African Journal of Biotechnology Vol. 11(2), pp. 273-279, 5 January, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2272
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Exposure to red light, temperature and exogenous gibberellins influenced germination of some winter weeds

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Accepted 7 December, 2011

Red light, temperature and gibberellins are well known for their capacity to induce higher germination in dormant seeds of several plant species. In the current study, we investigated the effect of various temperature (10, 13, 16, 19, 22 and 25°C) and gibberellic acid (GA_3) and GA_{4+7} concentrations (0.1, 1, 10, and 100 uM) on germination of *Poa annua*, *Alopecurus aequalis* and *Stellaria aquatica* seeds incubated under continuous dark or treated with red light pulse of 80 µ mol m^{-2} s^{-1} red light (660 nm) for 10 min after 12 h of dark period. We found that incubation temperature and gibberellins (GAs) significantly enhanced seed germination in red light treated seeds, as maximum germination was observed for *A. aequalis* (67.3% at 13°C), *P. annua* (61.3% at 13°C), and *S. aquatica* (42% at 19°C) in such treatments. Exogenous GA_{4+7} significantly promoted germination of *P. annua* and *A. aequalis* seeds, while GA_3 induced higher germination in *S. aquatica* seeds. It was observed that GAs were more effective in the presence of red light as least germination stimulation was recorded for GAs under dark condition and no germination was found in weed seeds at control and 0.1 mM GA_3 and GA_{4+7} treatments. It can be concluded that lower temperature (13°C) and GA_{4+7} in conjunctions with red light pulse, induced maximum germination in dormant weed seeds.

Key words: Germination, temperature, nitrogen-containing compounds, red light, gibberellins.

INTRODUCTION

Seed dormancy could be considered simply as a block to the completion of germination of an intact viable seed under favorable conditions, although to some researchers, it is one of the least understood phenomena in the field of seed biology (Hilhorst, 1995; Bewley, 1997a). In the last years, significant progress has been made in understanding seed dormancy; however, there have also been some potential sources of confusion that have been reported in the literature (Finch-Savage and Leubner-Metzger, 2006). In ecological studies, there has been confusion reported between seed dormancy and persistence in soil (Thompson et al., 2003; Fenner and Thompson, 2005; Walck et al., 2005). This has resulted in part from different views on dormancy, such as whether light terminates dormancy or induces germination.

Environmental factors are believed to regulate seed dormancy and germination. Temperature substantially affects the dormancy level of weed seeds (Forcella,

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Abbreviation: GA_3, Gibberellic acid.

1998), as diurnal and seasonal temperature fluctuation and mean temperature affect the breaking of dormancy and thus the germination rate. Some weeds require periods in which the temperature either increases or decreases slowly, whereas other species require daily temperature fluctuations or chilling to break dormancy (Baskin and Baskin, 2004). Likewise temperature, light is another environmental factor regulating seed dormancy and germination. Such regulation is mediated by photoreceptors called phytochromes, which initiate or stop physiological processes in the seed depending on the characteristics of the light to which it is exposed. The phytochromes change their form depending on the wavelength of the light to which they are exposed. The phytochrome Pr absorbs red light (R) and is biologically inactive, while Pfr absorbs far-red light (FR) and is considered the active form that promotes germination (Benech-Arnold et al., 2000). The wavelength, flux density, and exposure duration of light determine the extent of germination induction and breaking dormancy (Ballare and Casal, 2000).

Plant-hormones are naturally occurring organic substances, which influence physiological processes at very low concentrations either in distant tissues to which they are transported or in the tissue where synthesis occurred (Davies, 1995). Due to their structural simplicity, plant hormones are not specific enough to match the variety of controlled reactions (Canny, 1985). Contrary to this, it has been suggested that hormones only provide "turn on" or "turn off' signals and that the actual information's are provided by the cell. Gibberellins are considered important in the regulation of seed dormancy. Gibberellins are tetracyclic diterpenes, which are reported as necessary for seed germination in many plant species (Sawada et al., 2008; Calvo et al., 2004). Gibberellic acid (GA_3) is also reported to reprogram plants to higher growth and significantly ameliorate the adverse effects of salt stress and rescue the productivity and quality of soybean (Hamayun et al., 2010).

