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Influence of pre-germination treatments and temperature on the germination of crambe seeds (*Crambe abyssinica* Hochst)

*Influência de tratamentos pré-germinativos e de temperaturas na germinação de sementes de crambe (*Crambe abyssinica *Hochst)*

Lima Deleon Martins[1], *Felipe Pianna Costa*[2], *José Carlos Lopes*[3], *Wagner Nunes Rodrigues*[4]

ABSTRACT

The objective of this study was to evaluate the influence of pre-germination treatments and temperature on the germination of crambe seed (*Crambe abyssinica* Hochst). The completely randomized design was used in a 3 x 3 x 2 factorial (physical treatments x chemical treatments x temperature). The chemical treatments consisted of paper soaked in gibberellic acid (GA_3), potassium nitrate (KNO_3) and water. The physical treatments consisted of maintaining the seed intact, mechanical scarification and seed coat removal. Temperatures of 25 and 30 ºC were used in the completely randomized design. The variables studied were germination speed index, germination percentage, seed length and seedling dry mass. In the studied conditions, the percentage and rate of germination, length and seedling dry weight of seeds of *C. abyssinica* Hochst were enhanced by removal of the integument, in soaking with GA_3 at 25 ºC.

Key words: gibberellic acid, potassium nitrate, seed tegument removal, scarification and temperatures.

RESUMO

Objetivou-se com o presente trabalho, avaliar a influência de tratamentos pré-germinativos e de temperaturas na germinação de sementes de crambe (Crambe abyssinica *Hochst*)*, em esquema fatorial 3 x 3 x 2 (tratamentos químicos x tratamentos físicos x temperatura). Sendo os tratamentos químicos: ácido giberélico (GA_3), nitrato de potássio (KNO_3) e água. E os tratamentos físicos: semente intacta, escarificação mecânica e a retirada do tegumento. Foram utilizadas as temperaturas de 25 e 30 ºC em delineamento inteiramente casualizado. Estudou-se as seguintes variáveis: índice de velocidade e porcentagem de germinação, comprimento e massa seca das plântulas. Nas condições de estudo, a porcentagem e o índice de velocidade de germinação, o comprimento e a massa seca de plântulas das sementes de* C. abyssinica *Hochst foram incrementados com a remoção do tegumento, sob o umedecimento com GA_3 na temperatura de 25 ºC.*

Palavras-chave: *ácido giberélico, nitrato de potássio, remoção do tegumento, escarificação.*

Introduction

Currently there is a constant search for energy models based on sources of clean and renewable energy such as biodiesel. The oilseed crambe (*Crambe abyssinica* Hochst), with a grain yield between 1,500 and 1,900 kilograms per hectare and oil content up to 38%, stands out in this scenario due to its great quality for the production of biodiesel. The crambe seed has a spherical shape and is surrounded by a tegument structure called the pericarp (Ruas *et al*., 2010).

The presence of the pericarp, in some species, can cause irregular or no germination due to the impermeability of the seed coat to water and gases. Thus methods for breaking dormancy are important to monitor the viability of seeds (Alves *et al*., 2007).

[1] Engenheiro Agrônomo, Mestrando em Produção Vegetal, Dep. de Produção Vegetal, Universidade Federal do Espírito Santo, Centro de Ciências Agrárias, Alto Universitário s/n, Caixa Postal 16. 29500-000 Alegre, ES, Brasil.
[2] Engenheiro Agrônomo, Mestrando em Produção Vegetal, Dep. de Produção Vegetal, Universidade Federal do Espírito Santo, Centro de Ciências Agrárias, Alto Universitário s/n, Caixa Postal 16. 29500-000 Alegre, ES, Brasil.
[3] Engenheiro Agrônomo, D.Sc. em Ciências Biológicas, Dep. de Produção Vegetal, Universidade Federal do Espírito Santo, Centro de Ciências Agrárias, Alto Universitário s/n, Caixa Postal 16, 29500-000 Alegre, ES, Brasil. E-mail: jcufe@bol.com.br.
[4] Engenheiro Agrônomo, M. Sc. Produção Vegetal, Dep. de Produção Vegetal, Universidade Federal do Espírito Santo, Centro de Ciências Agrárias, Alto Universitário s/n, Caixa Postal 16. 29500-000 Alegre, ES, Brasil.

