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GERMINATION OF SOME IMPORTANT WEEDS INFLUENCED BY RED LIGHT AND NITROGENOUS COMPOUNDS

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Abstract

Seed dormancy is a major constraint in the eradication of weeds from agriculture fields. Seeds of *Amaranthus retroflexus*, *Echinocloa crus-galli* and *Digitaria adscendens* were collected from cultivated fields, dried and then treated with different nitrogen containing compounds i.e., potassium nitrate, ammonium chloride, ammonium nitrate and sodium nitrite. Some seeds were kept under dark while others were irradiated with red light for 10 min., after 12 hr of inhibition. The N-compounds were applied @ 1, 5, 10, 25 and 50 mM, while the strength of red light pulse was maintained at 80μmols^{-2}m^{-1}. It was observed that red light significantly improved germination rates of *A. retroflexus, E. crus-galli* and *D. adscendens*. Nitrogenous compounds significantly improved germination of weeds and maximum germination was induced by ammonium nitrate. However, exposure of seeds to both red light and N-compounds provided significantly higher germination as compared to singular application of either of them. *E. crusgalli* recorded highest germination rates in response to red light and N-compounds, while *D. adscendens* provided least values for the same treatments. Application of N-compounds in conjunction with red light significantly improves germination rates of selected weed species by breaking their dormancy.

Introduction

Seeds of many annual weeds enter a state of dormancy even after maturation and desiccation in plant (Bewley, 1997). Seed dormancy could be thus considered as a block to the completion of germination of seed under favorable conditions (Finch-Savage & Leubner-Metzger, 2006). Various chemicals have been known to show breaking of seed dormancy. Nitrogen containing compounds such as nitrate, nitrite, ammonium, cyanide and azide are elaborated to be effective in germination of dormant seeds of weed species (Li *et al*., 2005; Balouchi & Sanavy, 2006; Bethke *et al*., 2006; Bethke *et al*., 2006; Oracz *et al*., 2008). However, favorable role of light in breaking dormancy of seed has been reported for few species only (Kettenring *et al*., 2006). For instance, germination rates of lettuce (*Lacatuca sativa*), tobacco (*Nicotiana attenuata*) and tomato (*Lycopersicon esculentum*) enhance with light treatment (Borthwick *et al*., 1954; Wills, 1959; Mancinelli *et al*., 1966; Hayes & Klein, 1974). It has been reported that in some species an exposure to red light in combination promote germination compared to application of synthetic chemicals alone (Bungard *et al*., 1997).

Several studies reported that *Amaranthus retroflexus* L., *Echinochloa crus-galli* (L.) Beauv. var. crusgalli and *Digitaria adscendens* (*ciliaris)* (Retz.) Koel., are the three noxious and widely occurring weeds in agriculture fields (Gadamski *et al*., 2000*;* Kobayashi *et al*., 2004; Khan *et al.*, 2009; Khan *et al.*, 2010). Complete eradication of these weeds is impossible as the seeds of these weed species become dormant after maturity. There is little research done to elaborate the causal factors and mechanism influencing seed dormancy of these species. Different factors have been reported to improve seed germination by breaking seed dormancy. In barnyardgrass, germination can be stimulated by applying solutions of several anaesthetic substances (Taylorson, 1988), CO_2 (Yoshioka *et al*., 2002) and high temperatures (Taylorson & Dinola, 1989). Germination rates of *Amaranthus* species were enhanced with red light exposure (Gallagher & Cardina, 1998), ethylene (Egley, 1980) and both GA_{4+7} and ethylene (Kepczynki, 2006). However, there is no previous report elaborating the role of chemicals and red light on promoting seed germination of *Digitaria* species. Efforts have been underway to control notorious weeds. Information regarding the species specific light dependency for seed germination can lead the way to understand the plant behavior especially in noxious weeds (Kettering *et al*., 2006**)**. However, characteristics of germination vary among various species. Understanding and elaborating the role of light and chemicals can play a vital role in weed management at agricultural fields. In current study, we examined the influence of nitrogenous compounds and red light on the seed germination of some elite weed species.

Materials and Methods

General procedure: Mature and desiccated seeds of *A. retroflexus*, *E. crus-galli* var. *crusgalli* and *D. adscendens* were collected from fallow fields in the vicinity of Kyungpook National University, South Korea. Weed seeds were dried under green house conditions for two weeks at 24ºC. Dried seeds were gathered in polythene bags and stored in cold room at 4ºC.