The first objective of the current study was to investigate the effect of red light and temperature on seed germination rate and dormancy release of *Poa annua*, *Alopecurus aequalis* and *Stellaria aquatic*. These are common weeds of agriculture fields during winter season. The second objective was to observe the stimulating effect of bioactive GAs on seed germination, and compare the effectiveness of GA_3 and GA_{4+7} in the regulation of seed germination of these three weeds.

MATERIALS AND METHODS

General procedure

Seeds of *P. annua*, *A. aequalis* variety *amurensis*, and *S. aquatica* were collected from agriculture fields in Suwen, South Korea. Seeds were then properly dried for two weeks at green house and kept in air-tight polyethylene containers at cold room at 4℃. The seeds were surface sterilized by immersion in 0.5% (w/v) NaClO for 5 min and washed with sterile distilled water. 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 25℃. Seeds germination was observed after one week of red light treatment and emergence of radical (2 mm) was the criterion for germination (Andersson et al., 2002). Each treatment comprised 3 replicates and was repeated twice.

Application of red light, temperature and gibberellins

For red light treatment, seeds were irradiated with red light for 10 min after 12 h of imbibition. Light emitting diode (LED) lamp (Mil-R18: Sanyo Biomedical) with irradiation of 660 nm, with irradiation of 660 nm (80 μ $mol.m^{-2}$ s^{-1}) was used as light source for seed germination throughout the experiment (Tang et al., 2008). Treated seeds were transferred back to incubator. The temperature treatment comprised temperatures ranging from 10 to 30℃ at 3℃ intervals. For investigating GAs effect, seeds were incubated in the dark at 25℃ and treated with 0.1, 1, 10 and 100 uM of GA_3 and GA_{4+7}. GA_3 and GA_{4+7} are commercially available bioactive gibberellins. GAs (GA_3 and GA_{4+7}) were dissolved in 1 N NaOH and then solutions were made with distilled H_2O.

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 AND DISCUSSION

Influence of varying temperatures on germination of winter weeds

Viable seeds are dormant when their germination is prevented by a block within the seed, so that they cannot germinate in an otherwise favorable environment (Bewley and Black, 1982). Temperature has a double effect on seed germination. Both the final germination percentage and the rate at which this percentage is reached are affected by temperature. Generally, the maximum final germination percentage is reached in a broad temperature range, whereas the germination rate has a sharp optimum temperature (Garcia-Huidobro et al., 1982).

We observed that the seeds of *P. annua* and *A. aequalis* variety *amurensi* germinated at 10℃ and the germination rate increased with temperature and maximum seed were germinated at 13℃ in both red light and control treatments. However, with a further increase in temperature, the germination gradually declined and least germination was observed in seeds kept at 25℃. Current results showed that *P. annua* seeds failed to germinate below 10℃ and above 25℃ (Figures 1 and 2). Addition

