by nitrogen containing compounds like $NaNO_2$, $KNO_3$, $NH_4Cl$ and $NH_4NO_3$. We observed that seed germination increased significantly with 25 mM $KNO_3$ and 10 mM $NH_4NO_3$ in dark condition, while $NaNO_2$ and $NH_4Cl$ enhanced seed germination under red light irradiation. It was concluded that red light alone or synergized with alternating temperatures, stratification and nitrogen compounds, especially nitrite and ammonium enhanced seed germination of *C. album*. Thus, the red light can play a vital role in present and future weed management strategies.

Key words: *Chenopodium album*, nitrogenous compounds, red light, seed dormancy, temperature, weed management

## Introduction

Seed dormancy aids to the survival and propagation of plant species through withstanding unfavorable climatic and edaphic conditions and thus providing the plant an opportunity to germinate under favorable conditions. The timing of germination in seeds is regulated by dormancy-inducing and releasing mechanisms and by mechanisms involved in the initiation of germination (Voesenek and Blom 1996). In general, these mechanisms are sensitive to environmental factors, such as light, temperature, the duration of seed storage (after ripening) and some chemicals (Bewley 1997). Seed dormancy is influenced by light, temperature, stratification and nitrogen containing compounds. Light signals are among the most important environmental cues regulating plant development (Franklin and Whitelam 2004). The impact of light on germination is controlled by the photoreversible pigment phytochrome (Pons 1992). Phytochrome undergoes reversible photo-conversion between red (Pr-inactive form) and far-red ($P_{fr}$-active form) light absorbing forms upon light irradiation (Butler et al. 1959). The temperature influences the percentage and rate of germination through its effects on seed deterioration, loss of dormancy and the germination process itself (Roberts 1988). It was observed that alternating temperatures profoundly enhanced breaking of seed dormancy in barnyardgrass, common lambsquarters and redroot pigweed, when soil water content was high enough for germination (Martinez-Ghersa et al. 1997). Stratification at lower temperatures for 8 weeks or more resulted in almost complete germina-

* **To whom correspondence should be addressed**
In-Jung Lee
E-mail: ijlee@knu.ac.kr
Tel: +82-53-950-5708

tion of seeds of *Pittosporum eugenioides* and *P. obcordatum* and increased germination in seeds of *P. tenuifolium* (Moore et al. 1994). Seed germination stimulated by nitrogenous compounds, such as nitrate and ammonium, has been documented for several species (Adkins and Adkins 1994; Hartmann et al. 1997; Teasdale and Pillai 2005). Nitrate as a signal compound is more effective in breaking seed dormancy and a major source of N for many plant species.

Lambsquarters (*Chenopodium album* L.) is a fast-growing, upright, weedy annual species of Chenopodiaceae and ranked among the most serious weeds of several major crops throughout the world (Bassett and Crompton 1978). The exact relationships between the response of *C. album* seeds to temperature, stratification, nitrogenous compounds and their requirement of red light to break seed dormancy are not clear yet. Present research investigated the germination response of lambsquarters to red light, alternating temperature, cold stratification, nitrogenous compounds and also to the synergistic effect between red light and other factors.

## Methods and materials

### Seed collection and preparation

Seeds of lambsquarters were collected from different locations in Daegu, South Korea. The seeds were dried for one week at room temperature, cleaned to approximately 50% purity and sealed in transparent polystyrene containers at 4°C in a dark room. The seeds were surface sterilized by immersion in 0.5% (w/v) NaOCl for 5 min and then washed with sterile distilled water (Khan and Ungar 1997). The seeds were then placed in 9 cm diameter Petri-dishes with two Watman #2 filter papers moistened by 4 ml of treatment solutions, using autoclaved distilled water and analytical grade chemicals, and wrapped in aluminum foil. Light emitting diode (LED) lamps with irradiation of 660 nm, 80 $mol.m^{-2}\ s^{-1}$ were used as light source for seed germination throughout the course of experiment. The experimental set up was done under very dim green light (maximum florescence of $4\times10^{-6}$ $mol/m^2$), as dim green light had no effect on dark germination or on the germination induced by a subsequent experimental irradiation (Taylor et al. 2004). All subsequent manipulations, except for the explicitly described experimental irradiations, were carried out in complete darkness. Seeds germination was observed one week after treatment and emergence of radicle (2 mm) was the criterion for germination (Andersson et al. 2002). The experiment comprised of multiple treatments, 3 replicates of 50 seeds each per treatment. All the experiments conducted were repeated at least twice.

