International Journal of Farming and Allied Sciences
Available online at www.ijfas.com
©2014 IJFAS Journal-2014-3-7/722-728/ 31 July, 2014
ISSN 2322-4134 ©2014 IJFAS

The optimum condition under light and Media for Seed germination of *Withania coagulans*

Leila Edalatifard[1], Seyed Ali Mohammad Modares Sanavy[2*] and Hossain Askari[3]

1,2. Department of Agronomy, Tarbiat Modares University, Tehran, Iran,
3.Department of Biotechnology, Faculty of New Technologies Engineering, Shahid Beheshti University, Tehran, Iran

Corresponding author: Seyed Ali Mohammad Modares Sanavy

ABSTRACT: *Withania coagulans,* a traditional medicine plant in Southern Iran has become relatively scarce due to over collection. Natural propagation of this species is by seed, but to improve poor germination and promote the germination of this medicinal herb, the effects of media and light conditions for seed germination were investigated. Experimental factors were included 12 ecotypes of *W. coagulans* which have been collected from the native area; three germination media included B5 Media, Plain agar and filter paper, and two light conditions (Sunlight lamps and complete darkness). Factorial experiment (with randomized complete block design) in three replicates applied for statistical analysis and factors such as germination percentage as well as Daily seed germination rate were assayed. During the experiments, some ecotypes germinated earlier than others and seed germination was dependent on media and light condition. Although seed germination was varied, maximum germination was obtained in filter paper and under complete darkness. On the other hand light treatment completely inhibited seed germination of *W. coagulans*.

***Keywords*:** seed germination, daily germination rate,day to germination, Withania coagulans

INTRODUCTION

Plant establishment depends on successful dispersal in a suitable habitat and the ability to survive in interaction with the surrounding environment (Goode, 2009). Sowing seeds that will not grow or be low in viability is a loss of money and time. To save both, we need the laboratory germination test which is designed to indicate as closely as possible the proportion of seeds that can be expected to germinate and develop into strong plants. Seed germination is frequently sensitive to environmental conditions such as adequate moisture, favorable temperatures, gas exchange and, for some species that can lead to extremely high seed germination (Abe, 2008). The technique of seed germination by in vitro culture was first introduced by Knudson (1922). In vitro seed germination protocols have been established for many species, and a number of media and salts have been used for germination and propagation (Arditti and Ernst 1993 and Benson, 2000). Seeds of some species are sensitive to light to induce germination. Some seeds germinate similarly in light and darkness (Baskin & Baskin, 1988), while others do it more readily either under light or dark conditions (Colbach, 2002), also Khanna and (2013) conveyed that photoperiod has a significant role in *Withania somnifera* seed germination*. Withania coagulans* is a traditional medicinal plant in Southern Iran and important in Ayurvedic and indigenous medicine for over 3000 years (Mirjalili, 2009). *W. coagulans* is an erect, perennial shrub with densely velvety stems and leaves. The pharmacological properties of the plant are diverse, including anti-stress, anti-inflammatory, anti-arthritic, anti-oxidant, and anti-tumor agent (Gupta, 2007). The major constituents of these biological activities are the withanolides, a group of naturally steroidal lactones in the leaves (Kambizi , 2006). Unfortunately, the populations of this plant species have decreased and become scattered in the wild, which can be attributed to low rates of natural regeneration from

seeds (Vakeswaran and Krishnasamy, 2003, Kumar, 2001). Karnick (1978) and Vakeswaran and Krishnasamy (2003) reported that the seed germination of the *Withania* is poor and mortality rate of seedlings is high under field conditions. According to Kothari, (2003), effective methods must be determined to obtain a uniform, rapid seed germination of *W. coagulans* to develop and conserve this plant as a cultivated crop. The plant has a high potential for commercial cultivation (Kothari, 2003), before this goal, achieving the best conditions for seed germination is important. Herein, the purpose of this research was to clarify the optimal in vitro conditions for seed germination of 12 *W.coagulans* ecotypes based on their germination responses to light and germination media.