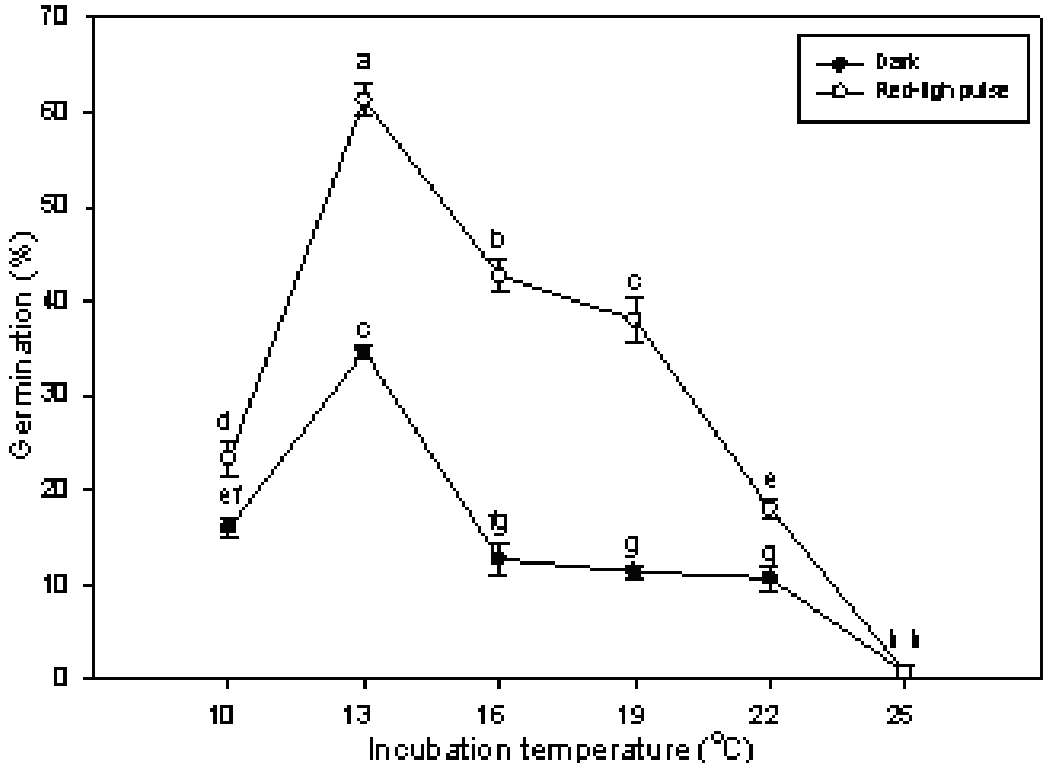

Figure 1. Germination of *P. annua* seeds at different temperatures. Seeds were treated with red light for 10 min after 12 h of dark, while the control seeds were kept under dark throughout.

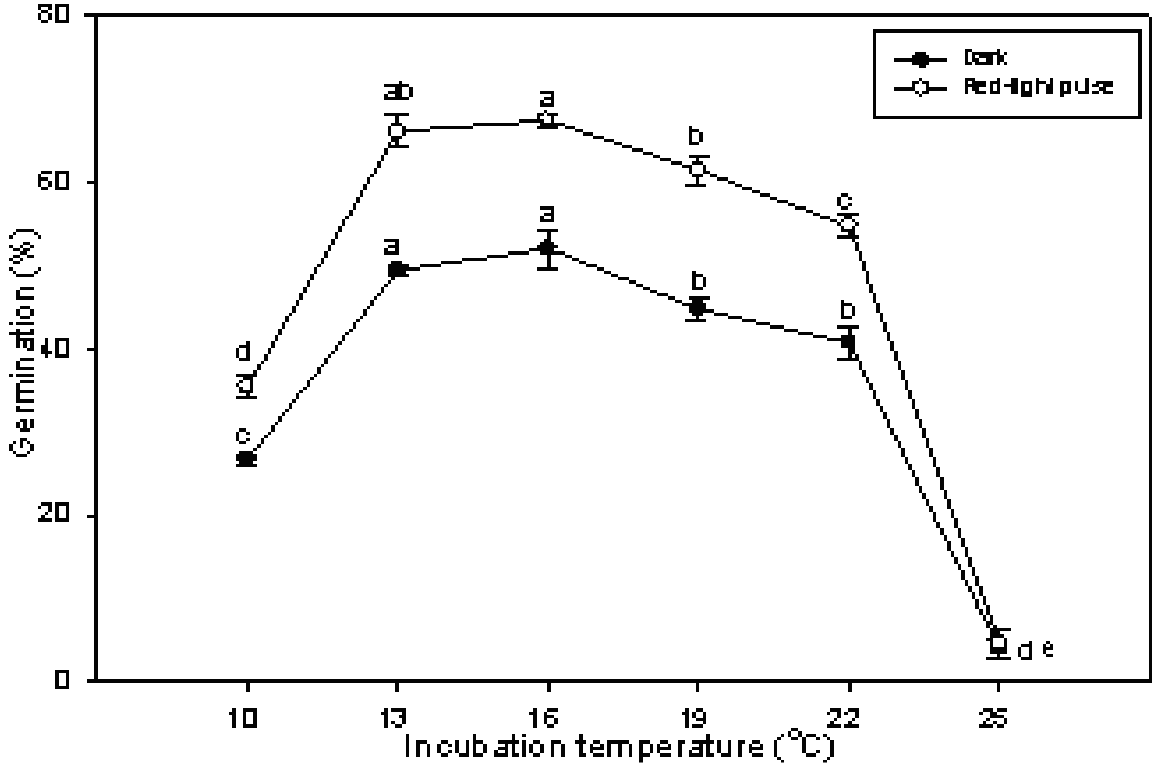

Figure 2. Germination of *A. aequalis* var. *amurensi* seeds at different temperatures. Seeds were treated with red light for 10 min after 12 h of dark, while the control seeds were kept under dark throughout.