### Red light duration and frequency

The lambsquarters seeds were imbibed for 12 hours in darkness, divided into 4 batches and each group was irradiated for 1, 5, 10 and 20 min with red light separately. Batches of seeds were given red light treatment at 12 h intervals over a period of 60 h (Botto et al. 1996; Shinomura et al. 1994). After irradiation each time, the petri-dishes were wrapped immediately and returned to 25°C growth chamber (Sanyo Model, MIR 253, Sanyo Electric Biomedical Co. Ltd., Japan).

### Red light and alternating temperature

The seeds were imbibed for 12 h in an incubator in dark, and then moved out to irradiate with red light for 10 min and immediately move to growth chamber with alternating temperature 25±1°C and 5±1°C for 12 h each per day for 7 days (Khan and Ungar 1997). Treatments without light irradiation were considered as control.

### Red light and cold stratification

The effect of stratification was studied by placing seeds at low temperature (5°C) for 5 to 15 days, after which the seeds were incubated at 25°C or irradiated under red light for 10 min before transferred to 25°C growth chamber.

### Red light and nitrogenous compounds

The role of nitrogen in promoting seed germination rates were evaluated by using different N containing compounds i.e. $NaNO_2$, $KNO_3$, $NH_4Cl$ and $NH_4NO_3$. These N sources were applied at the rates of 1 mM, 5 mM, 10 mM, 25 mM and 50 mM. Seeds were irradiated for 10 min after 12 h incubation in darkness and returned to 25°C growth chamber (Shinomura et al. 1996)

### Statistical Analysis

The means and standard error values for all treatments were compared using analysis of variance (ANOVA SAS release 9.1; SAS, Cary, NC, USA) in order to define whether the differences were significant. For interaction of light and other factors, mean percentage germination was graphically compared through Sigma Plot software (Sigmaplot 9.0, Systat Software Inc., 2004). Values included in the same group indicate that the differences among the values are not significant. But, values divided into the different groups means that the differences are significant according to the unreliability (% age) that is mentioned in each test.

## Results

### Action of red light on seed germination

The optimum condition of red light irradiation requirement for germination induction in *C. album* was investigated through applying red light pulse with different durations and frequencies. It was observed that an increase in length of red light irradiation promoted seed germination (Fig. 1).

Our study confirmed that seed germination of *C. album*, significantly enhanced, depending on red light exposure duration and frequency of red light application. Maximum seed germination (20%) was recorded in treatments exposed to 5 doses of red light with 10 min duration each time. Increase in seed germina-

tion was insignificant in treatments that received one dose of 1, 5 and 10 min of red light, although single dose irradiation for 20 min significantly increased seed germination from 5.33% to 10.67%. It was observed that application of red light pulse for 1 min gradually enhanced seed germination till 3 doses, but it reduced with further doses. Five min red light pulse significantly enhanced germination when applied 3, 4 and 5 times and 10 min pulse promoted seed germination gradually with 1, 2, 3 and 4 doses but recorded a very high germination percentage with 5 doses. Also, 20 min pulse increased germination with an increment in doses, although the increases were not pronounced between first four treatments. Red light irradiation for 1 min (3 doses), 5 min (3 doses), 10 min (5 doses) and 20 min (5 doses), promoted germination of *C. album* seeds by 14%, 16%, 20% and 18.67%, respectively. The results indicated that both irradiation exposure length and frequency significantly promoted germination of seeds and thus contributed to reduce seed dormancy of *C. album*.

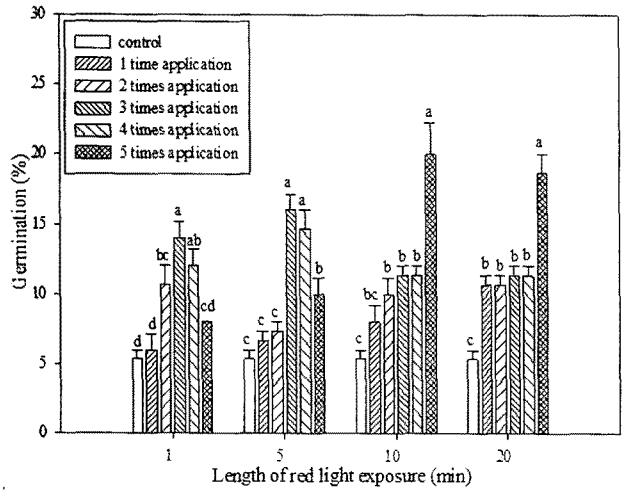


**Fig. 1.** Influence of red light irradiation length and frequency on seed germination of *C. album*. Values in each column having the same letter are not significantly different at $P < 0.05$ (Duncan's multiple range tests). Error bars represent mean values ± SE.

