(DOI): 10.5935/PAeT.V7.N3.05

This article is presented in English with abstracts in Spanish and Portuguese

Brazilian Journal of Applied Technology for Agricultural Science, Guarapuava-PR, v.7, n.3, p.39-47, 2014

Scientific Paper

Test of electric conductivity on the assessment of physiological quality of crambe seeds(*Crambe abyssinica* Hochst)

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Abstract

The study aimed to assess present the effects of water volume and imbibition periods on the application of electric conductivity test on crambe seeds. We used crambe (*Crambe abyssinica* Hochst) seeds of 5 lots, FMS Brilhante cultivar, provided by the MS Foundation, from properties located in different locations of Mato Grosso do Sul State, produced on the 2012 harvest. We used randomized block designs with four replicates. For the characterization of the lots, we conducted germination tests and emergence speed index. Posteriorly, we performed the electric conductivity test, using 25ºC as imbibition temperature, four volumes of water (20mL, 30mL, 40mL and 50mL) and 5 imbibition periods (30, 60, 120, 240 e 360 minutes). We observed that the higher the volume the lower the quantity of electrolytes liberated by the seeds. The electric conductivity test was efficient in order to assess the vigor of crambe seeds. The volumes of 20 and 30mL, with imbibition period of 240 minutes, were the most favorable conditions in the conduction of our test.

Keywords: Vigor test, standardization, water volume, imbibition period.

Prueba de conductividad elétrica en la evaluación de la calidad de fisiológi de la semilla de crambe (*Crambe abyssinica* Hochst)

Resumen

Este estudio tuvo como objetivo evaluar los efectos de volumen de agua y los períodos de imbibición en la aplicación de la prueba de conductividad eléctrica en semilla de crambe. Se utilizaron semillas de 5 lotes de crambe (*Crambe abyssinica* Hochst) cultivar FMS brillante proporcionados por la Fundación MS, provenientes de las granjas ubicadas en diferentes lugares del Estado de Mato Grosso do Sul, producidas en 2012. Fue utilizado diseño de bloques al azar con cuatro repeticiones. Se hicieron prueba de germinación e índice de velocidad de emergencia para la caracterización de los lotes. Posteriormente se efectuó la conducción de la prueba de conductividad eléctrica usando 25 °C como temperatura de imbibición, cuatro volúmenes de agua (20 ml, 30 ml, 40 ml y 50 ml) y 5 tiempos de imbibición (30, 60, 120, 240 y 360 minutos). Se observó que cuanto mayor el volumen, menor será la cantidad de electrolito liberado por la semilla. La prueba de conductividad eléctrica fue eficiente para la evaluación del vigor de las semillas de crambe. Los volúmenes de 20 a 30 ml, con tiempo de imbibición de 240 minutos, fue la condición más favorable para la realización de esta prueba.

Palabras clave: Análisis de Vigor, normalización, volumen de agua, tiempo de imbibición.

Received at: 02/04/14 Accepted for publication: 30/11/14

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Teste de condutividade elétrica na avaliação da qualidade fisiológica de sementes de crambe (*Crambe abyssinica* Hochst)

Resumo

O presente estudo teve como objetivo avaliar os efeitos do volume de água e períodos de embebição na aplicação do teste de condutividade elétrica em sementes de crambe.. Foram utilizadas sementes de 5 lotes de crambe (Crambe abyssinica Hochst), cultivar FMS Brilhante fornecidos pela Fundação MS, oriundos de propriedades localizadas em diferentes locais do Estado de Mato Grosso do Sul, produzidas na safra 2012. Foi utilizando delineamento em blocos casualizados com quatro repetições. Foram feitos, teste de germinação e índice de velocidade de emergência para caracterização dos lotes. Posteriormente foi efetuada a condução do teste de condutividade elétrica utilizando 25ºC como temperatura de embebição, quatro volumes de água (20mL, 30mL, 40mL e 50mL) e 5 tempos de embebição (30, 60, 120, 240 e 360 minutos). Foi observado que quanto maior o volume, menor a quantidade de eletrólitos liberados pelas sementes. O teste de condutividade elétrica foi eficiente para avaliação do vigor de sementes de crambe. Os volumes de 20 e 30mL, com período de embebição de 240 minutos, foi a condição mais favorável a realização deste teste.

Palavras chave: Testes de vigor, padronização, volume de água, tempo de embebição.