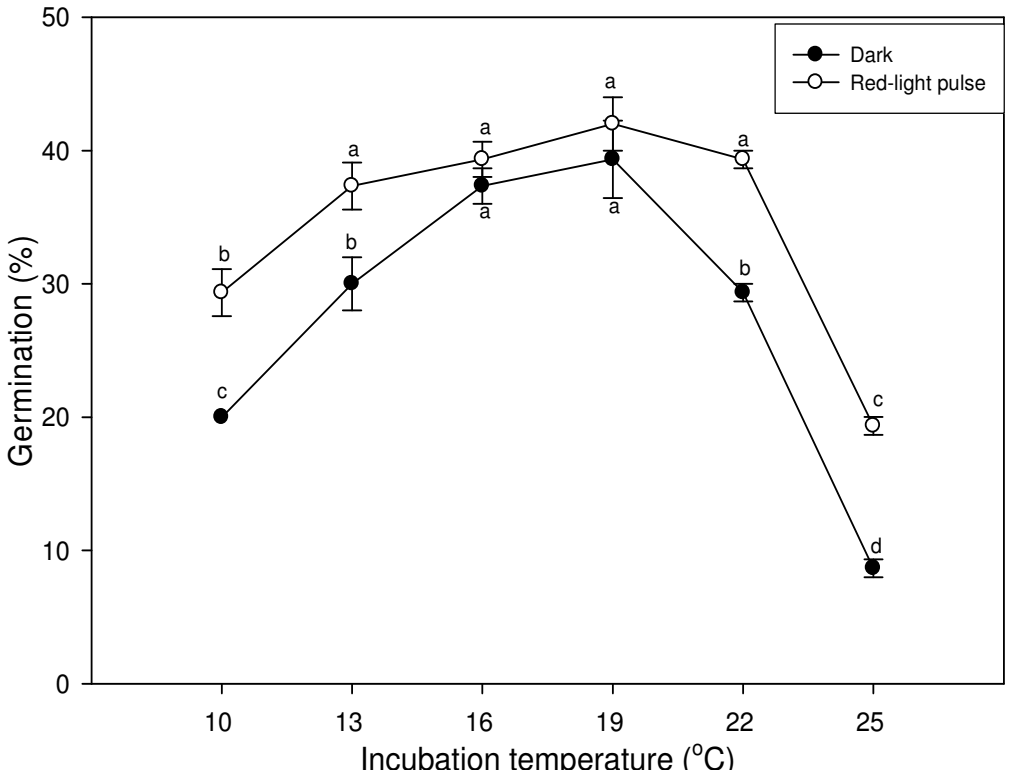

Figure 3. Germination of *S. aquatica* seeds at different incubation temperatures. Seeds were treated with red light for 10 min after 12 h of dark, while the control seeds were kept under dark throughout.

of red light significantly stimulated seed germination at temperatures ranging from 10 to 22°C as compared to dark treatments, but it failed to induce germination below 10°C and above 25°C. The overall germination capacity of *A. aequalis* variety *amurensi* was found higher than *P. annua* under similar environmental condition. It was previously reported that at temperatures below the optimum, germination rate increases linearly with temperature (Hegarty, 1973; Garcia-Huidobro et al., 1982); however, the current study showed that in weeds, at temperatures above the optimum, germination rate decreases linearly an increase in temperature.