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The impermeability can be eliminated, totally or partially, by the structural alteration of the pericarp with methods such as scarification with abrasive material (Alves *et al*., 2007); sectioning or removal of the seed coat (Costa, Martins & Lopes, 2010); electrical or pressure treatments (Nascimento, 1982); chemical treatment with sulfuric or hydrochloric acid (Pacheco *et al*., 2007), bases such as sodium hydroxide (Oliveira *et al*., 2006), salts such as potassium nitrate (Faron *et al*., 2004) or organic solvents such as ether, alcohol and acetone (Silva *et al*., 2011); immersion in water (Ruas *et al*., 2010); wet or dry heat (Dutra *et al*., 2007); and even storage (Costa *et al*., 2012).

Among the methods used to break the dormancy of the tegument, scarification is often used because it is simple, effective and has low cost (Costa, Martins & Lopes, 2010). However, this technique needs to be performed carefully to avoid damage to the vital tissues of the seed.

In addition to mechanical scarification, soaking seeds in gibberellic acid and potassium nitrate can also increase germination levels and make seedling emergence more uniform. Gibberellin is responsible for the induction of seed germination, and also for the elongation of the hypocotyl and stem (Taiz & Zeiger, 2004). Potassium nitrate can cause structural changes in the seed, decreasing the influence of the pericarp in water absorption, thereby increasing germination (Faron *et al*., 2004).

Germination can also be influenced by external factors, temperature being a major factor. Temperature variations affect the speed, rate and uniformity of germination; the optimum temperature is the one that enables the most efficient combination of speed and percentage germination (Marcos Filho, 2005).

The aim of the present study was to evaluate the influence of pre-germination treatments and temperatures on the germination of crambe (*C. abyssinica* Hochst).

Material and Methods

The experiment was conducted in the laboratory of technology and seed analysis, in the department of crop science of the Centro de Ciências Agrárias of the Universidade Federal do Espírito Santo (CCA-UFES). The seeds of crambe (*C. abyssinica* Hochst) used in this study were provided by the MS Foundation, located in Maracujá-MS, from a harvest in October, 2009, which were benefited by eliminating damaged and immature seeds. The water content of the seeds was maintained at 10%; seeds were packed and stored in a refrigerator (3 °C) before the experimental phase.

The chemical treatments were performed in paper soaked in gibberellic acid (GA_3), potassium nitrate (KNO_3) and water. The solution concentration used was 500 mg L^{-1} for GA_3, 0.2% for KNO_3 and distilled water as control.

The physical treatments consisted of: intact seeds as control; mechanically scarified seeds with manual rubbing on sandpaper nº 120-59B; and seeds without tegument whose removal was performed manually with the aid of forceps, without damage to the embryo of the seed.

The experiment studied temperatures of 25 and 30 ºC. The plates were kept in BOD type germination chambers, adjusted to 25 ºC and 30 ºC, equipped with fluorescent white and cold lights, with 8-16 hours of photoperiod (light-dark) (Brazil, 2009). Each replication contained 25 seeds for each treatment, distributed in Petri dishes with diameter of 11 cm, covered with filter paper with specific weight of 80 g m^{-1} and porosity of 3 µ, moistened with 2.5 times the equivalent weight substrate.

The experimental design was completely randomized with 18 treatments and 4 replications, in a factorial scheme 3 x 3 x 2 (chemical treatment x physical treatment x temperature). The verification of the proportion of germinated seeds (%) was performed daily for 12 days, germination being considered effective when primary root protrusions of at least 2 mm length were observed. During the same period, daily counts were performed to determine the germination speed index (GSI), according to Maguire (1962). After 12 days, the length (SL-cm) and dry mass (SDM-g $plant^{-1}$) of the normal seedlings were measured. An engineer's scale was used to measure the hypocotyl and determine seedling length. To obtain the dry mass the seedlings were placed in paper bags and dried in an oven at 70 ºC, and after 48 hours weighed in an analytical balance.

The data were subjected to variance analysis ($p \leq 0.05$) using the statistical program SISVAR 4.0 (Ferreira, 2008), and the means were compared using the Tukey test ($p \leq 0.05$).

Results and Discussion

Figure 1 shows the means of germination percentage, germination speed index, seedling length and seedling dry weight obtained for seeds of *C. abyssinica* Hochst influenced by physical treatments for each chemical treatment and temperature.

In general, tegument removal promoted an increase of germination speed, percentage of germination, seedling length and seedling dry weight. The effect of the coat removal was positive, causing higher means in all treatments to break dormancy, using each chemical agent and also for both temperatures (Figure 1).