Introduction

The biodiesel emerged as a promising substitute to fossil fuels, and/or a new option of renewable energy, for it is a clean and renewable energy with attractive characteristics, such as economic viability and social inclusion. The Brazilian government launched in 2004 the National Program for the Production and Use of Biodiesel (PNPB), which aims to implement a sustainable way of producing and using biodiesel (NAE, 2004).

Although it has progressed very efficiently, exceeding the initial goals and reaching 5% biodiesel in the petroleum diesel three years ahead of schedule, the Program still faces the strong dependency of a singular source of raw material, which is the soybean oil. It is crucial for the sustainability of the PNPB to identify the main bottlenecks of the chains of alternative oilseeds and work the solution of these obstacles.

In the Midwest region, where the biodiesel production is still incipient, there is no diversification of raw material sources, even though it is a region with elevated potential for alternatives oilseeds. Among the identified alternatives, we can cite the crambe (*Crambe abyssinica* Hochst), which is becoming a promising source of vegetal oil for biodiesel production. It belongs to the Crambe genus (Brassicaceae), with species known and distributed along four geographic regions: Macaronesia, Mediterranean, East Africa and Southeast Eurosiberia, and it is a species that has high oil level in its seed (approximately 35%), and presents good productivity on dry season, evidencing to be a great alternative for off season and winter (PITOL et al. 2010).

Considered as main factors for arousing interest of technicians, researchers and producers, are the good performance presented in experimental fields, low production cost, rusticity, drought resistance, not requiring specific machines or equipment for cultivation and the facility of extracting the oil, which eliminates the need of using solvent.

The productive chain of crambe finds itself in structuring phase in Brazil and little is known about the methods that assess the physiological qualitity of the seeds of this species. It is a necessity for companies and laboratories and research institutions to develop fast, easy and reliable methods to assess the viability of the seeds (CARVALHO et al., 2009).

There are several tests that assess the seed vigor, and one of those tests that stand out is the electric conductivity test. It is a fast and objective test that is being used successfully in programs of seed quality assessment, of various vegetal species (SOUZA et al., 2009).

The electric conductivity test is based on the indirect assessment of the organizational state of cellular membranes through the determination of the leachate amount liberated by the seeds in the imbibition solution (VIEIRA and KRZTZANOWSKY., 1999). In the seeds, the cellular membranes are the last structures to organize during the maturation process; however, they are the first to present signs of deterioration after the seeds reach the physiological stage of maturation.

For the developing and establishing of vegetal species in field on the most diverse environmental conditions, it is essential that seeds of guaranteed quality are produced, and that standard methods are set in order to compare the quality of the lots.

For crambe, there are no recommendations for the methodology of electric conductivity test, having the need for establishing procedures and obtain a standardization for the conduction of this test.

In this sense, the present study aimed to assess the effects of water volume and imbibition periods at the application of electric conductivity test in crambe seeds.

Methodology

The present study was developed in the seed laboratory of the Catholic University Dom Bosco, in Campo Grande, Mato Grosso (20° 23′ 11.47″, 54° 36′ 26.04″).

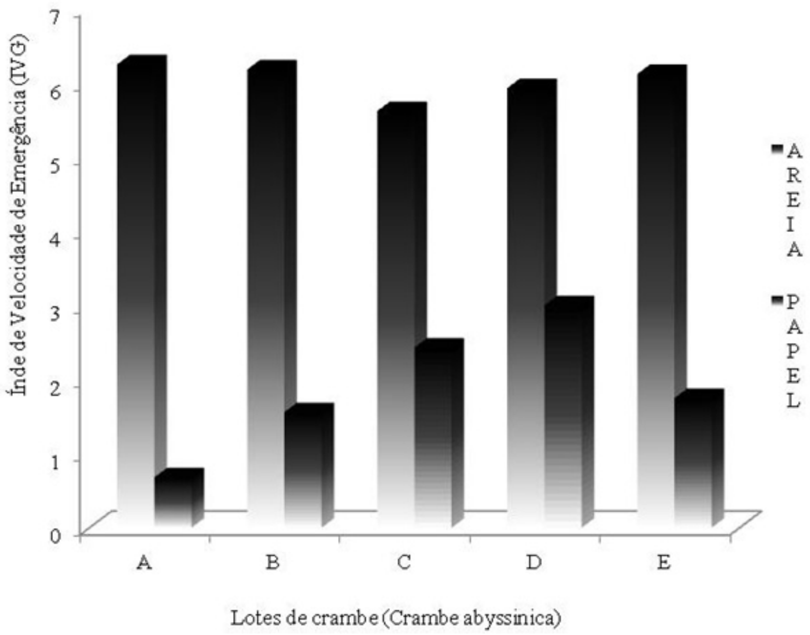
We used crambe seeds (*Crambe abyssinica*) of 5 lots, FMS Brilhante cultivar, provided by the MS Foundation, from properties located in different places of Mato Grosso do Sul, and produced in the crop of 2012.