Seeds of *S. aquatica* germinated at low temperature (10°C), and further increase in temperature regime stimulated germination till maximum germination was attained at 16°C. However, a gradual further increase in temperature caused marked reduction in germination of *S. aquatica* seeds (Figure 3). Similar to the other two weed studied, red light provided an additive stimulation for seed germination, although at extreme lower and higher temperatures, the red light failed to induce any germination. Current results proved that treatment of weed seeds with red light for a brief period significantly enhance germination percentages of treated seeds. It is because the red light promotes phytochrome biosynthesis which induces seed germination. Our current findings confirm previous report on favorable role of red light in seed germination (Tang et al., 2008).

Influence of GA_3 and GA_{4+7} on germination of winter weeds

Physiologically active GAs is known for inducing seed germination in a wide range of plant species, as they induce hydrolytic enzymes. The hydrolytic enzymes weaken the barrier tissues such as the endosperm or seed coat, inducing mobilization of seed storage reserves, and stimulating expansion of embryo (Bewley and Black, 1994). In the current study, exogenous GA_3 and GA_{4+7} applications induced higher germination in *P. annua*, *A. aequalis* var. *amurensi*, and *S. aquatica*, while higher temperature favored seed dormancy (Figure 4). Moreover, red light exposure showed an additive effect to seed germination of weed species in-conjunction with these GAs. However, the effect of GA_3 and GA_{4+7} varied for different weed species as GA_3 seems to show lesser effect on *P. annua* seeds as compared to GA_{4+7} treatments (Figure 4). The stimulatory effect of GAs was enhanced when there was an increase in GAs concentration from 0.1 to 100 µM (Figures 4, 5 and 6). In case of *A. aequalis* var. *amurensi*, either of GA_3 and GA_{4+7} significantly prompted seed germination (Figure 5), which was similar to that of *P. annua*. On the contrary, lower

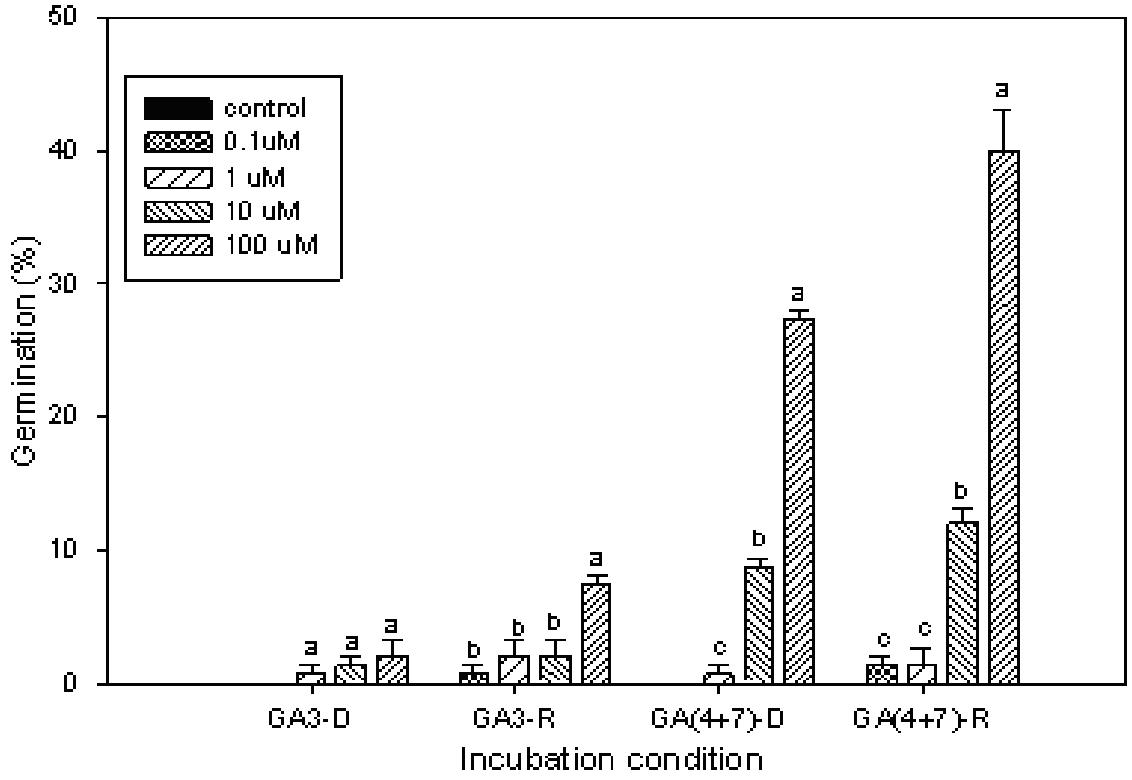

Figure 4. Germination (%) of *P. annua* seeds treated with elevated GA_3 and GA_{4+7}. Seeds were treated with red light for 10 min after 12 h of dark, while the control seeds were kept under dark throughout. D stands for dark, while R stands for red light.