Similar results were found by Ruas *et al.* (2010). The authors observed higher germination speed index in crambe seeds without tegument. However, according to Barros *et al.* (2009), tegument removal of seeds of *C. abyssinica* Hochst did not increase germination, which was similar to that of intact seeds.

This fact occurs because some species have seeds with diminished germination capacity due to the tegument being impermeable because of excessive presence of minerals or the presence of fatty substances (Popinigis, 1985). Thus, despite the fact that the pericarp of the seeds of *C. abyssinica* Hochst being permeable (Ruas *et al.*, 2010), its removal is beneficial because in addition to promoting better contact of the seed directly with water, oxygen and minerals, it can also allow the growth of the embryo without major physical impediments.

Figure 2 shows the means of germination percentage, germination speed index, seedling length and seedling dry weight obtained from seeds of *C. abyssinica* Hochst influenced by temperature, for each physical and chemical treatment.

Overall, for the variables studied, the temperature of 25 ºC caused higher means compared to 30 ºC, for all physical and chemical treatments used to break dormancy (Figure 2). except in the treatment using peeled seeds moistened with KNO_3, where the temperature of 30 ºC was more effective, producing higher seedling dry weight in comparison with 25 ºC (Figure 2D).

These results agree with those observed by Panno & Prior (2009), who found higher values for germination of *C. abyssinica* Hochst at 25 ºC.

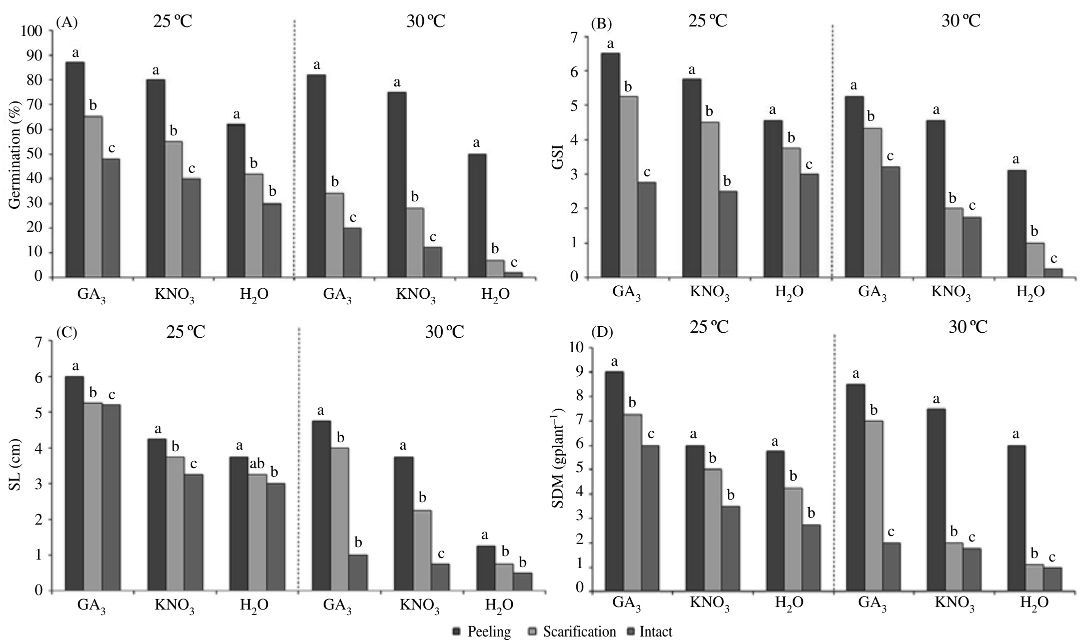

Figure 1. Means of germination percentage (A), germination speed index (B), seedling length (C) and seedling dry weight (D), obtained from seeds of *C. abyssinica* Hochst subjected to different temperatures and chemical methods for breaking dormancy, considering three physical methods (peeling, scarification and intact, respectively).
Means followed by the same letter for each physical treatment, within each chemical treatment and temperature, do not differ by the Tukey test at 5% probability.

