Brazilian Journal of Biology
ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

https://doi.org/10.1590/1519-6984.228777
Original Article

Light quality and dormancy overcoming in seed germination of *Echium plantagineum* L. (Boraginaceae)

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Received: September 16, 2019 – Accepted: March 3 2020 – Distributed: Aug. 31, 2021
(With 3 figures)

Abstract

Light is considered a factor that influences the seed germination of many weed species, and it can signal whether the environmental conditions are favorable or are not favorable for germination. We aimed to study if there is an influence of light quality and dormancy overcoming in seed germination of *Echium plantagineum* L. We carried out a 2 x 6 factorial experiment, with and without dormancy overcoming with potassium nitrate followed by immersion in gibberellic acid; six light qualities, obtained through the light filters: blue, green, red, far-red, white light and absence of light. The evaluations performed were germination speed index (GSI), average germination time (AGT), germination at the four and 14 days after seeding (DAS), accumulated germination and relative frequency of germination. We observed significant interaction among the light qualities and seed dormancy overcoming or not for the studied variables. There was no significant effect of light qualities, in the evaluated variables, when performing dormancy overcoming, presenting germination above 90% in all the light qualities. However, without dormancy overcoming, we observed greater GSI, germination at four and 14 DAS for the red light filter with 5, 4, 29 and 45%, respectively. When the seeds were submitted to the absence of light, and without dormancy overcoming, there was only 7% of germination at 14 DAS. The seeds of *E. plantagineum* presented greater germination under incidence of red light, without dormancy overcoming, being classified as preferably positively photoblastics, provided that the dormancy is not overcome.

Keywords: purple flower, photoblastism, phytochrome, cellophane paper, weed.

Qualidade da luz e superação de dormência na germinação de sementes de *Echium plantagineum* L. (Boraginaceae)

Resumo

A luz é considerada um fator que influencia a germinação das sementes de muitas espécies de plantas daninhas, podendo sinalizar se as condições ambientais são favoráveis ou não para a germinação. Objetivou-se estudar se há influência da qualidade da luz e superação de dormência na germinação de sementes de *Echium plantagineum* L. Realizou-se um experimento fatorial 2 x 6, com e sem superação de dormência com nitrato de potássio seguido pela imersão em ácido giberélico; seis qualidades de luz, obtidas através de filtros de luz: azul, verde, vermelho, vermelho-distante, luz branca e ausência de luz. As avaliações realizadas foram índice de velocidade de germinação (IVG), tempo médio de germinação (TMG), germinação aos quatro e 14 dias após a semeadura (DAS), germinação acumulada e frequência relativa de germinação. Observou-se interação significativa entre as qualidades de luz e a superação ou não de dormência das sementes para as variáveis estudadas. Não houve efeito significativo das qualidades de luz, nas variáveis avaliadas, ao realizar superação de dormência, apresentando germinação acima de 90% em todas as qualidades de luz. Todavia, sem superação de dormência, observou-se maior IVG, germinação aos quatro e 14 DAS para o filtro de luz vermelha com 5,4, 29 e 45%, respectivamente. Quando as sementes foram submetidas à ausência de luz, e sem superação de dormência, houve apenas 7% de germinação aos 14 DAS. As sementes de *E. plantagineum* apresentam maior germinação sob incidência de luz vermelha, sem superação de dormência, sendo classificadas como fotoblásticas positivas preferenciais, desde que não seja superada a dormência.

Palavras-chave: flor roxa, fotoblastismo, fitocromo, papel celofane, planta daninha.

1. Introduction

Echium plantagineum L., Boraginaceae, known in the South of Brazil as purple flower or "soagem", characterizes itself as an important weed of winter, infesting annual crop areas, as well as pastures and roadsides. This species is native from European countries near the Mediterranean and North Africa, being considered weed with economical importance in Australia, South Africa, Canada, New Zealand and South America (Weston et al., 2012; Florentine et al., 2018).

It presents fast growth, high leaf area production, aggressive root system, adaptability to water deficiency conditions, temperature variations and photoperiod, and also, elevated ability of competition for water and nutrients (Sharma and Esler, 2008; Konarzewski et al., 2012). Besides, it has phenotypic plasticity demonstrated by the variation of morphological characteristics, plant height, size and fruit mass, triggered in response to abiotic factors (Piggin, 1976; Konarzewski et al., 2012). Another form of adaptability to the environmental conditions is its capacity of seed production, which can exceed 6000 seeds per square meter in high infestations, presenting dormancy that contributes for the distribution of the emergence flows in time and permanence in soil seed bank (Piggin, 1976; Roso et al., 2017; Florentine et al., 2018). It characterizes itself by the scorpioid inflorescence and fruit denominated carcerulus (indehiscent dry fruit and one-seeded), whose seed remains united to the fruit, being a unit of dissemination and propagation (Moreira and Bragança, 2010; Souza and Lorenzi, 2012).