## Action of red light and alternating temperature on germination

It was observed that red light with alternating temperature also significantly affected seed germination in *C. album* as compared to control treatments. Germination percentage with red light pulse of 10 min and alternating temperatures of 25:5°Ê/12 hr increased significantly from 6% to 12% and 14%, respectively (see Fig. 2). Moreover, maximum germination of 26% was observed for treatments that were subjected to red light with alternating temperatures. Our results confirmed the importance of synergistic effect of alternating temperature and red light on breaking seed dormancy of *C. album*.

## Action of red light and stratification on germination

Our result showed that application of both red light (10 min)

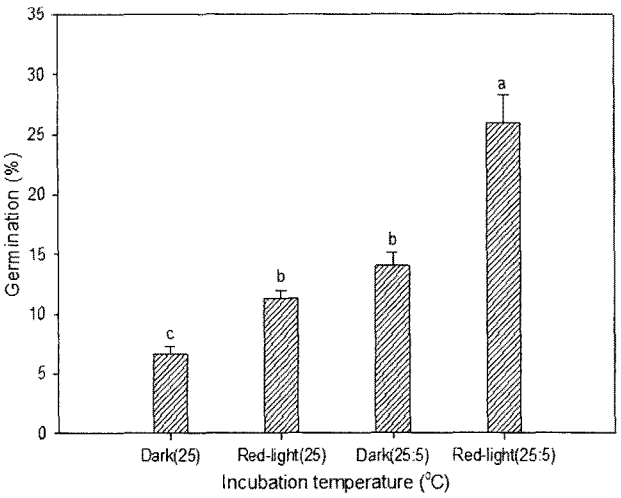


**Fig. 2.** Germination (%) of *C. album* incubated constant (25°C) and alternating temperature (25:5°C) with or without red light exposure (10 min). Columns having same letter within are not significantly different at $P < 0.05$ (Duncan's multiple range tests). Error bars represent mean values ± SE.

and stratification markedly improved germination rates in *C. album* (Fig. 3). Maximum germination was observed in treatments where seeds were exposed to red light irradiation after 15 days of stratification. Stratification of 5 days and 10 days alone did not increase seed germination significantly as compared to control. However, exposure of already stratified seeds to red light of 10 min duration sharply promoted seed germination in *C. album* and the germination percentage was directly correlated with an increase in length of stratification.

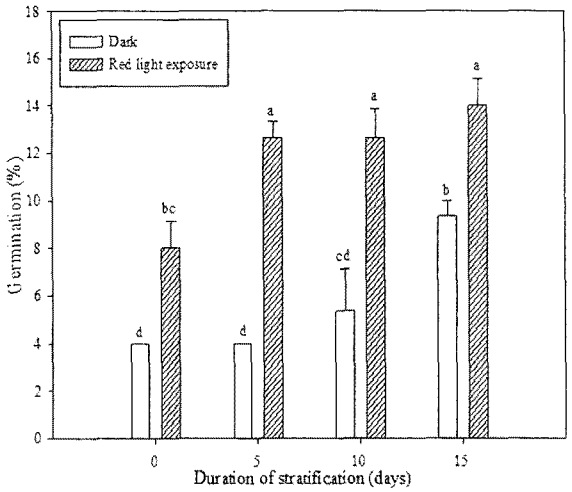


**Fig. 3.** Germination of *C. album* seeds as influenced by alternating temperature and red light. Columns having same letter are not significantly different at $P < 0.05$ (Duncan's multiple range tests). Error bars represent mean values ± SE.

