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RESEARCH ARTICLE

TEMPERATURE AND LIGHT ON GERMINATION AND VIGOR OF *CRAMBE ABYSSINICA* HOCHST SEEDS

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ABSTRACT

With the search for new sources of vegetable raw material for biodiesel production, the crambe (*Crambe abyssinica Hochst*) emerges as an alternative because of its high oil content, around 40%. Therefore it is necessary to know more about these species and the germination behavior of its seeds. Among the factors that affect germination, the main are temperature and light. The objective of this research was to evaluate the influence of temperature and light on germination and vigor of crambe seeds. Crambe seed of cv Brilhante FMS were used and submitted to three temperatures (15, 25 and 35 °C) and two light conditions (presence and absence of light). The following tests were performed: germination, first count of germination, germination speed index, percentage of abnormal seedlings and seeds no germinated. Data were submitted to analysis of variance. Means were compared by Tukey test at 5% and regression analysis was performed. The germination and vigor of crambe seeds was higher at 25 °C in the dark. The presence of light reduced the germination and seed vigor at 15 and 25 ° C. The temperature of 35 ° C does not promote germination in crambe seeds.

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INTRODUCTION

The crambe (*Crambe abyssinica Hochst*) is a plant with a high oil content, around 44.1%, with an expressive amount of erucic acid (a long chain fatty acid), around 37%, which does not make it suitable for human consumption, being available only for industrial purposes (Deleon Martins *et al.,* 2012). This fact makes crambe seeds a potential raw material for the production of biodiesel (Carlsson, 2009). In addition, crambe presents other advantages such as good adaptability to cold and drought conditions, and can be used as a fall / winter crop in the South and Southeast regions of Brazil, not competing with traditional summer crops such as corn and soybean (Souza *Et al.,* 2009, Reginato *et al.,* 2013). As a resultof this, researches are being carried out with the aim of knowing more about the crambe plant, especially the germinative capacity of its seed (Ruas *et al.,* 2010, Deleon Martins *et al.,* 2012, Lima *et al.,* 2015). Germination is a sequence of physiological events influenced by some factors, which may act by themselves or in interaction with others.

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Among the factors that directly affect germination and considered essential are temperature and light (Marcos Filho, 2005, Bewley *et al.,* 2012). In germination, the response to temperature depends on the species, variety, region of origin and storage time. The temperature modifies the speed of the chemical reactions that will trigger the unfolding and transport of the reserves and the resynthesis of substances for the seedling (Bewley et al, 2012). It also affects the germination process in the number of germinated seeds and the speed and uniformity of germination (Carvalho & Nakagawa, 2012). High temperatures initially stimulate the germination, until a certain point, when the effect reverses and the germination decreases. Light is one of the main controlling factors of seed germination, and its effect dependent on genotype and environmental factors during its formation, inducing dormancy or promoting germination (Válio & Scarpa, 2001). Seeds of a large number of species exhibit photoblastic behavior, so the germination can be promoted or inhibited by exposure to white light. Seeds that germinate in the presence of light are called photoblast positive (Yamashita *et al.,* 2011), while those in which germination is inhibited by light are called photoblast negative (Cardoso, 2010). There are also seeds that are indifferent to the luminosity conditions (Mayer and PoljakoffMayber, 1989; Bergo *et al.,* 2010). By means of the morphophysiological responses of plants to different conditions of luminosity it is possible to evaluate their competitiveness under different environmental conditions and their potential of occurrence and growth can still be determined (Socolowski *et al.,* 2010). A number of studies have shown that light and temperature interfere on the germination of seeds from different species such as in oilseeds (Galindo *et al.,* 2012), weeds (Mondo *et al.,* 2010, Yamashita *et al.,* 2011), and tree species (Rego *et al.,* 2009, Alves *et al.,* 2012, Nogueira *et al.,* 2012). *Crambeabyssinica* has a great potential to be used for agriculture, but it is necessary making further studies on the germination of its seeds as emphasized by Deleon Martins *et al.* (2012), Ruas *et al.* (2010) and Lima *et al.,* (2015). In view of the above, this study aimed to evaluate the germination capacity and vigor of crambe seeds under different temperature and light conditions.