The seed germination is the result of the balance between the intrinsic characteristics of the same and the favorable environmental conditions, which result in the resumption of the embryo growth originating a seedling (Orzari et al., 2013). In this sense, the reduction of germination and emergence can be attributed to the low vigor or dormancy of seeds and/or to critical environmental factors, such as: humidity, chemical substances, soil acidity, salinity, temperature and light (Luz et al., 2014). Thus, seeds of weed can remain viable in soil by long periods and germinate, since the dormancy is exceeded and the environmental conditions are favorable (Yamashita et al., 2011).

Many species of weed are photosensitive (Orzari et al., 2013), where the light can trigger or inhibit the germinative process of seeds. This is possible because the light is responsible by the activation of phytochromes, which is a pigment whose function is to capture light signals, and it can or cannot stimulate the germination in some species (Taiz et al., 2017). The response to light presents itself in a distinct way among the species, and the role of the phytochrome in dormancy overcoming is one of the few mechanisms totally known that act in germination (Vivian et al., 2008; Souza-Filho and Takaki, 2019).

The phytochrome mode of action depends on the type of incident radiation, and the light in the region of red (660 nm) and far-red (730 nm) promote alteration of the isomeric form of the phytochrome, modifying the balance between the active form (FVe) and inactive form (FV), respectively (Parreira et al., 2011). This way, high relation red/far-red (R/FR) can induce the phytochrome to assume the active form (FVe), triggering the germination of photosensitive seeds, while low R/FR relation can lead it to assume inactive form (FV), inhibiting the germination, remaining dormant (Vidaver, 1980; Vivian et al., 2008). With this, the seeds detect the environmental variations through the change in incident light quality, signaling whether the environmental conditions are or are not favorable for the germination, growth and development of the plant (Brancalion et al., 2008).

In general, long wavelengths (far-red), of the visible light spectrum, penetrate deeper into the soil, in its turn, short wavelengths (blue) are attenuated in the first millimeters of soil (Bliss and Smith, 1985). The effect of soil filtering the light spectrum provides in theory three different environments, important in germination of weed. Depending on the type and composition of soil, in the first millimeters occurs predominant effect of blue light; after the first millimeters this one disappears and increases the R/FR relation (phytochrome active form). However, in greater soil depths, the R/FR relation decreases (Barrero et al., 2012). In this sense, the light transmits information to the seeds, through its spectral composition and irradiance, which can orientate themselves in relation to the position in the soil profile (Batlla and Benech-Arnold, 2014), influencing the germination and composition of infestations of weed in agricultural cultivations.

The knowledge of germinative biology of *E. plantagineum* can help to develop correct strategies of management, and it is an alternative to the chemical control, or can permit to develop integrated management practices of weed. Before this, the present work aimed to study the influence of light quality and dormancy overcoming in seed germination of *E. plantagineum*.

2. Material and Methods

The work was conducted in Didactic and Research Laboratory for Seeds from the Plant Science Department of Federal University of Santa Maria, in Santa Maria, RS. The fruits of *E. plantagineum* were collected manually in crop with history of infestation by this weed (±20 plants m^{-2}), located in the municipality of Restinga Seca, RS (29° 51' 29" S and 53° 31' 41" W and 72 m of altitude). The collecting area characterizes itself by the soybean cultivation (*Glycine max* (L.) Merr.) in summer and ryegrass (*Lolium multiflorum* Lam.) in winter.

We collected only dark-colored fruits and that could easily detach from the mother plant, having, apparently, physiological maturity. The same ones passed through a manual cleaning process, dried at the shade for five days, and stored in Kraft paper bags at room temperature and dry place (laboratory) until carrying out the experiments.

The experiments were conducted in germination chamber of Biochemical Oxigen Demand (B.O.D.) type at temperature of 20 °C and photoperiod of 24 hours of artificial light inside the chamber (30 μmol m^{-2} s^{-1} de illuminance) (Brasil, 2009; Roso et al., 2017). We used the completely randomized experimental design (CRD) with four repetitions of 50 diaspores (carcerulus: fruit agglutinated with the seed) totaling 200 diaspores by treatment. The tests were conducted on a transparent acrylic gerbox (11.0 x 11.0 x 3.5 cm), under three sheets of germitest paper moistened with distilled water in quantity equivalent to 2.5 times the mass of dry paper (Brasil, 2009).

