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Chemical and physical treatments can change the germination and seed vigor of crambe (C*rambe abyssinica* Hochst). A case study

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Abstract

Crambe is an oleaginous plant with a high oleic content and a high potential for biodiesel production, offering important alternatives for farmers. Its seeds have a tegument structure (pericarp), which limits the uniformity of germination, and consequently, the plant height. This study evaluated seed germination and vigour of crambe seeds under the influence of physical and chemical treatments. The treatments were arranged in a 3 x 3 factorial scheme (three physical treatments × three chemical treatments) with four replicates of 50 seeds each. Physical treatments were as follows: intact seeds (no scarification), mechanically scarified seeds and chemically scarified seed. The chemical treatments consisted of the application of gibberellic acid (GA_3 – 50% concentration), potassium nitrate (KNO_3 – 0.2% concentration) and water. The traits evaluated were germination (%), germination speed index (GSI), seedling number, normal seedling shape, and seedling length and dry mass. The number of seeds germinating over a period of seven days was evaluated daily, and germination was considered effective when a 2-mm long radicle was observed. The physical treatments, when associated with gibberellic acid, resulted in a greater germination potential of the seeds, a higher GSI and a higher index of normal seedlings. The application of gibberellic acid (50% concentration) for seed imbibition at $25^{o}C$ as pre-germination treatment promoted a higher percentage of crambe seed germination and increased the biometric parameters of the seedlings.

Keywords: Biodiesel, oleaginous, gibberellic acid; potassium nitrate; physical scarification; winter cultivation.

Introduction

Crambe (Crambe abyssinica Hochst) originates from the Mediterraneans and belongs to the family Brassicaceae (Carneiro et al., 2009). It has been used for a long time as a pasture fodder plant but has recently attracted considerable attention because of its high oil content (35-60%), which exceeds the soybean (maximum 24%) (Lima and Peluzio, 2015). With its relatively short cropping cycle of 90 days, it is suitable for cultivation in in the off-season period, mainly because of its drought tolerance (Pitol, 2008). Therefore, Crambe can be considered a viable alternative biodiesel production in the fall/winter period (Aquino et al., 2018). The oil from crambe seeds is widely used in the production of biodiesel, lubricants, pharmaceuticals, cosmetics and plastic films, among others. It shows high levels of erucic acid (a long-chain fatty acid), which has, however, negative impacts on human heart health. Therefore, crambe plays no role in the food oil industry (Colodetti et al., 2012). Crambe fruits have the form of spherical-shaped silica particles and are distributed throughout the plant's branches. Thus, the seeds have a tegument structure, the so-called "pericarp", which acts as a barrier against microorganisms, protects against shocks and physical damage and provides greater resistance to storage for long periods. However, this structure may hinder the germination process or even to delay germination (Baskin and Baskin, 2008). In addition to limiting water absorption, the pericarp also affects the disruption of the hypocotyl-root axis (Ruas et al., 2010). This structure promotes seed dormancy, which may alter the seed´s ability to respond to environmental factors (Santos et al., 2015), thereby impairing germination even under suitable external conditions. It is therefore crucial to provide farmers with methods that enhance the germination of crambe seeds, thereby increasing the area of cultivation and the productivity of this oilseed (Faria et al., 2014). In this way, dormancy-breaking methods are important for uniform germination (Alves et al., 2007), and the removal of the pericarp or even abrasive processes on this structure may facilitate the germination of crambe seeds (Ruas et al., 2010). In this context, this study aimed to evaluate the influences of chemical and physical treatments to break crambe seed dormancy and to increase seed vigour.

Results and Discussion

Germination (first count) and total germination

Seeds on substrate moistened with gibberellic acid, regardless of the physical treatment (intact seed, mechanical scarification or chemical scarification), resulted in greater germination (first count), with an increase of up to 42% for