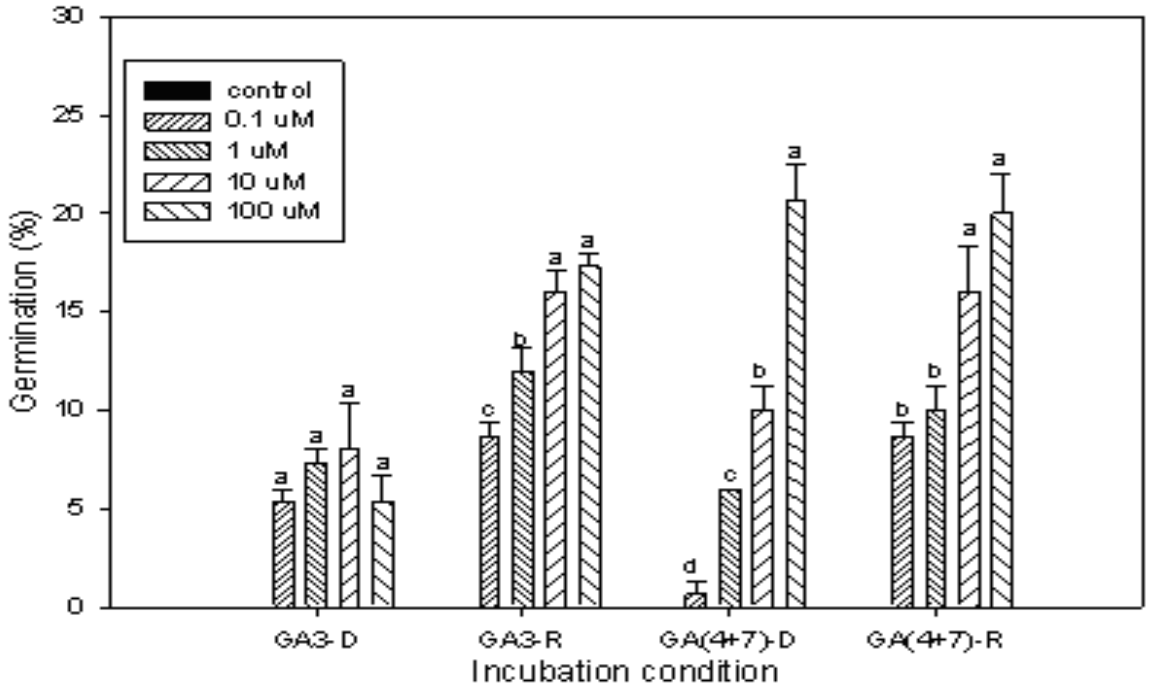

Figure 5. Germination (%) of *A. aequalis* var. *amurensis* treated with elevated GA_3 and GA_{4+7}. Seeds were treated with red light for 10 min after 12 h of dark, while the control seeds were kept under dark throughout. D stands for dark, while R stands for red light.