MATERIALS AND METHODS

The experiment was carried out at the Central Laboratory of Seed Analysis of the Federal University of Lavras, using crambe seeds of the cultivar FMS Brilhante. The tests were carried out in a germination chamber B.O.D type adjusted at constant temperatures of 15, 25 and 35 ° C. The lighting conditions imposed were constant light and no light. The chambers were equipped with a front light source composed of four 15W white fluorescent lamps each (GE, "daylight") to provide constant light. For studying in absence of light the daily counts were performed in dark rooms under green light. The following tests were performed:

Germination test

It was carried out in plastic boxes (11.0 x 11.0 x 3.0cm). For each repetition of the assay, we used 50 seeds on two sheets of blotting paper (10.5 x 10.5 cm), with a quantity of 0.2% potassium nitrate (KNO_3) corresponding to 2.5 times the mass of dry paper (Brazil, 2009). For the tests with absence of light, as plots were sown only in the presence of green light, to avoid the interference of light on the germination process (Noronha *et al.,* 1978). Then the boxeswere keptin transparent plastic bags in order to avoid a loss of water, and placed in B.O.D.s chambers with temperature control and light availability, according to each treatment. It carried out the rotation of the plastic boxes daily in the chamber that had lighting, in order to supply homogeneously luminous intensity for all as repetitions.At the end of the test, on the seventh day after the installation, normal, abnormal and non-germinated seedlings were separated and counted for the calculation of percentage of germination (total normal seedlings), abnormal seedlings and non-germinated seeds (dead and /or dormant seeds). Normal seedlings were considered for containing all essential structures intact and well developed.

First germination count: The vigor of the crambe seeds was evaluated on the fifth day after installation of germination test, by counting the number of normal seedlings to calculate the percentage of the first germination count.

Germination speed index

In conjunction with the germination test, daily and always at the same time, the protrusion of radicle was observed and annotated considering the length of 2 mm until the stabilization of the number of seeds germinated to calculate the germination speed index (GSI) according to the formula described by Maguire (1962) and also for the calculation of accumulated daily germination for vigor evaluation. Experimental design and statistical analysis - The experimental design was completely randomized. The treatments were distributed in a factorial scheme3 x 2, with three temperatures (15, 25 and 35 ° C) and two light conditions (constant light and absence of light), with 4 replications of 50 seeds. The datas were analysis of variance by the F test. The means were compared by the Tukey test at 5% of significance and regression analysis was alsoperformed.

RESULTS AND DISCUSSION

Germination was higher at 25 °C and in the absence of light (Table 1), similar results were obtained by Galindo *et al.* (2012) studying the effect of light and temperature on seeds of *Crataeva tapia* in which germination was favored by the absence of light at 25 °C. Lima *et al.* (2015) verified that the effect of the temperature of 25 °C on germination is also related to the initial quality of the batch of crambe seeds. The temperature of 35 °C was not favorable for the development of normal seedlings in crambe seeds, in the either presence or absence of light. However at the same temperature, there was seed germination with the formation of abnormal seedlings in the presence of light, which for the same condition did not differ statistically from the other temperatures. At the temperature of 15 °C there was a stimulus in the formation of abnormal seedlings in the absence of light (Table 1). At 25 °C there was no difference in the percentage of abnormal seedlings in both light conditions (Table 1). However, the constant presence of light inhibited the germination with formation of normal seedlings at that temperature when compared to the absence of light. Similar results happened at 15 °C. At the temperature of 35 °C, it was was observed the highest percentage of non-germinated seeds in relation to the other temperatures. According to Socolowski *et al.* (2010) light and temperature can determine seed dormancy intensity, as they are the main factors that promote germination in soils with adequate water availability. In this case the high percentage of crambe seeds that did not germinate is justified because it is a subtropical species, and it is expected a lower germination percentage at high temperatures (Deleon Martins *et al.,* 2012).

Table 1. Average of germination percentage, abnormal seedlings, non-germinated seeds, first germination count and germination speed index of crambe seeds submitted to different temperature and light conditions

	Germination (%)		
Luminosity	*Temperature*		
	15 °C	25 °C	35 °C
Constant	17 bB	42 aA	04 aC
Absent	37 aB	75 bA	00 aC
	Abnormal seedlings (%)		
Constant	18 bA	14 aA	12 aA
Absent	41aA	14 aB	00 bC
	Non-germinatedseeds (%)		
Constant	65 aB	44 aC	82 bA
Absent	22 bB	11 bC	100 aA
	Firstgerminationcount		
Constant	05 bB	21 bA	08 aB
Absent	25 aB	70 aA	00 bC
	GSI		
Constant	3.61 bB	5.43 bA	2.24 aB
Absent	8.49 aB	13.93 aA	0.08 bC