MATERIALS AND METHODS

Dried fruits were collected from wild populations growing in the Sistan Baluchistan province, Southern Iran, during June 2012 (Fig 1). The seeds were separated manually from dried fruits and were washed by tap water. Cleaned seeds were dried at room temperature for one day, then numbered from E-1 to E-12 and were placed in a Lab refrigerator at 4 °C. Germination experiment was done in the Biotechnology Laboratory of the Shahid Beheshti University of Iran. Seeds were sterilized by soaking in 70% alcohol for 30 seconds, followed by 5% sodium hypochlorite solution for 20 minutes. Later, the seeds were thoroughly washed with sterile water four times to remove any hypochlorite residue. After air drying for one minute, 15 seeds (Because of limit seed) of each Ecotypes were randomly placed in separate 9 cm Petri plates containing either two layers of Whatman No. 1 filter paper saturated with distilled water, B5 media (With the macro- and micronutrients, and vitamins) contain 0.7% agar and Plain agar (no nutrients) 0.7%. After seeding, all treatments were placed in a controlled environmental chamber (Noorsanat, Model STC 1300 Ltd. Iran) however, a set of petri plates was wrapped with aluminum foil to study seed germination in the complete darkness and part of petri plates were placed in light controlled as daylight (sunlight lamps were used to produce a photosynthetic photon flux density of 900 foot-candles at 16/8 h light/dark). The optimum temperature for germination and Humidity (25°C and 65%) were maintained during the experiment. Germination was monitored daily for each ecotype for analyzing of the daily germination rate dynamics of individual ecotype. Seeds were considered germinated when the emerging radicle reached 2mm in length. Germination percentage under each treatment and rate of germination was calculated following the procedure of Czabator (1962). The germinated seeds (radical emergence) were removed after the daily count. Final germination percentages were calculated after incubation for 25 days where no further germination was observed. All germination percentage data were arcsine transformed before statistical analysis. One-way analysis of variance was performed using SAS version 9.2 (SAS GLM, $P < 0.05$; SAS Institute, 2009) Treatments were arranged in a factorial experiment (with randomized complete block design) in three replicates. Duncan's test was used to evaluate differences among the means. Significance was reported at $P < 0.05$.

Figure 1. Location of Sistan Baluchistan province, Southern of Iran

RESULTS AND DISCUSSION

Results

Day to germination experiment

According to experimental results are presented in Table 1, although media, light regimes, ecotypes and interaction between them were affected the germination initiation time ($p = 0.001$), days to Germination was significantly longer when seeds were exposed to light condition. At dark condition seeds germinated seven days, whereas seeds germinated within ten days in a light condition (Fig. 2). On the other hand, ANOVA revealed that there was a significant difference observed in the days taken for initiation of germination among all the ecotypes (p

= 0.001). The result showed that all ecotypes in dark condition germinated earlier than light condition. At dark condition maximum seed germination occurred between 6 and 10 days, whereas required time for initiation germination at light condition was between 8 to 13 days (Fig. 3a). Interaction between different media and light regimes on days to germination showed that the number of seeds germinated in filter paper under complete darkness was higher than the other treatments (Fig.3b).

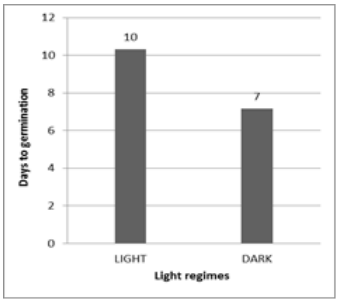

Figure 2. The influence of Light/Dark condition on Days of germination after sowing in ecotypes

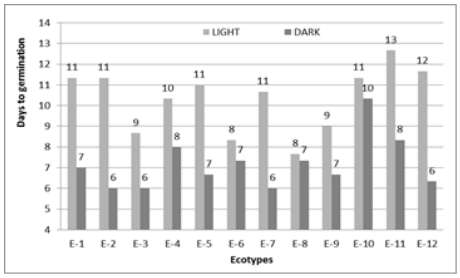

Figure 3a. Mean Days of germination after sowing in ecotypes

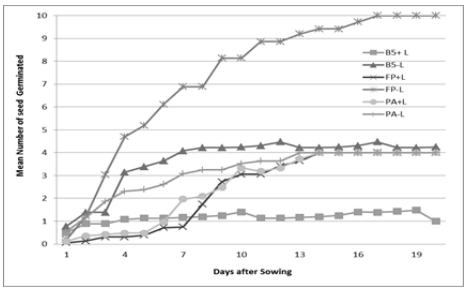

Figure 3b. Mean Days of germination after sowing in different media and light regimes