## Action of red light and nitrogen on germination

In dark condition, 25 mM of $KNO_3$ and 10 mM of $NH_4NO_3$ markedly stimulated seed germination 8.67% and 10%, respectively, as compared to control (5.33%), although no significant differences were observed for $NaNO_2$ and $NH_4Cl$ applied treatments. Exposure of seeds to red light irradiation of 10 min

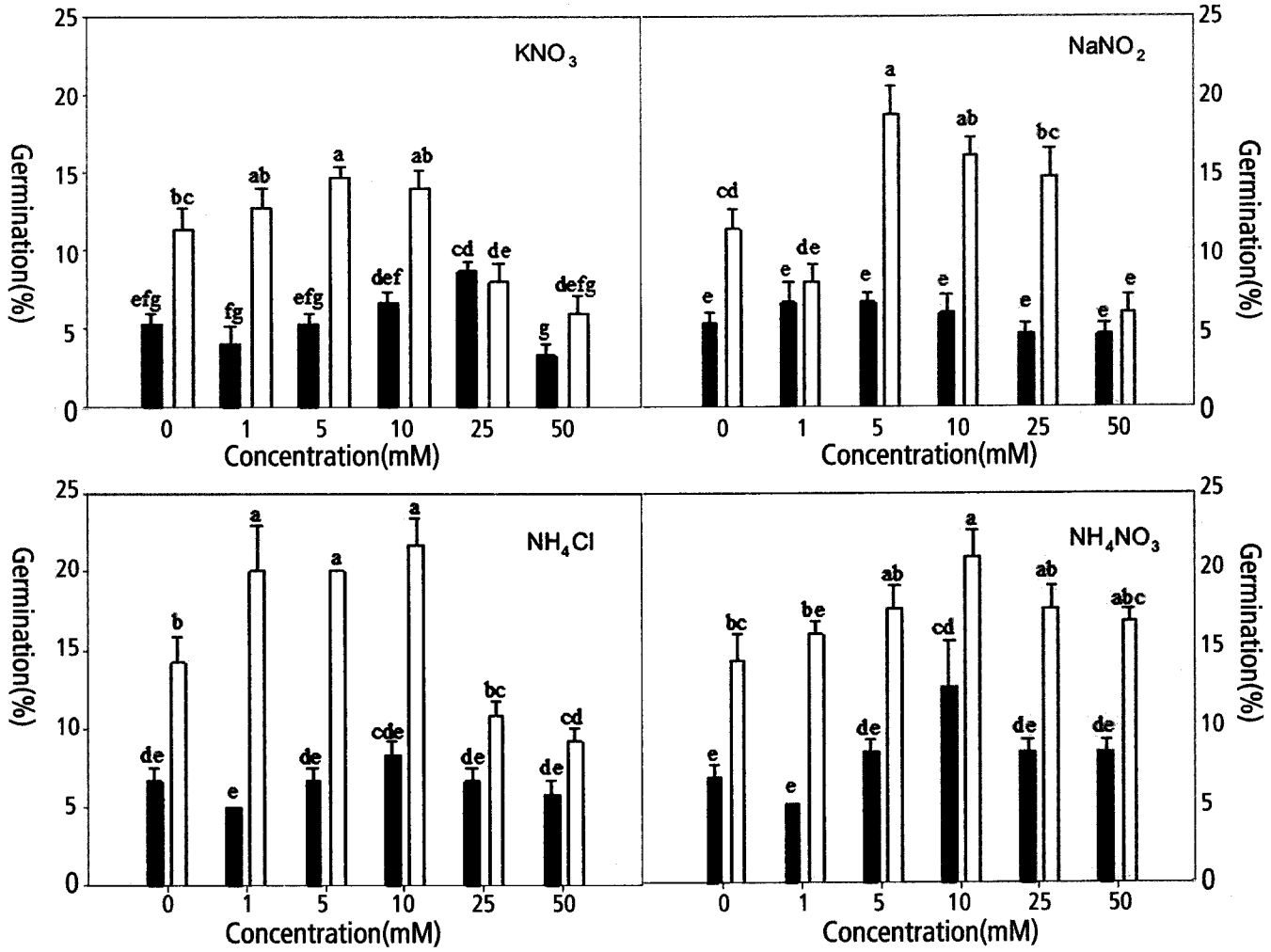


**Fig. 4.** Germination of *C. album* incubated in solutions containing four different nitrogenous compounds, with different concentrations and then exposed to 10 min of red light irradiation (white bars) or dark (black bars). Columns having the same letter are not significantly different ($P < 0.05$) (Duncan's multiple range tests). Error bars represent mean values ± SE.

