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The influence of light spectra, UV-A, and growth regulators on the *in vitro* seed germination of *Senecio cineraria* DC.

Cristiane Pimentel Victório^{1,*}, Nina Cláudia Barbosa da Silva², Maria Aparecida Esquibel³, Alice Sato⁴

ABSTRACT

This study was carried out to investigate the effects of light spectra, additional UV-A, and different growth regulators on the *in vitro* germination of *Senecio cineraria* DC. Seeds were surface-sterilized and inoculated in MS medium to evaluate the following light spectra: white, white plus UV-A, blue, green, red or darkness. The maximum germinability was obtained using MS0 medium under white light (30%) and MS + 0.3 mg L⁻¹ GA₃ in the absence of light (30.5%). *S. cineraria* seeds were indifferent to light. Blue and green lights inhibited germination. Different concentrations of gibberellic acid (GA₃) (0.1; 0.4; 0.6; 0.8; 1.0 and 2.0 mg L⁻¹) and indole-3-acetic acid IAA (0.1; 0.3 and 1.0 mg L⁻¹) were evaluated under white light and darkness. No concentration of GA₃ enhanced seed germination percentage under white light. However, when the seeds were maintained in darkness, GA₃ improved germination responses in all tested concentrations, except at 1.0 mg L⁻¹. Under white light, these concentrations also increased the germination time and reduced germination rate. Germination rate, under light or darkness, was lower using IAA compared with GA₃.

Key words: Auxin, gibberellin, light spectra, plant tissue cultures, ultraviolet-A.

RESUMO

Germinação *in vitro* de sementes de *Senecio cineraria* DC.: Efeitos de espectros de luz, UV-A e reguladores de crescimento

Este estudo foi conduzido para investigar os efeitos dos espectros de luz, adição de UV-A, e diferentes concentrações reguladores de crescimento sobre a germinação *in vitro* de *Senecio cineraria* DC. As sementes foram desinfestadas e inoculadas em meio MS para avaliação das seguintes qualidades de luz: branca, branca acrescida de UV-A, azul, verde, vermelha ou ausência de luz. A maior germinabilidade foi obtida usando meio MS0 sob luz branca (30%) e MS + 0,3 mg L⁻¹ de AG₃ no escuro (30,5%). Sementes de *S. cineraria* foram indiferentes à luz para germinar. As luzes azul e verde inibiram a germinação. Diferentes concentrações de ácido giberélico (AG₃) (0,1; 0,4; 0,6; 0,8; 1,0 e 2,0 mg L⁻¹) e ácido indolacético (AIA) (0,1; 0,3 e 1,0 mg L⁻¹) foram avaliadas sob luz branca e no escuro. Sob luz branca, nenhuma concentração de AG₃ aumentou a taxa germinativa. As concentrações de AG₃ aprimoraram as respostas de germinação para as sementes mantidas no escuro, exceto na concentração de 1,0 mg L⁻¹. Essas concentrações também aumentaram o tempo médio e reduziram a velocidade de germinação. A taxa de germinação, sob condições de luz e no escuro, foi menor quando se utilizou AIA em comparação com AG₃.

Palavras-chave: Auxina, cultura de tecidos vegetais, espectros de luz, giberelina, ultravioleta-A.

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INTRODUCTION

Senecio cineraria DC. (syn.: *Cineraria maritima* L.) (Asteraceae) is a Mediterranean plant widely distributed in cold areas and cultured for ornamental and medicinal purposes (Comes & Abbott, 2001; Tundis *et al.*, 2005). It is used to treat conjunctivitis, cataracts, corneal opacity, eye ache and inflammatory diseases (Srivastava & Bagchi, 2006). In Brazil, some industries use this species as raw material for the production of phytomedicines, such as *Cineraria* eyedrops.

Plant tissue culture is a valuable biotechnological resource that optimizes the production of standardized plant species in a short time. It also guarantees mass production of seedlings and the quality of raw plant material for phytotherapeutic production and ornamental markets. Large-scale production using micropropagation to obtain raw plant material to supply herbalists has recently been studied (Victório *et al.*, 2008; 2010). With this same aim, the present study has established tissue cultures of *S. cineraria* to test the *in vitro* germination of this species under the effects of different light spectra, UV-A radiation, and hormonal growth factors.