Table 1. Analysis of variance of some characteristic in *W. coagulans*

Source of variation	DF	Day to germination	Seed germination	Daily germination rate
Block	2	26.041**	0.259**	389.157**
Media	2	104.013**	1.343**	299.379**
Light regimes	1	532.041**	27.050**	104.230
Ecotypes	11	21.718**	0.604**	42.328
Media * light regimes	2	38.097**	0.896**	419.529**
Media * ecotypes	22	15.827**	0.146**	29.124
Light regimes * ecotypes	11	14.294**	0.258**	36.395
Media * light regimes * ecotypes	22	27.395**	27.881	27.881
Error	142	0.045	7371.463	51.911
Total	215			

Seed germination

Light regimes had a significant effect on the germination percentage. On the other hand, the highest germination percentage was recorded on dark condition in ecotype seven (E-9) with 98% and the lowest germination belonged to ecotype ten (E-10) with 67% (Figs. 4). Also on light condition, the highest germination percentage was belonged to ecotype seven (E-7) 63% and the lowest germination observed in ecotype ten (E-10) with 5%.

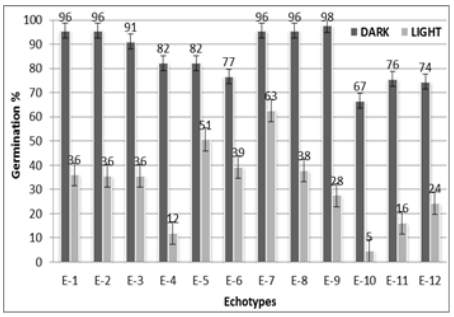

Figure 4. The influence of Light/Dark condition on germination percentage of ecotypes

Effect of media on seed germination of *W. coagulans* under light/dark conditions was presented in Table 1 (p = 0.001). Result showed B5 media and Plain agar had no effects on germination regardless light conditions (Fig. 5), whereas that B5 media contains macro and micro-nutrients, and vitamins compared to plain agar media. The mean germination percentage in B5 media and Plain agar was 56% (data did not show). According to results filter paper had the highest seed germination than B5 media and Plain agar. The mean germination percentage in filter paper condition was 70% in as same light condition as B5 media and Plain agar.

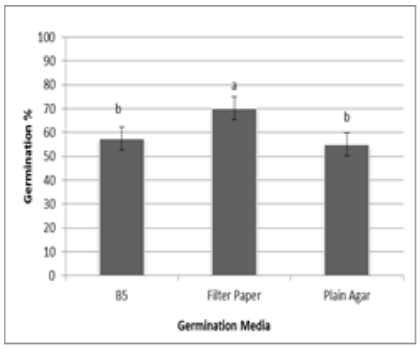

Figure 5. The influence of media culture on germination percentage

Interaction between germination media and light regimes showed that mean germination percentage of *W. coagulans* seeds in the light condition were lower than those of seeds under different media (Fig. 6). Although filter paper had the highest germination percentage (94%) at dark condition, less than 50% of the seeds were able to germinate under light conditions at both B5 media and Plain agar, on the other hand, at light condition germinated seeds in filter paper (46%) were more than B5 media (25%) and Plain agar (37%).

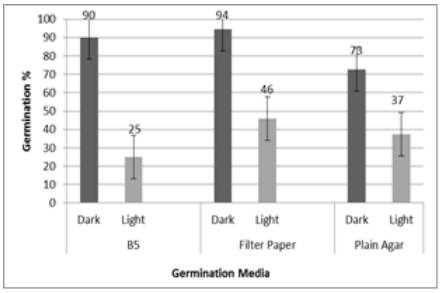

Figure 6. The influence of media culture and Light/Dark condition on germination percentage

Daily germination Rate

The media had a significant effect on the mean germination rate (Table 2). The daily germination rate in B5 media and plain agar was 1.25 and 1.30 respectively, while filter paper had the highest daily germination rate (1.40), (Fig 7). The light had no significant effects on the daily germination rate, but interaction of light and media was significant (Table 2). Although filter paper within light condition had the least germination rate (0.5), at the dark condition had the highest germination rate (2.30) compare to other media (Fig. 8).