The light quality in germination was evaluated by a 2 x 6 bifactorial experiment, with and without dormancy overcoming and six qualities of light, which were obtained through light filters with different wavelengths: blue (450 nm), green (500 nm), red (700 nm), far-red (760 nm), white light (380 to 760 nm) and absence of light. According to the adapted methodology by Yamashita et al. (2008), for obtaining different light qualities (blue, green and red) the gearbox type boxes were involved by two layers of cellophane paper with colors corresponding to the used treatment. In the far-red treatment, the boxes were involved by two layers of red cellophane and two blue ones. In the treatment with white light, the gearbox boxes were not involved with cellophane paper, and the treatment with absence of light was obtained through the involvement by two layers of aluminum foil. During the evaluations, so that there was no interference of white light in germination, the evaluations were carried out in dark room in presence of green light (Yamashita et al., 2008).

We verified passage of light through the cellophane papers, performing readings of transmittance percentage using a UV-Visible spectrophotometer model Nova® 2000 UV, according to the adapted methodology by Yamashita et al. (2008). As reading cell, we used quarzto cuvette with optical path of 10 mm, where the papers were placed as used during the germination involving the gearbox boxes, performing readings in different wavelengths (Figure 1).

In the treatments submitted to the dormancy overcoming, we performed the soaking of the diaspores in potassium nitrate solutions (0.2%) for 12 hours, followed by immersion in gibberellic acid (500 mg L^{-1}) for 48 h, occurring the washing of the diaspores after each period of immersion, for 10 min in running water (Roso et al., 2017).

The analyzed variables were: germination at four (4) and 14 DAS (days after seeding) and germination speed index (GSI) during14 days, considering germinated seed when there was the first root protrusion (≥ 2 mm) according to Bewley and Black (1994). The percentage of germination at four and 14 DAS was calculated according to the methodology described by

Labouriau and Valadares (1976). Yet, the GSI was calculated by the equation according to Maguire (1962) and the average germination time (AGT) was determined according to Furbeck et al. (1993). From the results of daily germination, used for GSI, we calculated the relative germination frequency, through methodology by Labouriau and Valadares (1976) and demonstrated accumulated germination.

The data was submitted to variance analysis by F test ($p<0.05$) through SISVAR statistical program (Ferreira, 2011). The averages were compared by Scott-Knott test in 0.05 of probability. For the variables in percentage, the data was transformed for arc sine √%/100.

3. Results and Discussion

According to the results of transmittance readings of cellophane papers, used as light filters, we observed that the methodology used is in accordance with the expected (Figure 1). The cellophane papers played the role of

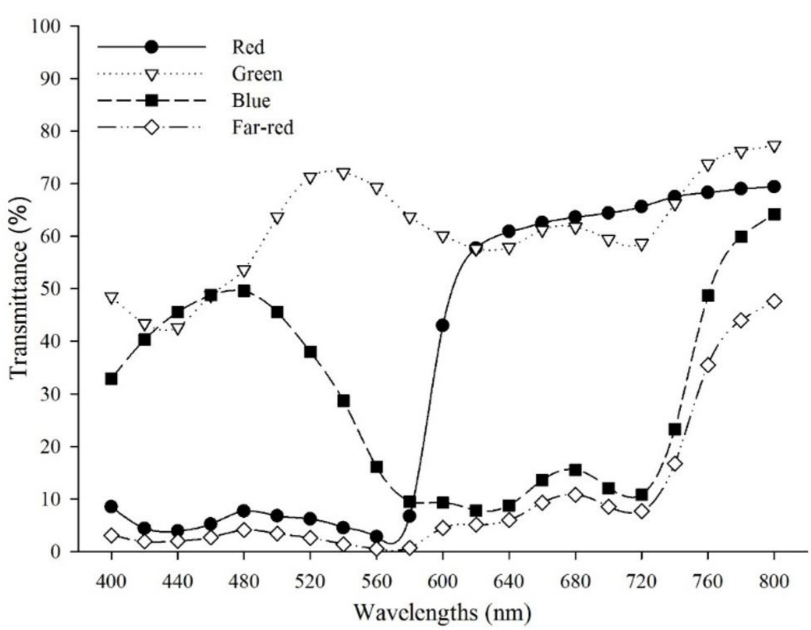

Figure 1. Values of transmittance (%), in different wavelengths (nm), obtained in the cellophane papers used as light filters.

filtering the wavelengths of the visible light spectrum, presenting peaks of transmittance in the regions of blue, green, red and far-red.