The quality, intensity, direction and duration of light all affect different phenomena in plant development, including seed germination (Victório *et al.*, 2007; Tlig *et al.*, 2008; Victório & Lage, 2009a; 2009b; 2009c). Phytochrome and cryptochrome families are the main group of photoreceptor proteins responsible for light absorption (Kerbaui, 2008). Classical studies show the effects of red and blue light on germination (Sullivan & Deng, 2003; Kerbaui, 2008). However, little is known about the effects of UV-A radiation on plant development. Despite the presence of the ozone layer, plants are exposed daily to UV-A which affects their physiological processes. Previous studies showed that UV-A is related to the repair of DNA damage, thereby enhancing photosynthesis and flavonoid production, as well as altering leaf anatomy by the concomitant changes in the thickness of epidermal and parenchyma layers (Wang *et al.*, 2006; Victório *et al.*, 2007).

Different gibberellins are also involved in plant development (Ge *et al.*, 2007). These plant hormones promote germination, supporting the synthesis of specific proteins and mRNA (Bewley, 1997). Additionally, gibberellic acid (GA_3) may accelerate seed germination of some species by stimulating glucose synthesis, plant hormone production, or by increasing amylolytic activities, an essential stage of seed germination in which α -amylase converts starch to soluble sugar in the endosperm of some seeds (Lonvegrove & Hooley, 2000; Noguchi, 2008). Our preliminary studies indicated that seed germination of *S. cineraria* was very low under

white light conditions or treatment with vernalization periods (Silva *et al.*, 2002). Therefore, to identify the causal factors, this study aimed to study the effects of light spectra and plant hormones on the germinability of *S. cineraria*.

MATERIAL AND METHODS

Plant material and seed sterilization

Senecio cineraria commercial seeds, cultivar *Candidissima*, were surface sterilized by immersion in 70% ethanol (v/v) for 1 min, commercial detergent solution for 15 min, and 30% commercial sodium hypochlorite (2.3%) and rinsed with sterile distilled water between treatments. Fractions of seeds from two lots, containing 50 g each, were used in the experiments. The viability of each lot was about 85% according to producers.

Tissue cultures

Seeds were placed in Petri dishes containing 15 mL of MS medium (Murashige & Skoog, 1962) supplemented with 30 g L⁻¹ of sucrose, 1.3 μ mol L⁻¹ of thiamine-HCl, 3 μ mol L⁻¹ of pyridoxine, 4.1 μ mol L⁻¹ of nicotinic acid, 0.6 mmol L⁻¹ of myo-inositol and solidified with 7.8 g L⁻¹ agar, pH rated to 5.8 \pm 0.1, then sterilized in an autoclave at 120 °C and 1.1 Kg cm⁻². Seeds were kept in a growth room at 25 \pm 2 °C in the dark or light under a photoperiod of 16 hours. Light intensities were measured by a quantameter (Biospherical Instruments Inc., QSL-100). Darkness was ensured by wrapping the Petri dishes in two layers of aluminum foil. All treatments were arranged in a completely randomized design with four replicates of 25 seeds per treatment.

Treatment with light spectra and growth regulators

White light plus UV-A was tested using MS0 (control) and MS + 0.3 mg L⁻¹ of GA_3 . The chosen concentration was based on previous results with *S. cineraria* tissue cultures (Silva, 2001). Light intensities varied from 14 to 20 μ mol m⁻² s⁻¹, and one lamp per shelf was used for each light spectrum. Light spectra were obtained from one fluorescent lamp (Sylvania®, 20 W T 12) in white (control, 20 μ mol m⁻² s⁻¹), blue (17 μ mol m⁻² s⁻¹), green (14 μ mol m⁻² s⁻¹) and red (14 μ mol m⁻² s⁻¹) (Figure 1). Treatment with white light supplemented by ultraviolet A (UV-A) was adjusted to provide the same light intensity of white light (20 μ mol m⁻² s⁻¹).