Germination conditions: Fifty seeds of each weed species were placed in 9 cm Petri plates with two layers of Whatman No. 1 filter paper and moistened with 4 ml of distilled water. The Petri plates were wrapped in aluminum foil, covered with a dark cloth and transferred to incubator set at 25ºC. For red light treatment, seeds were irradiated with red light (80 μ mol s^{-2} m^{-1}) for 10 min., after 12 hr of imbibition. Light emitting diode (LED) lamp with irradiation of 660 nm, was used as light source for seed germination throughout the experiment. Treated seeds were transferred back to incubator. Seeds germination was observed after one week of red light treatment and emergence of radical (2 mm) was the criterion for germination. Each treatment comprised 3 replicates and was repeated twice.

Application of nitrogenous compounds: N-containing compounds i.e., $NaNO_2$, NH_4NO_3, KNO_3 and NH_4Cl were applied in absence or presence of red light pulse. Concentration gradients of 1, 5, 10, 25 and 50 mM solutions were used for seed germination induction.

Statistical analysis: The means and standard errors 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. 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 otherwise.

Results

The results revealed that germination percentage of *A. retroflexus* was significantly improved by applying various concentrations of KNO_3, NH_4Cl, NH_4NO_3 and $NaNO_2$ in the presence of red light. On the other hand, germination rate of *A. retroflexus* was lower under dark condition, as in the absence of red light pulse, mean germination rate increased from 3.3% to 18.6%, 28.6%, 30.0%, and 42.67% (Fig. 1). Seeds treated with any of KNO_3, NH_4Cl, NH_4NO_3, $NaNO_2$ and red light provided germination of 31.33%, 44%, 46% and 46% respectively (Fig. 1). NH_4NO_3 and $NaNO_2$ provided maximum germination percentages as compared to NH_4Cl and KNO_3. The combination of ammonium and nitrate showed additive effect to seed germination and combination of red light and nitrates showed high germination percentage. Best results were obtained when N-compounds were applied in the concentrations of 10 mM (KNO_3, NH_4Cl, NH_4NO_3) and 25 mM ($NaNO_2$) along with red light (Fig. 1).