Initially, we determined the humidity level of the seeds through the greenhouse method at 105+3°C for 24 hours (BRASIL, 1992), with 2 replicates of 10g of seeds, the results being expressed in percentage of humid weight.

The electric conductivity test was conducted after the characterization of the initial quality of the lots (germination test in sand, germination test in paper and first count of the germination test and emergence speed index – IVE).

At the conduction of the conductivity test we used only one temperature of imbibition, 25°C, 4 water volumes (20mL, 30mL, 40mL and 50mL) and 5 imbibition periods (30, 60, 120, 240 and 360 minutes).

The seeds were removed at random from the samples, discarding those with visible damage on the seed coat; subsequently, we weighed the seeds on an analytical scale with precision of 0.0001g. After, 50 seeds were placed in plastic cups for imbibition in distilled water. Passed the programmed time for the imbibition, we determined the electric conductivity of the solution with a conductivity meter, model Marconi CA 150. After the readings, we



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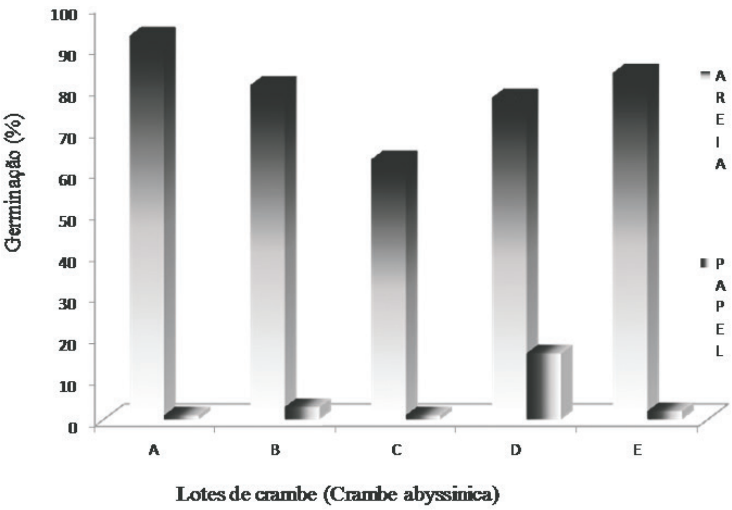
(vertical: Germination Speed Index (IVG), Sand, Paper | horizontal: Crambe lots (Crambe abyssinica))

Figure 1. Average values of the Germination Speed Index (IVG) for crambe seed lots. Campo Grande, MS, 2013.



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(vertical: Germination (%), Sand, Paper | horizontal: Crambe lots (Crambe abyssinica))

Figure 2. Average values of germination through the Standard Germination Test (TPG) on paper and sand for crambe seed lots. Campo Grande, MS, 2013.

conducted the calculation of the electric conductivity values per gram of seed placed for imbibition, and the results expressed in $\mu S\ cm^{-1}\ g^{-1}$ (VIEIRA and KRZYZANOWSKI., 1999).

We used the random block design with four replicates. We used 5 lots of crambe, 4 volumes of distilled water (20mL, 30 mL, 40mL and 50ml) and 5 imbibition periods (30, 60, 120, 240 and 360 minutes), distributed in factorial scheme.

After the obtaining of data, we conducted the variance analysis at 5% probability by the F test for all treatments. For the average of the lots, we applied the Tukey test at 5% probability and the averages of temperature, volume and interactions were assessed through the regression analysis.

Results and Discussion

Initially, we conducted the characterization of the lots used in this study, regarding the test of germination speed index – IVG (Figure 1) and the germination pattern test – TPG (Figure 2). When comparing the tests performed under different substrates, we observed that in the paper substrate the seed germination was inadequate.

In Figure 1, we can observe that all lots, when subjected to germination in sand substrate, presented elevated germination percentage and IVG.