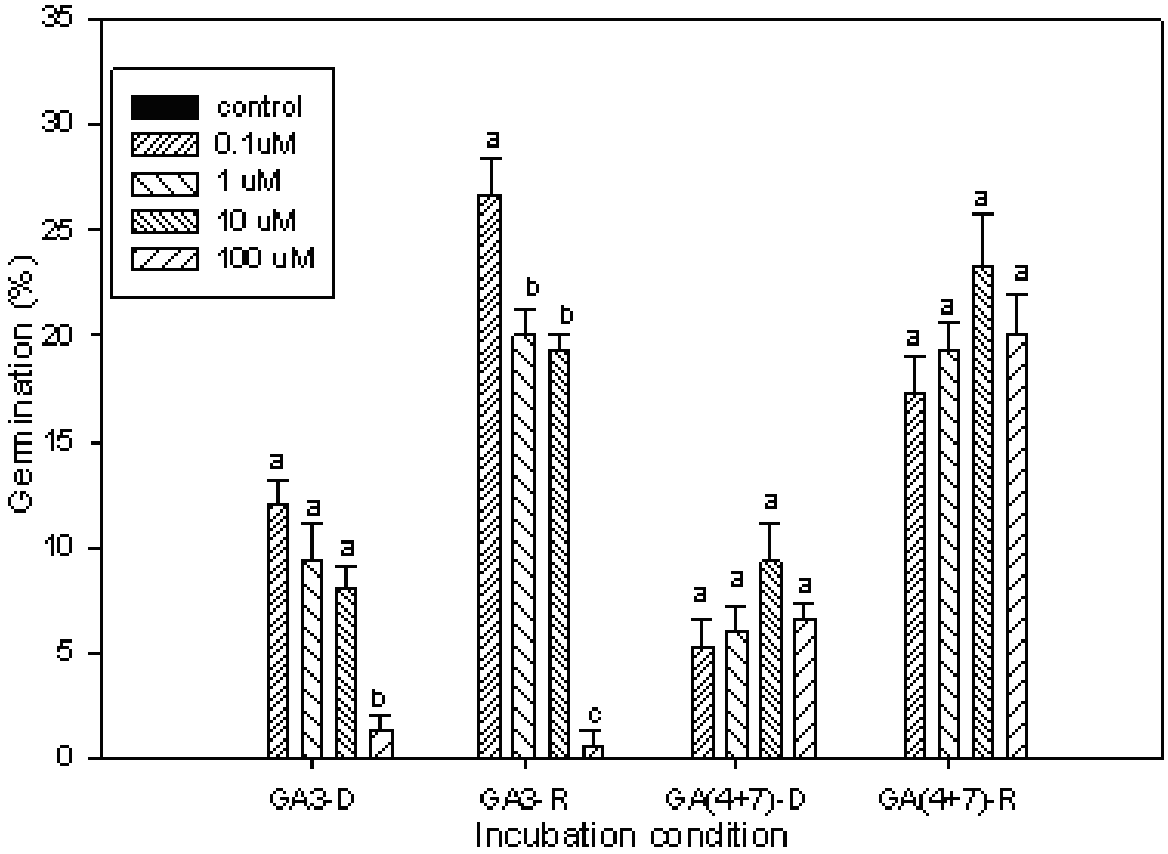

Figure 6. Germination (%) of *S. aquatica* treated with elevated GA_3 and GA_{4+7}. Seeds were treated with red light for 10 min after 12 h of dark, while the control seeds were kept under dark throughout. D stands for dark, while R stands for red light.

concentrations of GA_3 and GA_{4+7} induced seed germination of *S. aquatica,* as maximum germination was achieved at 0.1 µM GA_3 and 10 µM GA_{4+7} (Figure 6).

Conclusion

Weed eradication is a difficult task as weed seeds possess differential dormancy levels and can survive under the soil for many years. An efficient eradication of these undesired plants can be achieved by adopting a two pronged strategy, that is, either to induce permanent dormancy in these seeds or to break the existing varying degrees of seed dormancy, so that all seeds germinate at the same season and can be effectively eradicated by applying chemicals or through traditional weeding practices. Our study is linked to the latter part and narrates about the favorable role of red light and N-compounds in germination induction or breaking of seed dormancy. The current study explains how interactions between environmental factors have a great impact on the germination of weeds. The variable responses of weeds to environmental factors and their interaction could account for differences in seed germination and seedling emergence timing, which represent differences in adaptation and survival strategies. This study would help in understanding the favorable role of red light, temperature and exogenous GA_3 and GA_{4+7} in germination induction of weed seeds, as weed eradication from crop fields is highly desirable, which will increase crop production and reduce the use of herbicides. However, further research is needed on the topic in order to understand and elaborate the germination induction mechanism of weed seeds.

ACKNOWLEDGEMENTS

This research work was supported by the Agenda Program (Project No.PJ007522), Rural Development Administration, Republic of Korea.

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