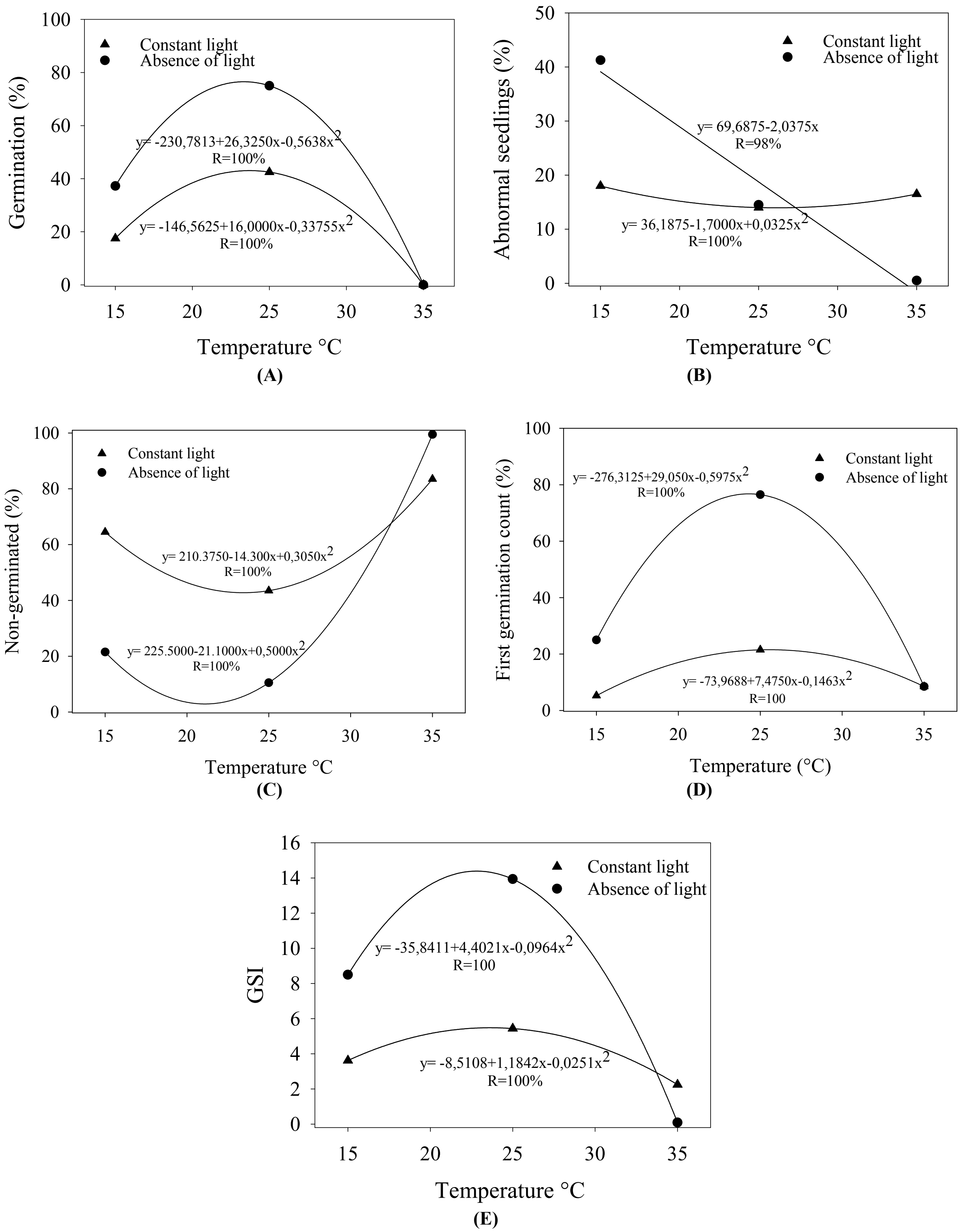

Figure 1. Regression graphs of germination (A), abnormal seedlings (B), non-germinated seeds (C), first germination count (D) and germination speed index (GSI) (E) of crambe seeds submitted to presence or absence of light at different temperatures

Means followed by the same letter, lowercase in the column and upper case in the row, do not differ from each other at the level of 5% of probability by the Tukey test. The results described above corroborate with the low rates of germination (Table 1) at the same condition. In general the GSI was higher in absence of light when the seeds were submitted to 15 and 25 °C. The first germination count (FGC) was an indirect way of evaluating the germination speed and the results of this test were similar to GSI, observing the superiority at 25 °C at the absence of light and the inferiority at the temperature of 35 °C.