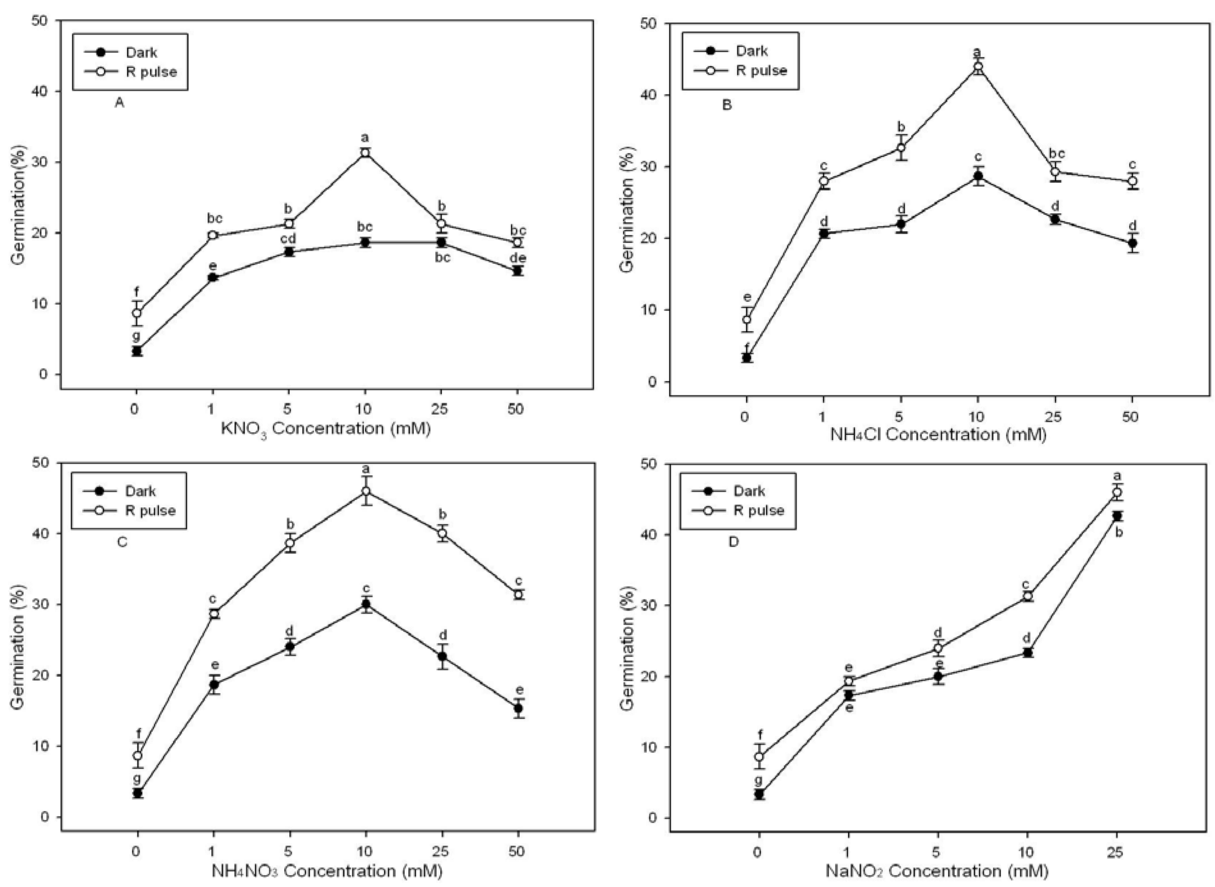

Fig. 1. Germination (%) of *Amaranthus retroflexus* seeds in response to N-compounds and red light. D represents dark condition, while R pulse indicates red light irradiation for 10 min.

Current results showed that all the over germination in *E. crus-galli* var. *crusgalli* was higher than *A. retroflexus* and *D. adscendens*. Combined application of red light and N-compounds significantly promoted seed germinations. In this weed, the best concentrations for N-compounds were 50 mM (KNO_3), 5 mM (NH_4Cl), 50 mM (NH_4NO_3, NH_4NO_3) and 25 mM ($NaNO_2$). Similar to *A. retroflexus*, NH_4NO_3 and $NaNO_2$ provided maximum germination percentages (Fig. 2). Nitrite induced seed germination of 64.67%, which was lower than nitrate (76.67%) (Fig. 2).

In *D. adscendens*, seed germination was markedly influenced by red light, while the effect of N-compounds was secondary (Fig. 3). Best results were recorded in treatments that received KNO_3 (25 mM), NH_4Cl (5 mM), NH_4NO_3 (25 mM) and $NaNO_2$ (10 mM) along with red light pulse of 10 min. Over all NH_4NO_3 provided maximum germination percentage. However, in comparison with other species the germination percentage was much lower in *D. adscendens* (Fig. 3).

Discussions

Weeds compete with crop plants, reducing yield and crop quality, and take the space of native bush-lands. Some can also taint milk and others could be poisonous to humans and animals. Still others have attributes like thorns and spines, which cause physical injury. They may act as host plants for parasitic insects or diseases, while yet others can be parasitic on other plants. Through either direct and indirect effects, weeds often increase the cost of farming and decrease the productivity of land. Our dislike for weeds is reflected in the global figures from agrochemical sales. In 2005 alone, approximately 33600 million US dollars were spent on agrochemicals, globally, of which 45.8% was spent on herbicides (Anon., 2006).

The results showed that combined application of nitrogenous compounds and red light exhibit strong role in germination induction than their singular treatment. Ammonium and nitrates affected germination differently as nitrates induced higher germination than ammonium. It is due to the fact that nitrates being a major source of nitrogen for many plant species are assimilated *via* reduction by nitrate reductase (NR) and other enzymes, which ultimately lead to the production of amino acids and nitrogen compounds. In addition to its role as nutrient, nitrates are shown to act as a signal molecule in plant development and metabolism besides assimilation controls (Scheible *et al*., 1997; Wang *et al*., 2003). However, the mechanism of nitrate in germination induction is still not fully understood. In *S. officinale*, stimulation of germination depends on the simultaneous presence of nitrate. The effect of both factors is completely blocked by tetcyclacis, an inhibitor of gibberellin (GA)-biosynthesis. However, Hilhorst & Karssen (1989) concluded that nitrate reduction does not play a role in the induction of germination**.** This conclusion was supported by lack of inhibition of seed germination by sodium chlorate and sodium tungstate in spite of an inhibition of nitrate reduction. Ammonium was proved to inhibit or promote seed germination dependent on species type (Singh & Amritphale, 1992) . Current results demonstrated that the response of seed germination to nitrogen containing compounds were quite different among different weed species. Our study also confirmed the effectiveness of red light in the breaking of dormancy and induction of germination. It proved that treatment of weed seeds with red light for a brief time significantly enhance germination percentages of weed seeds. Current study confirmed previous report of Tang et al. (2008), which observe that red light and N-compounds significantly enhanced germination of *Chenopodium album*.