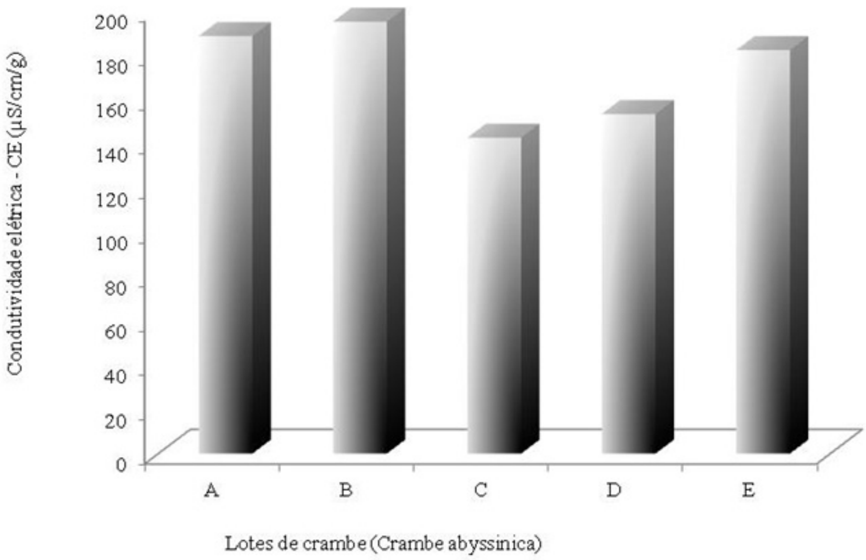
The C lot presented a germination percentage average inferior to the others, with an equivalent of 63% of germinated seeds (Figure 2).

Since for the conduction of this research we used seeds produced in the 2012 crop, the seeds belonging to lot C probably presented some level of dormancy, characteristic of the species, which can last from 6 months to 1 year, after the harvest.

In Figure 3, we can observe the difference between the average electric conductivity of the lots, being lots A, B and E with higher quantity of electrolytes liberated by the seeds during the imbibition, and lots C and D with lower quantity of liberated electrolytes, indicating to be these the most vigorous ones, when subjected to the electric conductivity test.

By comparing the standard germination test (Figure 2) and the electric conductivity test (Figure 3), lot C did not present elevated germination percentage, with an average of 63% of germinated seeds; when this lot was assessed by the electric conductivity test, comparing with the others, it was the one that presented lower quantity of liberated electrolytes in the imbibition solution. Such result suggests that the seeds belonging to lot C are younger and presented dormancy; however, it is nonetheless a vigorous lot, evidenced by the electric conductivity test.

The crambe seeds are harvested and stored



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(vertical: Electric conductivity – CE (µS/cm/g) | horizontal: Crambe lots (Crambe abyssinica)

Figure 3. Average values of electric conductivity for crambe seed lots assessed by the Tukey test. Campo Grande, MS, 2013.

with the fruit, factor that can favor the prolongation of the dormancy (TOKUMASU and KATO 1987). It is important to highlight that in this study all conducted tests were performed without removing the seeds from the fruits.

According to BOTELHO et al. (2010), the stage of development or maturation of the seed at the moment of harvest is one of the factors that can also influence the results of the electric conductivity.

It is possible to highlight that on the electric conductivity test, the lower the electrolyte liberation, the better the seed vigor will be and, consequently, the greater its emergence and germination potential will be.

DUTRA and VIEIRA (2006), in a study performed with electric conductivity of eggplant seeds, observed that, regardless the combination of water quantity and temperature used, the information provided by the electric conductivity test results did not confirm the results of the initial assessment, when it was determined the germination percentage and the vigor through the tests of the first count, percentage and emergence speed of the seedlings, corroborating with the results observed by this study.

Figure 4 describes the outspread of the interaction between the imbibition period X water volume for the five crambe seed lots assessed in this study. For all assessed volumes we verified quadratic responses. We observed an elevated quantity of liberated electrolytes in solution, until the period of 240 minutes of imbibition, with possible stabilization on the liberation from this period, regardless the water volume tested. MAGRO et al. (2011), using the electric conductivity test for assessing the physiological potential of broccoli seeds, observed that the test proved to be efficient in detecting the differences regarding the physiological potential in the periods of 60 and 120 minutes of imbibition, and concluded that only in these two readings differences were found among the tested lots in the essay.