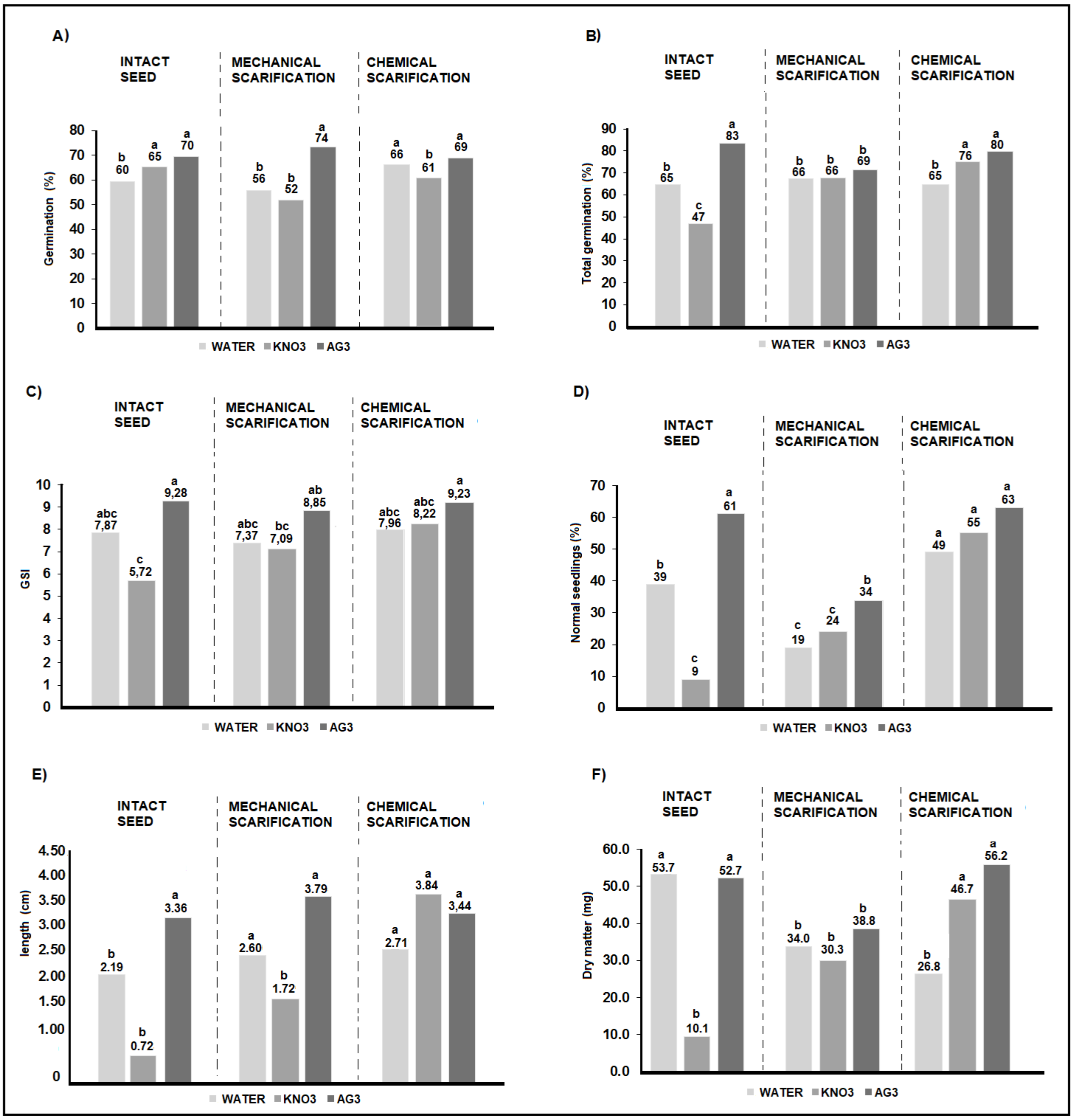

Fig 1. First germination count (A), percentage of total germination (B), germination speed index – GSI (C), normal seedlings % (D), seedling length (E), seedling dry matter (F), after the germination test of crambe seeds (*Crambe abyssinica* Hochst) submitted to pre-germinating tests under laboratory conditions. Means followed by the same letter in each chemical treatment do not differ by the Scott-Knott test at $p < 0.05$.