Significant interaction we observed ($p<0.05$), among the light qualities and the dormancy overcoming for the GSI variables, average germination time (AGT) and germination at four and 14 DAS. For these variables, there was no significant effect of the light qualities used when performing the dormancy overcoming of seeds. Nevertheless, there was significant difference of the light qualities when this was not performed (Table 1). In general, the dormancy overcoming provided greater values of GSI and percentage of germination at four and 14 DAS and lower AGT values, indicating fast germination, when compared to treatments without overcoming. We verified germination above 83 and 90% at four and 14 DAS respectively, when performing dormancy overcoming, in all the light qualities (Table 1).

When the seeds were not submitted to dormancy overcoming, in red light quality, we observed greater GSI and germination at four and 14 DAS, with 5, 4, 29 and 45%, respectively, and it was not different from white light (24%) for germination at four DAS. When the seeds were submitted to light absence and without dormancy overcoming, we observed lower GSI and germination at four and 14 DAS, 0.6; 3.0 and 7.0%, respectively, and greater AGT (7.8 days) which indicates that the germination occurred in a slow way (Table 1). These results demonstrate that the seeds of *E. plantagineum* present dormancy, which can be overcome in a efficient way using potassium nitrate (0.2%) and gibberellic acid (500 mg L^{-1}), independent from the light quality. And, also, in a less efficient way, without dormancy overcoming, exposing the seeds to red light conditions, possibly by the greater conversion of the inactive form of phytochrome (Fv) to active form (Fve) (Yamashita et al. 2011; Batlla and Benech-Arnold, 2014), triggering the germinative process. The responses obtained by the germination induction when exposing the seeds to the red light, can be related to the gene expression regulation of the biosynthesis of gibberelline by the active phytochrome, which act directly in the germination promotion (Toyomasu et al., 1998).

According to Bewley and Black (1994), for preferably photoblastic seeds, light wavelengths between 650 and 700 nm (red) promote greater percentages of germination, yet wavelengths greater than 730 nm (far-red) inhibit the germination. Similar results were observed in this study, in which red light filters provided greater germination (45%) of seeds of *E. plantagineum*, in comparison to the far-red light filters (22%), when the seeds were not submitted to dormancy overcoming. As well as *E. plantagineum*, most of the seeds of weed species respond to light to germinate, because they are not domesticated species, and the germination process is regulated by the phytochrome, and depends on adequated wavelengths to trigger the germination (Yamashita et al., 2011). The importance of red light in dormancy overcoming of seeds, and the inhibition of germination by the blue and far-red light was reported for several species in literature, among them *Lolium rigidum* Gaud. (Goggin et al., 2008), *Murdannia nudiflora* L. (Ferraresi et al., 2009), *Conyza canadensis* L. and *C. bonariensis* L. (Yamashita et al., 2011) and *Brachypodium distachyon* L. (Barrero et al., 2012).

Analyzing the data of accumulated germination of seeds with dormancy overcoming of *E. plantagineum* (Figure 2A), we observe that there was fast germination in all the light qualities, inclusive continuous dark, complementing the results of GSI, with germination above 83% on the fourth day after seeding. These results indicate that, once the dormancy is overcome, the germinative process is triggered, independent of light stimulus. This way, the dormancy overcoming treatments using gibberellic acid can have substituted the red light effect in germination, for this reason the seeds treated with gibberellic acid germinated independent of the light quality. According to Carvalho and Nakagawa (2000), the light is one of the natural agents of dormancy overcoming of seeds in some species; however, after this is overcome by the light action or by influence of other factors, the germination will occur both in the presence and absence of light. Nevertheless, depending on the species, the light will promote the germination

Table 1. Germination at four and 14 days after seeding (DAS), germination speed index (GSI) and average germination time (AGT) of *Echium plantagineum* L. submitted to different light qualities, with (CS) and without (SS) dormancy overcoming by the soaking of the seeds in potassium nitrate (0.2%) and gibberellic acid (500 mg L^{-1}).