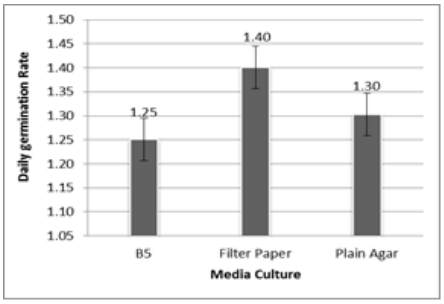

Figure 7. The influence of culture media on daily germination rate of ecotypes

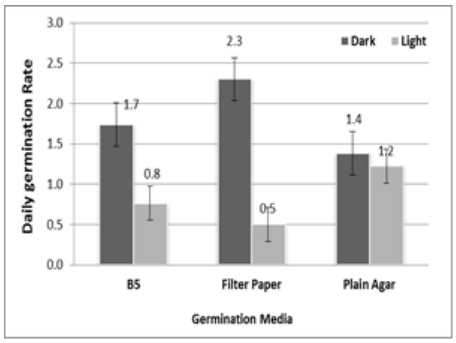

Figure 8. The influence of media culture and Light/Dark condition on daily germination rate of ecotypes

DISCUSSION

Among different ecotype seeds at completely dark conditions, ecotype seven (E-9) and ecotype ten (E-10) had the maximum and minimum of germination percentage and radicle was not observed until after at least a week and took 14 days to reach 50% germination. Therefore ecotype nine could be used for restoration purposes when rapid seed germination and establishment are needed. Seed germination of ecotypes was determined in vitro under light regimes, but, in nature seeds have placed on the soil and receiving light by seed is possible. In this situation selected ecotype should be has the most germination in both dark and light condition so ecotype seven is the best one. Critical examination of the data showed that variation in germination in *W. coagulans* is mainly due to type differences. Barring a few types overall seed germination in *W. coagulans* is low. It is indicated that the seeds

of some species are sensitive to light for inducing germination, germination percentage of *W. coagulans* seeds decreased under light conditions. Incubation of seeds in complete darkness enhanced seed germination in *W. coagulans* seeds and ecotypes of different areas at the same media, preferred darkness to germinate. Lai (2010) reported that light almost totally inhibited germination of *Citrullus colocynthis* seeds at 20°C, while they germinated to 100% in darkness. Therefore, light as an important variable for some seeds, is not required for initiating germination in some spices including *W. coagulans*.

Besides darkness, the lack of significant difference between B5 media and Plain agar at the same condition indicated that macro and micro elements were not necessary for germination and seed germination was highly affected by water. Our study showed germination could be affected by culture medium because of water availability. According to the results, seeds had a high percentage of germination in Filter Paper compare to B5 media and Plain agar. Maybe access to water in filter paper is more than two other media; on the other hand, water availability in B5 media and Plain agar were less than in filter paper. Fuller and Ritchie (1967) noted that water is essential for enzyme activation, breakdown, translocation, and use of reserve storage material during the germination. On the other hand, continuous supply of water is needed to start and complete germination and elements were merely deprived of enough water supplies to germinate (Katembe, 1998). Klimaszewska (2000) also reported that raising the concentration of the gelling agent increased medium gel strength but decreased water availability. Therefore, with decreasing in moisture content in B5 Media and Plain Agar, reduction of germination percentage was recorded in all ecotypes. Moreover, *W. coagulans* could serve as reservoirs of valuable genes for future medicinal plant improvement, it is important to conserve the observed ecotype diversity.

ACKNOWLEDGEMENT

The authors are thankful Dr. Craker and Dr. Imanoe. The authors thank Faculty of New Technologies Engineering, Shahid Beheshti University for providing constructs.

REFERENCES

Afolayan AJ, Grierson DS, Kambizi L, Madamombe I and Masika PJ. 2002. In vitro antifungal activity of some South African medicinal plants. South African Journal of Botany 68, 72– 76.

Baskin CC and Baskin JM. 2001 Seeds: ecology, biogeography, and evolution of dormancy and germination. 2001 . Academic Press, San Diego, CA. Pp. 5-20.

Baskin CC & Baskin JM. 1988. Germination ecophysiology of herbaceous plant species in a temperate region. American Journal of Botany 75: 286-305.

Baskin JM and Baskin CC. 1973. Plant population differences in dormancy and germination characteristics of seeds: heredity or environment? American Midland Naturalist 90:493-498.