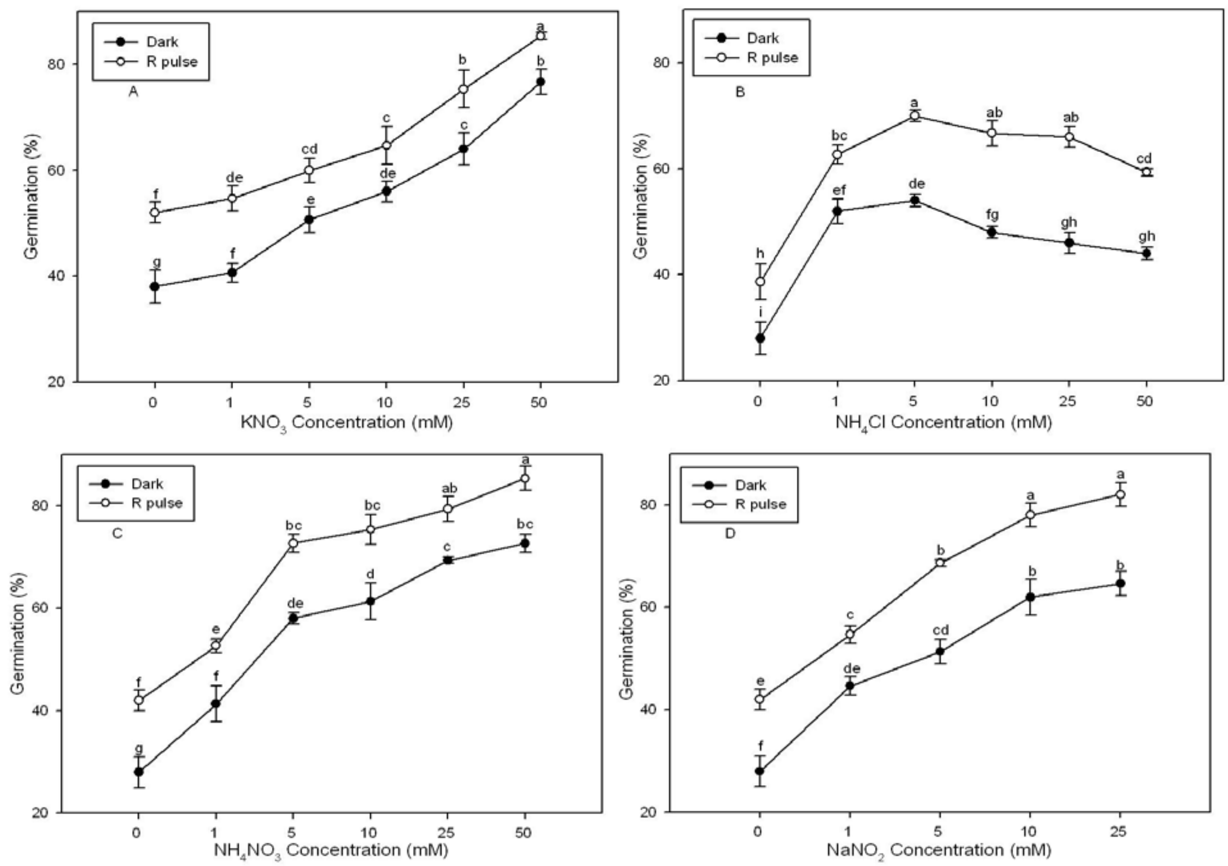

Fig. 2. Germination (%) of *Echinochloa crusgalli* seeds in response to N-compounds and red light. D represents dark condition, while R pulse indicates red light irradiation for 10 min.