mechanical scarification, compared to KNO3 treatment and 17% compared to the control (water) (Fig. 1A).
In the chemical treatment with gibberellic acid (AG_3), we found above 69% germination of the seeds, compared to physical treatments. Gibberellic acid further promoted over 60% of normal seedlings from intact seeds and chemically scarified seeds.
In terms of the total germination parameters in intact seeds, AG_3 resulted in a 28% higher germination rate compared to the control (water) and a 77% higher rate compared to the treatment with KNO_3 (Fig. 1B). Mechanical scarification, independent of the chemical treatment, did not increase total germination. In contrast, chemical scarification along with chemical treatment (KNO_3 and AG_3) resulted in higher total germination, when compared to water treatment and mechanical scarification. However, chemical scarification led to the same results compared to the treatment of intact seeds with AG_3, indicating that scarification, whether mechanical or chemical, had no effect on seed germination, when compared to the treatment using moistening with gibberellic acid. Apart from this, seed scarification is a rather expensive technique. In terms of intact seeds, seedling

length (3.36 cm) and dry mass (52.7 mg) were higher when the seeds chemically treated with gibberellic acid, compared to the other chemical treatments.
These results are in agreement with those obtained by Cardoso et al. (2014) and Santos et al. (2013), who reported improvements in the germination of seeds of several species with the application of gibberellic acid.

Index of germination speed (IVG) and percentage of normal seedlings

In relation to the germination speed index (IVG), the treatment of mechanically-intact seeds with gibberellic acid showed the highest average (9.28) compared to those treated with water and potassium nitrate (5.72) with an increase of the IVG by 52% (Fig. 1C). The application of KNO3 in mechanically-intact seed reduced IVG by 27% compared to control (water) and 62% compared to AG_3 application. In the germination phase, gibberellic acid increases the speed of emergence and consequently promotes greater seed germination (Lopes et al., 2008).
Regarding the percentage of normal seedlings, we observed an increase of more than 500% using gibberellic acid treatment, compared to KNO_3. In mechanically-intact seeds, the index of plant normality was considerably higher in the treatment using gibberellic acid, compared with the treatment using only water, in which the increase was 56% for intact seeds (Fig. 1D). Chemical scarification resulted in the highest percentage of normal seedlings when the three solutions, namely water, KNO_3 and AG_3, were applied, leading to 49, 55 and 63% of normal seedlings, respectively (Fig. 1D). Beckmann-Cavalcante et al. (2012) also observed better results in the germination of juçara seeds after treatment with scarification, which was not verified in açai seeds.
Mechanical scarification with the application of water and KNO_3 and the use of mechanically-intact seeds with the application of KNO_3 application reduced the percentage of normal seedlings by 51, 38 and 77%, respectively (Fig 1D). The application of AG_3 to intact seeds provided the highest mean (61), with a 56% increase in normal seedlings, compared to the control (water). The results did not differ significantly from the treatment with additional chemical scarification (63). These results showed that the physical treatments were not efficient, compared to applying water or chemical treatments where the seeds were only moistened with AG^3. The physical scarification was detrimental to the development of the seedlings, resulting in the smallest means. For chemical scarification, we observed statistically similar results.
Similarly, Cardoso et al. (2014) observed that the pre-soaking of crambe seeds in gibberellic acid resulted in increased seed germination and vigour. Several authors have reported that the application of gibberellic acid breaks seed dormancy in a number of plant species (Santos et al., 2010; Silva et al., 2013).

Seedling length and dry mass

Application of gibberellic acid resulted in greater seedling heights (3.36 cm) and, consequently, 67 and 466% larger seedlings in comparison to the control In mechanically-intact seeds, (water - 2.19 cm) and the application of KNO_3 (0.72), respectively (Fig. 1E). In addition, we found no differences to the seeds submitted to scarification, either chemical or physical, except that mechanical scarification and the application of KNO_3 which presented the lowest mean values. Seeds submitted to chemical or physical scarification and moistening with either water, KNO_3 or AG_3 showed no statistically significant differences, but presented higher values than intact seeds treated with water (2.19 cm), with the exception of mechanically scarified seeds and seeds treated with KNO_3.
The application of KNO_3 to mechanically-intact seeds reduced seedling dry mass (10.1 mg) by 81% compared to the control (53.7 mg). Statistically similar results were obtained for mechanical scarification (plus application of water, KNO_3 and AG_3) and chemical scarification with water application. The highest average dry mass was obtained for the treatment with gibberellic acid on intact seeds (52.7 mg), for chemical scarification (56.2 mg) of physically-intact seeds treated with water (53.7 mg) and for chemical scarification of intact seeds treated with KNO_3 (46.7 mg). Thus, we verified that seed moistening with KNO_3 resulted in a lower germination rate and reduced biometric parameters such as normal seedling and seedling length and dry mass, compared with physical treatment and mechanical scarification.
Martins et al. (2012) observed that the treatment of *C. abyssinica* seeds with chemical agents such as gibberellic acid resulted in an increase in the following parameters: germination, germination speed index as well as seedling length and dry mass, irrespective of the physical method (mechanical scarification, seeds without integument and intact seeds) at both temperatures (25ºC and 30ºC), suggesting that the pre-germination treatments had a significant effect on the following order $GA_3 > KNO_3 > H_2O$. These results are in accordance with those obtained in the present work.