The effects of different concentrations of GA_3 (0.0 (MS0 - control); 0.1; 0.3; 0.4; 0.6; 0.8; 1.0 and 2.0 mg L⁻¹) and IAA (indole-3-acetic acid) (0.0 (control); 0.1; 0.3 and 1.0 mg L⁻¹) were evaluated. Both hormonal treatments were conducted under white light (20 μ mol m⁻² s⁻¹) and in the dark.

Seeds maintained under light were evaluated every day for five weeks, recording the percentage of germination, average germination time (days) and average germination rate (days⁻¹). The average germination rate and average germination time were calculated according to Labouriau (1983). Seeds placed in the dark were evaluated after five weeks. Germination was considered to have occurred when the radicle protruded through the seed.

Statistical analysis

Data were analyzed in a completely randomized design using the Statistica® 6.0 program. The Least Significant Difference (LSD) at 5% probability level was used to test differences between germination percentage means.

RESULTS AND DISCUSSION

At the end of five weeks, the application of UV-A was not found to affect the germination percentage of seeds when compared with white light (control) in either medium. However, difference was found in mean germination time. Under white light plus UV-A, germination started during the first week using MS0 and during the second week using MS + GA₃ 0.3 mg L⁻¹, compared with white light treatment alone, indicating that additional UV-A induced germination more rapidly compared with other treatments. Addition of UV-A and GA₃ medium in combination did not produce significantly different responses in germination compared with MS0 under white light (Figure 2A-B).

Red light is considered as a stimulus for germination of several seeds that show positive photoblastic behavior. In addition, seeds require white or red light to reach maximum germinability (Figueroa *et al.*, 2007). However, in the present study, red light was not effective in improving *S. cineraria in vitro* germination (Figure 2A).

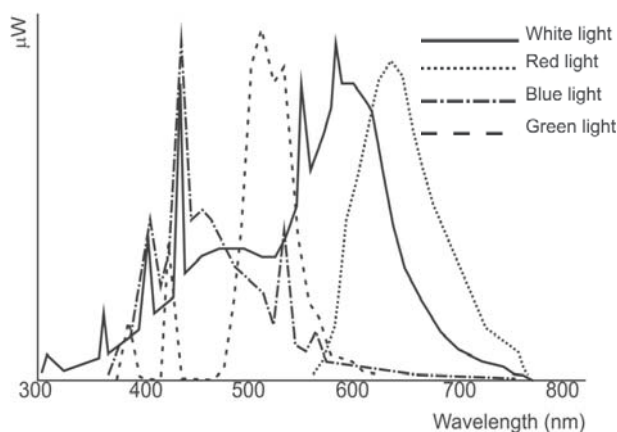


Figure 1. Spectral power distributions of used light sources (data provided by the Sylvania supplier).

It should be noted that works evaluating green light on plant development have only just emerged in the literature (Islam *et al.*, 1999). However, in this study, it was found that germination percentage under green light was significantly smaller than white light, in MS0 (Figure 2A). Islam *et al.* (1999) also reported low *in vitro* germination of *Cattleya walkeriana* under green light. This kind of light is used as security light in germination experiments. It was also found that germination percentages under red and green lights were very similar over the five-week experimental period. Under green light, these data indicate that seed germination may occur.

Seeds maintained in MS0 showed low germination under blue light (Figure 2). This finding agrees with results presented by Gopal & Sharma (1983) who described the inhibitory effect of blue light in *Typha angustata* seed germination. Interestingly, the same plant photoreceptors absorb blue light and UV-A (Sullivan & Deng, 2003). The