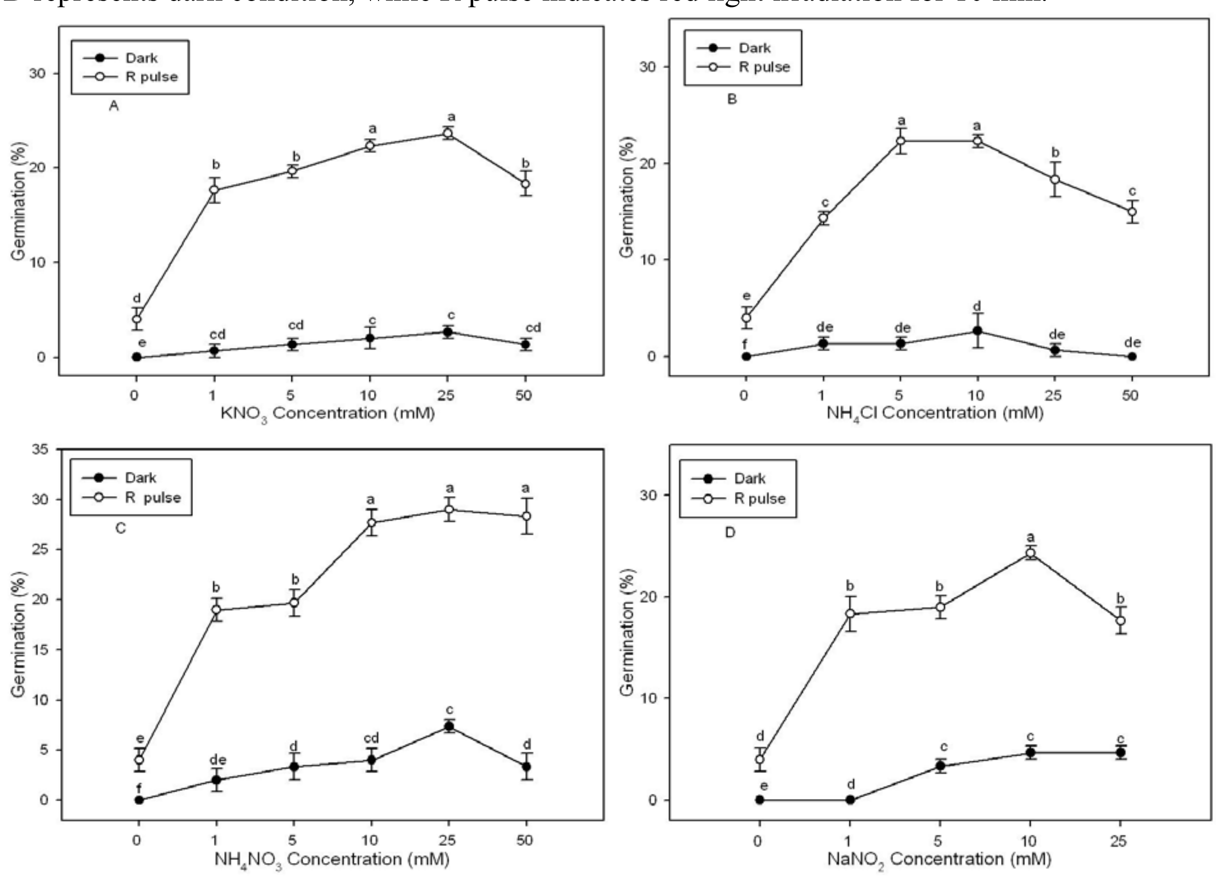

Fig. 3. Germination (%) of *Digitaria adscendens* seeds in response to N-compounds and red light. D represents dark condition, while R pulse indicates red light irradiation for 10 min.

The present study would help in understanding the favorable role of red light and N-compounds in germination induction of weedy seeds, as weeds eradication from crop fields is highly desired in order to increase crop production and reduce the extensive use of herbicides. However, weed eradication is a difficult task as weed seeds possesses differential dormancy levels and thus they can survive in the soil for many years. Thus an efficient eradication of these undesired plants can be achieved by adopting a two pronged strategy i.e. either to induce permanent dormancy in all seeds so that they fail to germinate or to break the existing varying dormancy levels, so that all seeds germinate and thus can be effectively controlled by applying herbicides or through traditional weeding practices. The present study is linked to the second part and narrates about the favorable role of red light and N-compounds in germination induction or breaking of seed dormancy. However, further research is needed to understand and elaborate the germination induction mechanism of weed seeds.

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