Materials and Methods

The experiment was conducted at the Fitotecnia Laboratory of the State University of Londrina (UEL, Londrina, Brazil). Crambe seeds (Crambe abyssinica Hochst), cultivar 'FMS Brilhante', were obtained from the Agronomic Institute of Paraná (IAPAR, Londrina, Brazil).

Experimental design and treatments

The experimental design was completely randomised with treatments arranged in a 3 x 3 factorial scheme (physical treatments x chemical treatments), with four replicates of 50 seeds each. We evaluated the following treatments: seed mechanically kept intact, mechanically scarified seeds, chemically scarified seeds, seeds treated with gibberellic acid (GA_3), seeds treated with potassium nitrate (KNO3) and seeds treated with water.

Scarification of seeds

Mechanical scarification of the seeds was carried out using a cylindrical glass container with a capacity of 100 g. The container was fitted with a sieve No. 120-59B, in which the seeds were introduced and stirred for one minute. Chemical scarification of the seeds was carried out with 50 seeds in a

50-mL glass beaker with 10 mL concentrated sulfuric acid (H_2SO_4) (98.08 g/mol), Sigma-Aldrich™. The seeds were immersed in the acid for a period of 30 seconds. Afterwards, the acid was drained with the aid of a fine-mesh stainless steel wire sieve and the seeds were rinsed under running water to neutralise the acid.

Chemical pre-germination treatments were constituted by moistening solutions. Two solutions were prepared, namely 0.2% potassium nitrate (KNO_3) and gibberellic acid (GA_3) in a concentration of 500 mg L-1. The control solution consisted of distilled water only. Subsequently, 50 seeds from each physical treatment were moistened with the respective solutions and distributed on two sheets of germitest germination paper inside plastic gearbox-type boxes (11.0 x 11.0 x 2.5 cm). The amount of the solution used for seed moistening was equivalent to 2.5 times the mass of dry paper (Brasil, 2009). After the application of the treatments, the seeds were kept in a germinator at 25°C (Brasil, 2009).

Characteristics assessed

The number of seeds germinated for seven days was evaluated daily and germination was considered effective when a ca. 2-mm long radicle was visible. The germination speed index (GSI) was calculated according to the formula described by Maguire (1962), and the percentage of total germination was determined. The first germination count was performed on the fourth day after sowing. The number of normal seedlings was determined on the seventh day according to Brazil (2009). Among the normal seedlings, 10 sub-samples were selected from each replicate to determine seedling length (cm). Subsequently, they were oven-dried at 60°C for 48 hours until constant weight and seedling dry mass (g) was evaluated.

Statistical analysis

The data were submitted to exploratory analysis, verifying the assumptions for analysis of the variance. Once the requirements were met, data were submitted to ANOVA (0.05%) and the means were compared by the Scott-Knott test ($P \leq 0.05$), using the SASM-Agri program (Canteri, 2001).

Conclusion

The application of gibberellic acid to crambe seeds increased total germination by 28%, compared to the control (water) and by 77% compared to treatment with potassium nitrate (KNO_3). Mechanical scarification, independent of the chemical treatment, did not result in increased germination. Mechanical scarification with the application of water or KNO_3 and the use of intact seeds with KNO_3 application reduced the percentage of normal seedlings by 51, 38 and 77%, respectively. In comparison to the treatment in which the seeds were only moistened with gibberellic acid, the physical treatments were not efficient, since physical scarification impeded seedling development. Similar results were found for chemical scarification. The application of KNO_3 to intact seeds reduced seedling dry mass by 81%. A similar result was found for mechanical scarification (plus application of water, KNO_3 and AG_3) and chemical scarification followed by the application of water. Seed wetting with gibberellic acid resulted in greater seedling heights compared to the control and application of KNO_3, but no differences were found to the seeds submitted to chemical or physical scarification. Based on our results, we recommend the use of gibberellic acid for the treatment of crambe seeds to improve germination and facilitate uniformity in the field, in addition to increasing the biometric parameters of the plants.

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