Benson EE, Danaher JE, Pimbley IM, Anderson CT, Wake JE, Daley S and Adams LK. 2000. In vitro micro propagation of Clemente, M., Haywood, V. Eds. , Conservation Techniques in Botanic Gardens. Koeltz Scientific Books, Koenigstein, pp. 27–32.

Cerabolini B, De Andreis R, Ceriani RM, Pierce S and Raimondi B. 2004 .Seed germination and conservation of endangered species from the Italian Alps: *Physoplexis comosa* and *Primula glaucescens*. Biological Conservation. 117:351–356.

Colbach N, Chauvel B, Dürr C & Richard G. 2002. Effect of environmental conditions on Alopecurus myosuroides germination. I. Effect of temperature and light. Weed Research 42: 210-221.

Czabator FJ. 1962. Germination value: An index combining speed and completeness of pine seed germination. Forest Science 8: 386 – 395.

Dissanayake P, George DL and Gupta ML. 2010. Effect of light, gibberellic acid and abscisic acid on germination of guayule (Parthenium argentatum Gray) seed. IND Crops Prod. 32, 111–117.

Du Y and Huang Z. 2008. Effects of seed mass and emergence time on seedling performance in *Castanopsis chinensis*. Forest Ecol. Manag., 255:2495-2501.

Estrelles E, Gumes J, Riara J, Boscaiu M, Ibars A and Costa M. 2010. Seed Germination Behavior in Siderites from Different Iberian Habitats. Not. Bot. Hort. Agrobot. Cluj 38 1 09-13.

Ford R. 1971. Terminology of forest science, technology, practice and products. Multilingual forestry terminology. series No.1. Society of American Foresters, Washington D.C.

Fuller HJ & Ritchie DD. 1967. General Botany. New York: Barnes & Noble, Inc.

Goode LK and Allen MF. 2009. Seed germination conditions and implications for establishment of an epiphyte, Aechmea bracteata (Bromeliaceae). Plant Ecol 204:179–188.

Harper JL, Lovell PH and Moore KG. 1970. The shapes and sizes of seeds. Annual Review of Ecology and Systematics 1, 327–356.

Kambizi L, Adebola PO and Afolayan AJ. 2006. Effects of temperature, pre-chilling and light on seed germination of Withania somnifera; a high value medicinal plant. South African Journal of Botany 72, 11 – 14.

Kambizi L and Afolayan AJ. 2003. Phytomedicinal studies of four selected plants used for the treatment of STIs in the Eastern Cape. Fort Hare Papers 12, 10– 24.

Karnick CR. Cultivation trails of Withania somnifera used in indigenous system of medicine. 1978. Journal of Research in Indian Medicine, Yoga and Homeopathy 13 (1): 50–56.

Kattimani KN and Reddy NY. 1999 .Effect of pre-sowing seed treatment on germination, seedling emergence, seedling vigour and root yield of Ashwagandha *Withania somnifera* . Seed Science Technology 27, 483– 488.

Khan ML. 2004. Effects of seed mass on seedling success in *Artocarpus heterophyllus* L., a tropical tree species of northeast India. Acta Oecol., 25:103-110.

Klimaszeska K, Bernier –Cardou M and Sutton BCS. 2000. Influence of gelling agents on culture medium gel strength, water availability, tissue water potential, and maturation response in embryo genic cultures of pinus strobus L. In Vitro Cell. Dev. Biol. Plant 36:279-286.

Kothari SK, Singh CP, Kumar YV and Singh K. 2003. Morphology, yield and quality of Ashwagandha *Withania somnifera* L. Dunal roots and cultivation economics as influenced by tillage depth and plant population density. The Journal of Horticultural Science and Biotechnology 78, 422– 425.

Lai L, Zheng Y, Bai H, Yu Y, An P, Li X, Rimmington GM and Shimizu H. 2010. Strong light inhibits germination of Artemisia sphaerocephala and A. ordosica at low temperature and its relevance to revegetation in sandy lands of Inner Mongolia. China. Ecol Res 25: 771–780.

Murashige T and Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15, 473–497.

Vakeswaran V and Krishnasamy V. 2003. Influence of plant growth regulators on germination of Withania somnifera Dunal seeds. Seed Technology 25, 207.