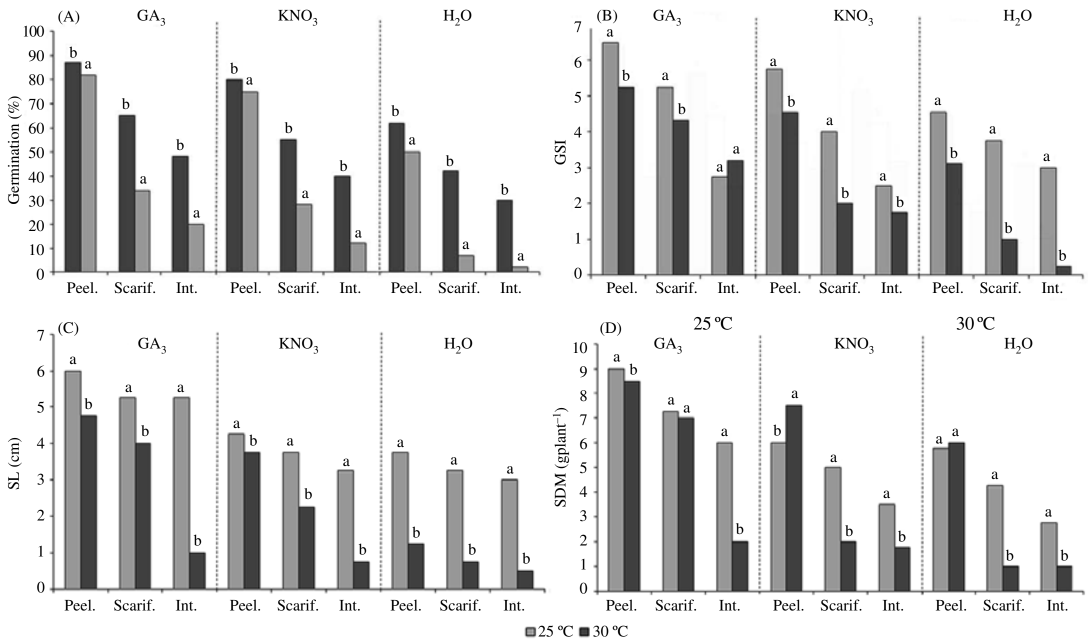

Figure 2. Means of germination percentage (A), germination speed index (B), seedling length (C) and seedling dry weight (D) obtained from seeds of *C. abyssinica* Hochst subjected to physical and chemical methods for breaking dormancy, considering two temperatures (25 and 30 ºC, respectively).
Means followed by the same letter for each temperature, within each chemical and physical treatment, do not differ by the Tukey test at 5% probability.

By contrast, Santos *et al*. (2009) found for the same species the best germination percentage at 30 ºC, but with low germination speed index. These results confirm those reported by Bewley & Black (1994), who claimed that the germination process, including germination speed, is affected by temperature.

Seeds have the ability to germinate in a certain temperature range, characteristic for each species. Lower temperatures generally prolong the germination period due to the decreased activity of enzymes involved in metabolism (Carmona & Martins, 2010). In contrast, excessively high temperatures cause a decrease in the supply of free amino acids, protein synthesis and anabolic reactions, which can denature proteins and change the permeability of membranes (Marcos Filho, 2005).

Studies on the influence of temperature on seed germination and early seedling development are essential to understand the biochemical and ecophysiological aspects of this process (Bewley & Black 1994).

Figure 3 shows the means of germination percentage, germination speed index, seedling length and seedling dry weight obtained from seeds of *C. abyssinica* Hochst influenced by chemical treatments for each physical treatment and temperature.

In the study of dormancy breaking of seeds of *C. abyssinica* Hochst with chemical agents, the presence of gibberellic acid (GA_3) in the wetting solution enhanced the germination, germination speed index, seedling length and seedling dry weight, regardless of the physical method employed in both temperatures (Figure 3). Overall, the order of significance was $GA_3 > KNO_3 > H_2O$. Except in a few treatments, as in the combination Intact/25 ºC for the variables SL and SDM (Figure 3C and 3D), and also for the combination Intact/30 ºC for GSI (Figure 3B) where the influence of wetting with GA_3 and KNO_3 were statistically similar.

This result can be explained by the fact that wetting of seeds with gibberellic acid used an endogenous enzymatic activator which affects the protein metabolism of the seed, stimulating the synthesis of enzymes such as alpha amylase, doubling the synthesis rate of proteins in seeds (McDonald & Khan, 1983) and facilitating the release of energy; this fact influences the resumption