Results that seed vigor was favored by absence of light was also reported in*Crataevatapi*a (Galindo *et al.,* 2012). Figure 1 shows the graphs of the physiological tests studied as a function of temperature and luminosity. The quadratic regression was the one that fit best, except for the percentage of abnormal seedlings in absence of light, in which the linear model was better adjusted. Observing the graphs,it shows the greater germination response in the absence of light at all temperatures in which normal seedling formation existed. Several researchers report the importance of knowing the germinative behavior of seeds as a function of light and temperature for the study of their ecophysiology (Bewley *et al.,* 2012, Carvalho and Nakagawa, 2012). Based on knowledge of this nature, it is possible to make decisions, aiming at reducing or increasing the germination of the species under study, taking into account the specific interest. Rego *et al.* (2009) studied the effect of light on seeds of *Blepharocalyxsalicifolius* and they noticed that the germinative capacity of those seeds is indifferent to the light condition at 25 °C, similar to Alves *et al.* (2012) had observed in seeds of *Clitoriafairchildiana*. Both those researchers agree that this mechanism is important for spatial distribution, in other words, they are capable of developing in the most varied environments or stages of vegetation, from open fields to developed sub-forests. Yamashita *et al.* (2010) studied two species of weeds of the Conizagenus,they are*C. canadensis* and *C. Bonariensis*. Both species are stimulated to germinate in the presence of light at 25 ° C.

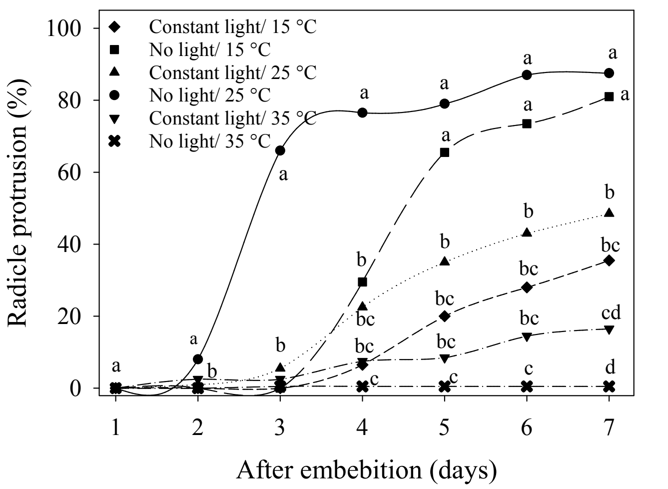

Figure 2. Accumulated daily germination of crambe seeds, considering a protrusion with 2 mm of length, submitted to different temperatures and light conditions

In the absence of light both species remain dormant. This is also used as a survival strategy as reported by Socolowisk *et al.* (2010) in seeds of *Cereus pernambucensis*. Positive photoblastic activity has also been reported in seeds of*Digitariaciliaris* (Mondo *et al.,* 2010). The mentioned authors affirm that the adhesion of the use of the no-tillage system in the agricultural crops can aid in the control of weeds with positive photoblast, considering that the vegetal cover does not allow the light to reach the seed to trigger the germinative process. In the crambeseeds a greater stimulation of germination was observed in absence of light at 25 °C. The requirement of light is the main reason why seed germination is restricted to the proximity of the soil surface (Toledo *et al.,* 1993), and temperatures near the soil surface can vary greatly among environments (Reginato *et al.* 2013). In order to understand the potential of this crop in its high oil content associated with the short cycle (Souza *et al.,* 2009; Carlsson, 2009), thegerminative knowledge allows us to infer that this species can be successful in agricultural crops by the No-tillage system. The sowing below the vegetation cover favors the germination due to the absence of light, tied to the soil temperature that remains constant and does not vary abruptly according to the incidence of sunlight. We also have considered the radicle protrusion of the seeds evaluated daily when submitted to the conditions of temperature and luminosity mentioned above, the highest germination speed is at 25 °C in the absence of light, followed by the temperature of 15 °C also in absence of light. However, the final germination (which is the radicle protrusion after seven days) did not differentiate statistically between them. The presence of light at both temperatures 15 and 25 °C induces low germination. According to Válio&Scarpa (2001) one of the main controlling factors of seed germination is light, inducing dormancy or promoting germination. In both conditions of luminosity when associated to 35 °C, there was a significant reduction of the germination (Figure 2). The effect of the temperature associated with luminosity is particular to each species and sometimes one of these factors predominates (Bergo *et al.,* 2010, Nogueira *et al.,* 2012).There was a harmful effect of high temperature in both conditions of light in crambe seeds.

Conclusion

The temperature of 25 °C and absence of light promotes greater germination and vigor incrambe seeds. The presence of light reduced seed germination and vigor at 15 and 25 °C. The temperature of 35 °C does not promote germination in this species.

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