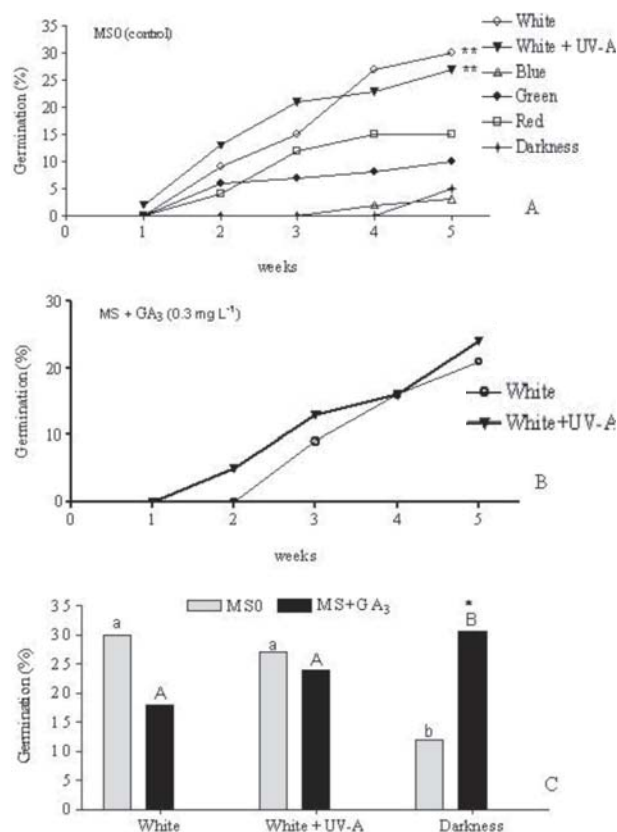


Figure 2. *In vitro* germination of *Senecio cineraria* DC. for five weeks under different light spectra and in the dark: A. MS0 (control), B. MS + GA₃ 0.3 mg L⁻¹. C. 5-week *in vitro* germination comparing MS0 and MS + GA₃ 0.3 mg L⁻¹ media under white light, UV-A supplementation and dark. Different letters indicate significant differences. Lower case letters compare the effects of light on seeds kept in MS0 medium. Upper case letters compare light spectra using MS + GA₃ 0.3 mg L⁻¹. **Indicates statistical differences compared with blue and dark (A). *Indicates statistical differences between culture media (MS0 and MS + GA₃) for each light spectrum (C), (p ≤ 0.05, 100 seeds/treatment).

results of the present study could shed light on this seeming paradox in that the absorption of these spectra by cryptochromes induce different responses, as also mentioned by Gyula *et al.* (2003), since results for *S. cineraria* seeds under blue light and UV-A were completely different. Compared to white light, all other light spectra showed a reduction of seed germination of *S. cineraria*, suggesting an inhibition of photoreceptors. The effect of GA₃ was not found to improve under white light + UV-A compared with MS0 (Figure 2B).

Experiments were performed under both light and dark, using different GA₃ concentrations. Under white light, results showed that GA₃ did not improve the germination percentage (Figure 3). Although germination time increased under white light, germination rate was reduced to 0.6 and 0.8 mg L⁻¹ of GA₃ (Figure 3A-B). In contrast to a

generally low germination rate induced by GA₃ in any concentration under light conditions, all concentrations of GA₃, except 1.0 mg L⁻¹, induced a higher germination rate in the dark. Higher GA₃ concentrations did not correspond to an increase of germination percentage (Figure 3C).

The highest germination percentage (a maximum 10 percent point increase compared with control medium) was obtained using 0.6 and 0.8 mg L⁻¹ of GA₃, but in the dark (Figures 2C and 3C). This result can most likely be explained by the replacement of light by GA₃, which is necessary to stimulate photoreceptors and, consequently, the synthesis of gibberellins involved in germination (Toyomasu *et al.*, 1998). Gibberellins have a well-known role in seed germination induction. In fact, GA₃ may be used as a means of breaking dormancy. However, according to Baskin & Baskin (2001), no seed dormancy was reported for *S. cineraria*. Moreover, based on germination studies of *S. vulgaris*, Lutman *et al.* (2008) found only limited results of dormancy in the dark. Finally, Silva *et al.* (2002) carried out studies using vernalization periods (1, 5, 15, 30 and 60 days) and observed that the germination of *S. cineraria* was less than 16 %. Similarly, we found only limited germination of *S. cineraria* seeds under dark conditions with no treatment. Importantly, however, when seeds were treated with GA₃ in the dark, we found, as noted above, a higher rate of germination than the same concentrations of GA₃ in the light. Therefore, it can be concluded that GA₃ is instrumental in breaking dormancy. Studies have shown that IAA is involved in the early stages of germination (Slavov *et al.*, 2004). Germination tests using IAA indicated that there was no increase in *S. cineraria* germinability, either in the light or dark, in comparison with MS0 medium. Moreover, under dark conditions, the germination rate was lower with the addition of IAA compared with white light (Figure 4) and therefore different from results found for GA₃ (Figure 3).