When the seeds were subjected to the volumes of 20 and 30 mL of distilled water, we observed that in the imbibition period of 60 minutes there was a reduction on the quantity of liberated electrolytes in the solution, and in the following periods we found an increase of liberated electrolytes, until the period of 240 minutes. For the volumes of 40 and 50 mL, the decrease on the quantity of electrolytes only occurred after 240 minutes of imbibition.

In a previous study, which served as a pilot

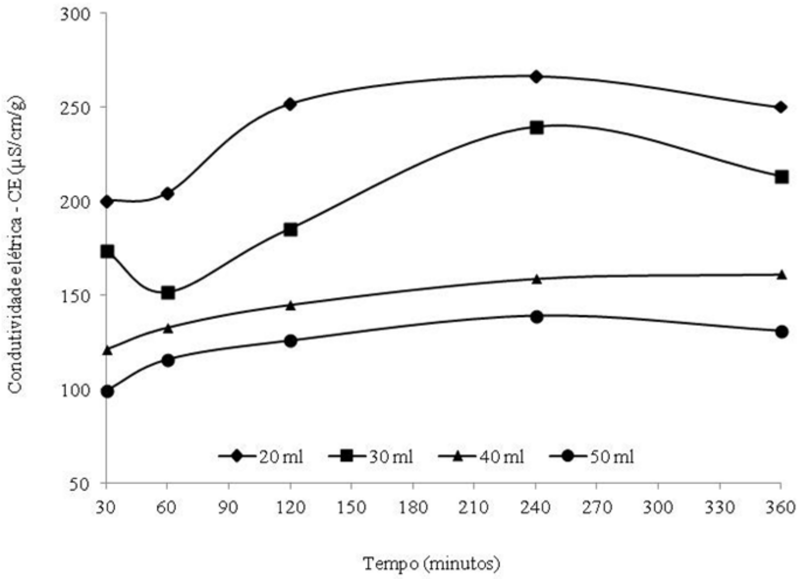

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Figure 4. Graphic representation of the regression equations calculated for the outspread of period X imbibition volume at the assessment of electric conductivity in crambe seeds. Campo Grande, MS, 2013.

Water volume	Equation	R^2
20	$y = -0.0016x^2 + 0.7765x + 173.65$	$R^2 = 0.95$**
30	$y = -0.0011x^2 + 0.6291x + 136.98$	$R^2 = 0.76$**
40	$y = -0.0005x^2 + 0.3185x + 113.7$	$R^2 = 0.99$*
50	$y = -0.0008x^2 + 0.3889x + 91.325$	$R^2 = 0.97$**

(vertical: Electric conductivity – CE (µS/cm/g) | horizontal: Period (minutes))

to the present essay, we assessed in crambe seeds the interference of different imbibitions temperatures (15, 20, 25, 30, 35 ºC) and water volumes (75, 150 and 250 ml) in replicates containing 50 seeds, and we found a higher electrolyte concentration in the solution of lower water volume (75 mL). The temperature variation did not interfere on the electric conductivity in relation to the volumes, that is, in the tested volumes the temperature did not present alteration on the conductivity. We concluded that different volumes of imbibition solution alter the quantity of liberated electrolytes, but it was not efficient on the indication of vigorous lots for crambe seeds.

In relation to the imbibiton period of the seeds, we were able to verify that in the several combinations a progressive increase occurred on the conductivity readings, as we increased the imbibitions time.

ALVES et al. (2012), in studies of electric conductivity with eggplant seeds, using the time periods of 1, 2, 4, 6, 12, 18 and 24 hours of imbibitions concluded that using replicates of 25 seeds, 50 mL of water and the imbibitions period of 4 hours, the test can be conducted for the species.

According to SANTOS and ROSSETO (2013), the electric conductivity test, conducted with 25 crambe seeds immersed in 50 mL of water at 20ºC, classified the lots in different vigor levels, similarly to the results obtained on the classification of the same lots through the seedling emergence test in sand, and emergence speed index of seedlings in sand and field.

In cauliflower (MIRDAD et al., 2006), cabbage (DEMIR et al. 2008) and forage turnip (NERY et al. 2009), it was possible to classify the seed lots through the electric conductivity test, employing 50 immersed seeds in 75 mL of water at 20ºC, and thus, SANTOS and ROSSETO (2013) observed that the results obtained using 25 seeds, immersed in 50 mL, indicate the possibility of reduction in the water volume and amount of seeds at the conduction of the test.