Light qualities	Germination (%)								GSI				AGT (days)			
	4 DAS[3]				14 DAS											
	CS		SS		CS		SS		CS		SS		CS		SS	
Blue	85	ns[4]A	11	bB	90	nsA	17	cB	12.7	nsA	2.0	cB	3.7	nsB	4.8	aA
Green	85	A[1]	10	bB	94	A	21	cB	13.0	A	2.3	cB	3.9	B	5.3	aA
Red	83	A	29	aB	91	A	45	aB	13.1	A	5.4	aB	3.7	B	4.8	aA
Far-red	89	A	15	bB	93	A	22	cB	13.7	A	2.7	cB	3.5	B	5.0	aA
White light	90	A	24	aB	93	A	37	bB	13.1	A	4.4	bB	3.6	B	4.7	aA
Absence of light	83	A	3	cB	90	A	7	dB	12.9	A	0.6	dB	3.7	B	7.8	bA
CV (%)[2]	9.86				5.89				4.85				7.41			

[1]Averages followed by different letters, lower case in the column and upper case in the line, differ from each other by the Scott-Knott test (p-valor<0.05); [2]CV: coefficient of variation; [3]DAS: days after seeding; [4]ns: not significant.

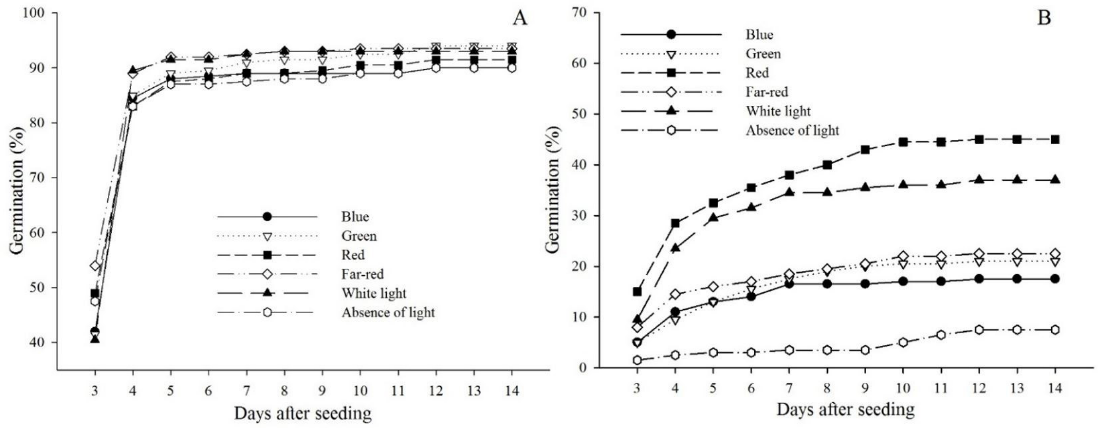

Figure 2. Accumulated germination of seeds of *Echium plantagineum* L. submitted to different light qualities with (A) or without (B) dormancy overcoming.

or induce the dormancy, being considered an important environmental sign in germination (Barrero et al., 2012; Batlla and Benech-Arnold, 2014).

However, without the dormancy overcoming, the light promoted the germination of seeds of *E. plantagineum*, even though in lower percentages. There was accumulated germination near to 30 and 25% on the fourth DAS, when using red and white light filters, respectively (Figure 2B). Gradual increase of accumulated germination occurred in these light filters until the 14 DAS, with final germination of 45 and 37%, respectively. The blue, green and far-red light filters also presented gradual increase in accumulated germination until the 14 DAS; however, we observed only 17, 21 and 22% of final germination, respectively. In the absence of light there was lower accumulated germination, 7% at 14 DAS.

The relative germination frequency confirms the results of GSI and accumulated germination, indicating fast germination when the seeds were submitted to dormancy overcoming. Also, we verified greater relative germination frequency on the third and fourth DAS, in all the light qualities, presenting more than 44 and 37% of germinated seeds, in these evaluations, respectively (Figure 3A). In the treatments in which the seeds were not submitted to dormancy overcoming, there was distribution of relative germination frequency throughout the evaluation period (Figure 3B). Nevertheless, we observed a peak of germination on the third and fourth DAS when exposing the seeds to some light condition. When the seeds were submitted to absence of light, we verified greater relative germination frequency near to 3rd and 11th DAS, indicating that in this condition, besides presenting lower germination, this one occurs in a slow way, and it is related to the dormancy mechanism.