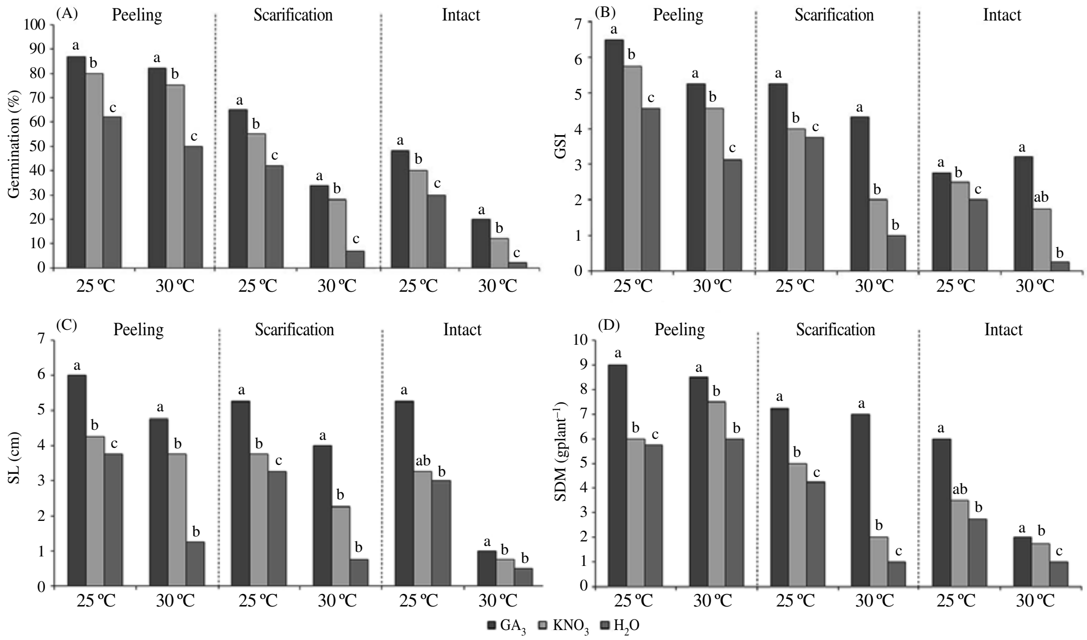

Figure 3. Means of germination percentage (A), germination speed index (B), seedling length (C) and seedling dry weight (D), obtained from seeds of *C. abyssinica* Hochst subjected to different temperatures and physical methods for breaking dormancy, considering three chemical treatments (GA_3, KNO_3 and H_2O, respectively).
Means followed by the same letter for each chemical treatment within each temperature and physical treatment do not differ by the Tukey test at 5% probability.

of embryo growth and subsequent germination (Taiz & Zeiger, 2004).

According to Ferreira *et al.* (2005), the use of biologically active chemicals such as gibberellic acid can overcome the effects of adverse factors in seed performance and can be used to stimulate the germination even in seeds with tegument.

Lopes & Souza (2008) and Ferreira *et al.* (2005) achieved effective results in germination of seeds treated with GA_3. According to these authors, the use of gibberellin in the germination phase can improve the vigor and germination of various species, especially under adverse conditions. This confirmation was also reported by Marcos Filho, Barzaghi & Komatsu (1987) for overcoming seed dormancy in *Helianthus annus*; this treatment was the most efficient in comparison with other tested methods such as soaking in KNO_3, pre-cooling and Ethrel.

For the variables SL and SDM (Figure 3C and 3D) there was effective influence of the treatment with GA_3. According to Taiz & Zeiger (2004), gibberellin promotes cell expansion and also stem and radicle elongation. This effect was observed in the seedlings of *C. abyssinica* Hochst used in this study, causing higher means for seedling length and dry matter, showing that probably the use of gibberellin on germination interferes in later seedling growth.

The wetting of the seeds of *C. abyssinica* Hochst with KNO_3 was not effective to break tegumentary dormancy in either the physical treatments or temperatures (Figure 3).

Although KNO_3 is used in laboratories to break dormancy of seeds, its mode of action is still debated. The use KNO_3 is recommended in species that have coats impermeable to gases, as it is believed that KNO_3, on contact with substances in the pericarp decreases the resistance of the coat and facilitates gas exchange (Frank & Nabinger, 1996).

In some seeds, moistening with KNO_3 seems to not work to break dormancy. This was confirmed by Gazziero *et al.* (1991), testing three concentrations of KNO_3 in seeds of *Sorghum halepense*. These authors reported that none of the tested concentrations of KNO_3 enhanced germination.

Conclusion

Under the studied conditions, the germination percentage, germination speed index, length and dry weight of seedlings of seeds of *C. abyssinica* Hochst were increased by the removal of the tegument under moistening with GA_3 at 25 ºC.

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