VIDIGAL et al. (2008) observed that the

electrolyte release was considerably higher in pepper seeds, regardless of the amount of seeds (25 or 50), immersed in 25 mL of distilled water. The authors considered that, in reducing the water quantity in the imbibitions, there was a greater electrolyte concentration.

At the outspread of the interaction between water volume X imbibition period (Figure 5), we verified a linear response for the periods of 30, 240 and 360 minutes and quadratic response for the periods of 20, 60 and 120 minutes of imbibitions.

We observed that as the water concentration in the imbibition was increased, there was a reduction on the leachate release in the solution. These results are similar to the ones observed by LOEFFLER et al. (1988), who concluded that it occurs greater dilution of leachates in higher water volumes.

HAMPTON et al. (1994), assessing the water volume of imbibition and the seed amount of radish, found that the increase on water volume minimized the effect of the seed amount, that is, when they worked with higher water volume (250 mL), the electric conductivity did not suffer interference from the seed amount (50 and 100), while in lower water volume (125 mL), there was a significant variation in function of the seed amount employed in this test.

RODO et al. (1998), assessing tomato seeds, observed that starting from 4 hours of imbibitions, the electric conductivity maintained the same tendency, which indicates the possibility of conducting the readings from the period of 4 hours, a results similar to the one obtained in this study, which can be confirmed by other authors, such as GILVELBERG et al. (1984) and GUIMARÃES et al. (1993), who, seeking the standardization of the electric conductivity test in small seeds of several vegetable species, obtained significant leaching in the periods of 3 and 4 hours of imbibitions.

We observed that the initial electrolyte

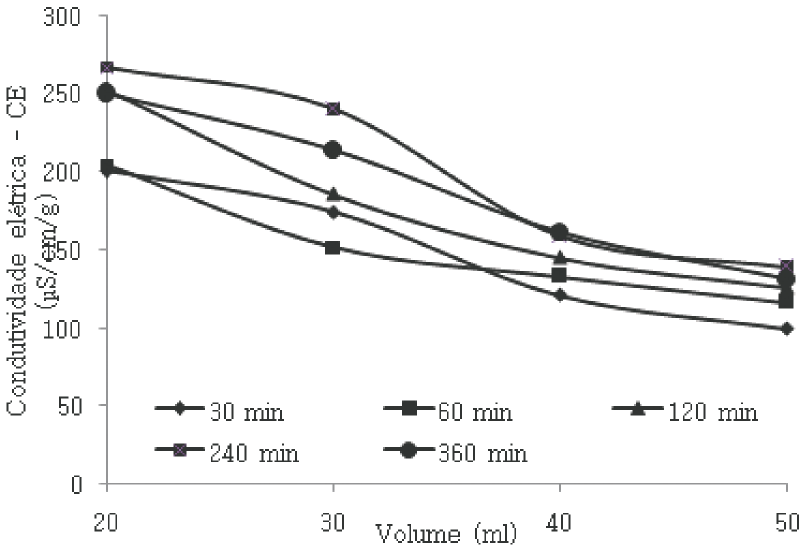

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Figure 5. Graphic representation of the regression equations calculated for the outspread between volume and imbibition period at the assessment of electric conductivity in crambe seeds. Campo Grande, MS, 2013.

	Equation	R2
30	y = -3.5568x + 273.21w	R^2 = 0.97**
60	$y = 0.0895x^2 – 9.107x + 349.17$	R^2 = 0.99**
120	$y = 0.1181x^2 – 12.446x + 453.28$	R^2 = 0.99**
240	y = -4.63x + 363.2	R^2 = 0.94**
360	y = -4.092x + 332.27	R^2 = 0.99**

(vertical: electric conductivity – CE (µS/cm/g) | horizontal: Volume (mL))

liberation was intense, both by the intact seeds as by the seeds with some damage, making it difficult the identification of the lots in the initial imbibitions periods. With the course of the process, the quantity of leached electrolytes stabilized due to the reorganization of the seed membranes.

According to MARCOS FILHO (2005), the imbibition period for small seeds can be inferior to those used for species that present bigger seeds, such as cereals and legumes, because the solute release is higher during the beginning of the imbibition, declining as the reorganization of the cell membrane system occur.

SÁ (1999), in studies with tomato seeds, observed that 75% of the leachates obtained occur in the first six hours of imbibition. This fact occurred with crambe after 4 hours of imbibition.

Conclusion

Volumes of 20 or 30 mL in the imbibition period of 240 minutes (4 hours) are considered adequate for the realization of the electric conductivity test in crambe seeds.

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