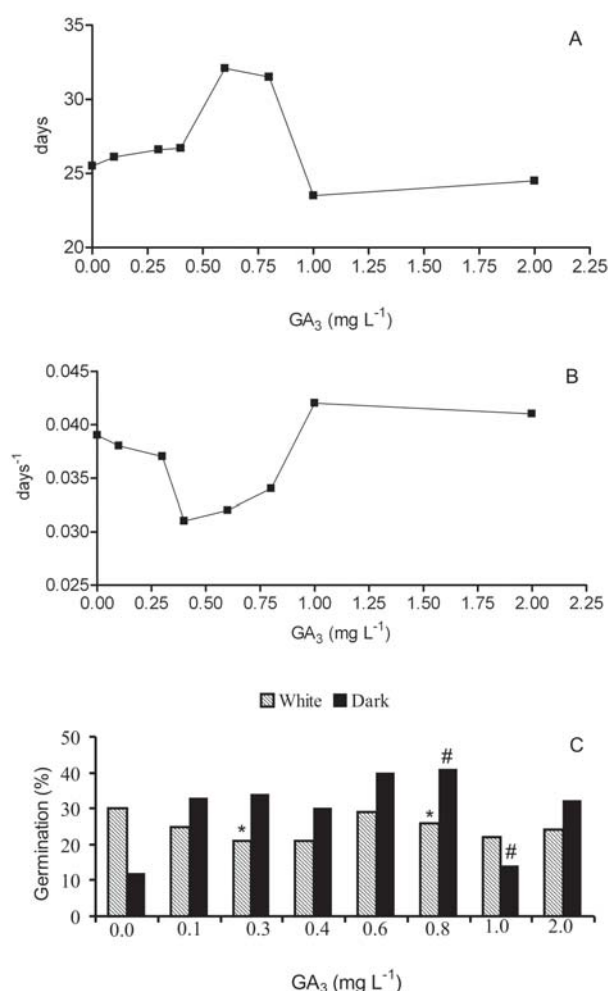


Figure 3. *Senecio cineraria* DC. mean time and mean rate of germination under white light in different GA₃ concentrations (mg L⁻¹) over 5 weeks. A. Mean time; B. Mean rate; C. Total germination under white light and in the dark. *Indicates differences between white light and dark, considering each concentration in mg L⁻¹. #Indicates statistical differences among concentrations, considering the dark control (p<0.05, 100 seeds/treatment).

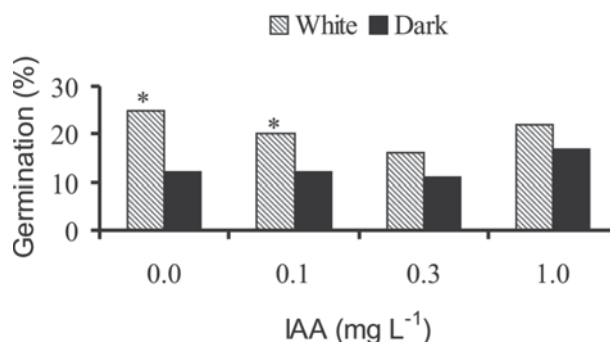


Figure 4. *Senecio cineraria* DC. germination using different IAA concentrations in mg L⁻¹, after 5 weeks. *Indicates statistical differences between white light and dark (p<0.05, 100 seeds/treatment).

CONCLUSION

The presented data showed a higher germinability of *S. cineraria* under light conditions compared with dark, but germination percentage was low which may be related to other parameters, such as temperature, an important determinant of seed germination. The addition of GA₃ enhanced seed germination in the absence of light.

Seeds of *S. cineraria* respond more to any light exposure when compared with dark, although the influence of the photoreceptors in germination seemed very slight by the few changes in different light spectra.

White light and white light supplemented with UV-A gave the best responses to *in vitro* germination using MS0 medium.

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