resulted in a gradual increase at 1 mM, 5 mM, and 10 mM of $KNO_3$, $NH_4Cl$ and $NH_4NO_3$, but germination rates reduced when these compounds were applied at the rates of 25 mM and 50 mM. Moreover, the germination percentages increased with red light and 5 mM, 10 mM and 25 mM $NaNO_2$. The combined effect of red light and N significantly enhanced germination percentage of *C. album* at 5 mM $KNO_3$ (14.67%), 5 mM $NaNO_2$ (18.67%), 10 mM $NH_4Cl$ (16%) and 10 mM $NH_4NO_3$ (17.33%) as compared to control (11.33%). Our experimental results showed that combined application of red light and N was more effective than separate application of red light or nitrogen.

## Discussion

Seed dormancy is a common character of many weedy species that favorably help the plants to survive under difficult circumstances. Seed dormancy can be broken by the help of certain factors, including light, incubation temperature, cold stratification and nitrogen. Light is a critical determinant for seed germination in some small-seeded plants such as lettuce (*Lactuca sativa*) and Arabidopsis (*A. thaliana* (Shinomura 1997). In these plant species, a red (R) light pulse promotes and a far-red (FR) light pulse reversibly inhibits germination of dark-imbibed seeds via phytochrome (Toyomasu et al. 1998; Yamaguchi et al. 1998). Seeds of *C. album* required relatively short exposure to red light pulse (in minutes) to trigger high germination percentages. Similar observations were also reported for other species (Sauer and Struik 1964; Schutz 2000; Scopel et al. 1994; Steadman 2004) though some species like Carex species required much longer exposure to white light to promote maximum seed germination (Kettenring 2006). Our study also confirmed the importance of red light frequency in breaking seed dormancy in *C. album*. Alternating temperature stimulate seed germination by activating certain physiological processes within the seed. Our study showed that alternating temperature was more favorable in breaking seed dormancy in *C. album* than a constant temperature. The combined effect of alternating temperatures and red light was much pronounced in *C. album*, indicating the importance of

synergetic action of these two factors. Steadman (2004) reported that seed germination was greater under fluctuating diurnal temperatures than constant temperatures.

*C. album* seeds required both light and cold stratification for high germination percentages as both of these factors recorded lesser stimulation of germination when applied alone. Cold stratification was also reported to increase *C. album* germination (Hock et al. 2006). Similarly, *Carex stricta, C. comosa* and *C. lacustris* showed very high germination response after wet or moist cold storage, while *C. lasiocarpa* and *C. rostrata* showed similar rates of germination after either wet-cold or dry warm storage (Budelsky and Galatowitsch 1999). Induction and promotion of seed germination in *C. album* may be due to gibberellins synthesis in seeds as GA biosynthesis is regulated by both light and cold temperature. The final step in active GA biosynthesis is catalyzed by gibberellic acid 3-oxidase ($GA_3ox$) and importantly, the expression of both seed-expressed $GA_3ox$ isoforms is promoted by both light and cold stratification (Yamaguchi et al. 1998, 2001, 2004). Nitrogen containing compounds are considered to be effective in breaking seed dormancy and thus induce seed germination. Our results showed that $KNO_3$, $NaNO_2$, $NH_4Cl$ and, $NH_4NO_3$ can effectively release seeds dormancy in *C. album*, although an addition of red light pulse can produced much better results than separate application of these two factors. Nitrates are reported to stimulate germination of many dormant seeds, as nitrate plays role as a cofactor in phytochrome action (Grubisic and Konjevic 1990). Nitrates may be associated with enhancing the number of $P_{fr}$-receptors (Hilhorst 1990) or may act as a $P_{fr}$ cofactor (Grubisic and Konjevic 1990) in positively photoblastic species. It was observed that nitrate provided exogenously or by mother plants to the produced seeds, acts as a signal molecule favoring germination (Alboresi et al. 2005; Hilhorst 1990). Although ammonium salts are usually ineffective (Hendricks and Taylorson 1974), our study confirmed the effectiveness of ammonium in stimulating seed germination in *C. album*. However, the mechanism of nitrogenous compound in regulating seed germination and breaking seed dormancy is poorly understood and more research work is needed to clear the existing ambiguities.

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