These results corroborated in part with the ones found by Roso et al. (2017), in which the authors classified *E. plantagineum* as preferably positively photoblastic, presenting greater germination when the seeds were exposed to light. In this work, we verified that the light provides stimulus to germination if the dormancy overcoming is not carried out; however, when overcoming the dormancy, the germination occurs independent of light stimulus, indicating that the dormancy of this species can be related to a combination of factors. In this sense, the potassium nitrate and the gibberellic acid can have substituted the effect of light quality in germination, which may not be the main cause of dormancy. The dormancy overcoming with potassium nitrate and gibberellic acid together provides greater germination than both isolated, indicating synergetic effect (Roso et al., 2017). Thus, the red light influenced positively in germination when there was no dormancy overcoming, but in lower percentage when compared to the treatments with dormancy overcoming, because the potassium nitrate can have helped in germination.

In this sense, the seed germination of most of weed species is restricted to the proximity of the soil surface, presenting variation among the species, due to the availability of reserves of seeds and mainly by the light requirement to trigger the germination process (Ferraresi et al., 2009; Barrero et al., 2012). Depending on the soil composition, this can play the role of filtering the light in different wavelengths, depending on the depth, promoting three distinct environments for germination, and it can have influence in seed dormancy (Barrero et al., 2012). The first millimeters of soil present greater influence of light in region of blue, and it can inhibit or decrease germination, disappearing after the first millimeters and the seeds find adequate germination conditions with high proportion of red/far-red (Bliss and Smith, 1985; Barrero et al., 2012). These same authors also affirm that in greater soil depths, the proportion of red/far-red decreases and the germination can be inhibited again in photosensitive seeds. According to Batlla and Benech-Arnold (2014) agricultural cultures or plants of soil coverage also filter the light when passing by the canopy, hitting the soil low proportion of red/far-red light, allowing the seeds of weed to detect the presence of vegetation, indicating the dormancy permanence of seeds and unfavorable conditions to germination.

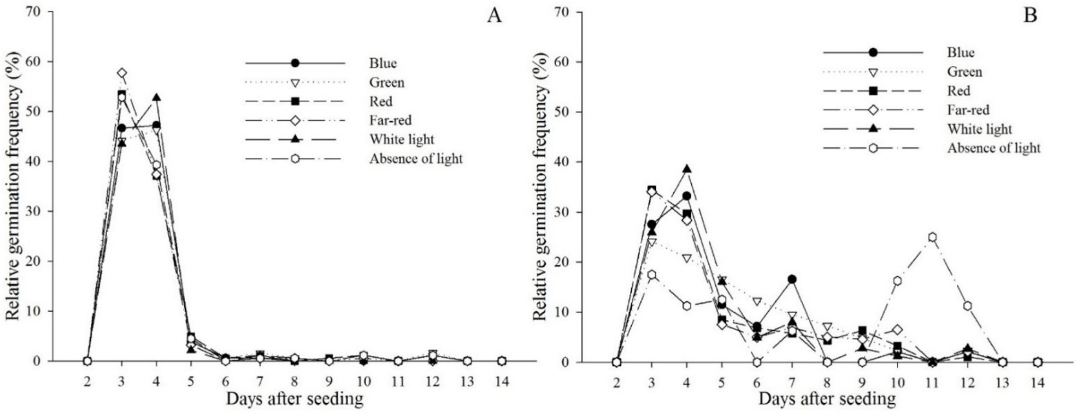

Figure 3. Relative germination frequency of seeds of *Echium plantagineum* L. submitted to different light qualities with (A) or without (B) dormancy overcoming.

Due to the sensitivity of seeds of *E. plantagineum* to light, when it is not submitted to dormancy overcoming, with greater response of germination under incidence of red light, and lower germination in absence of light, the use of cultures of soil coverage associated to other practices, can be an efficient management strategy, in order to decrease the germination of this species. Besides that, the possibility of predicting the seed germination, located next to the soil surface, when there are no coverage plants or upturned soil, it can help in the control planning of this invasive species or in the management of agricultural cultures.

4. Conclusion

The dormancy overcoming of seeds, with potassium nitrate (0.2%) for 12 hours, followed by soaking in gibberellic acid (500 mg L^{-1}) for 48 h provides the germination of *E. plantagineum*, independent of incidence and quality of light.

The seeds of *E. plantagineum* present greater germination under the incidence of red light, without dormancy overcoming.

Without treatment of dormancy overcoming with potassium nitrate and gibberellic acid, the seeds of *E. plantagineum* can be considered preferably positively photoblastics.

Acknowledgements

CNPQ - the PQ productivity scholarship teacher researcher Dr. Ubirajara Russi Nunes.

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