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pH of exudate test in the physiological quality of crambe seeds

Teste do pH do exsudato para avaliação da qualidade fisiológica de sementes de crambe

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ABSTRACT

Crambe is a rapeseed with high oil content and can be used as a winter cover or as a source of raw material for the production of biodiesel, however espite the growing interest in the culture, research on the subject is still incipient, especially concerning the seed production and analysis technology. The purpose of this study is to evaluate the physiological quality of crambe seeds, 'FMS Brilhante' cultivar, by testing the pH of exudate. Five seed lots were submitted to the determination of water content and the tests of germination and vigor (first count, emergence and tetrazolium). In the conduction of pH exudate test, temperatures (25 and 30ºC), and periods of seed imbibition in water (15, 30 and 45 minutes) were tested. The experiment was conducted in a completely randomized manner, with four replicates, and the mean values were compared by the Tukey test at 5% probability; Pearson correlation between the pH of the exudate and initial tests was also made. Testing the pH of exudate is promising for separating lots of crambe seeds and the following combinations of 25°C/30 minutes or 30°C/45 minutes can be used.

Key words: ***Crambe abyssinica*** *Hochst, viability, vigor*.

RESUMO

O crambe é uma brássica com alto teor de óleo, podendo ser utilizada como cobertura de inverno ou fonte de matéria prima para a produção de biodiesel, porém, apesar do crescente interesse pela cultura, as pesquisas ainda são incipientes, principalmente quanto à tecnologia de produção e análise de sementes. O objetivo neste trabalho foi avaliar a qualidade fisiológica de sementes de crambe, cultivar 'FMS Brilhante', pelo teste do pH do exsudato. Para tanto, cinco lotes de sementes foram submetidos à determinação do teor de água e a testes de germinação e vigor (primeira contagem, emergência e tetrazólio). Na condução do teste do pH do exsudato, foram testadas as temperaturas constantes de 25 e 30ºC e períodos de embebição das sementes em água de 15, 30 e 45 minutos. O experimento foi realizado no delineamento inteiramente casualizado com quatro repetições e as médias foram comparadas pelo teste de Tukey a 5% de probabilidade; também foi feita a correlação de Pearson entre o pH do exsudato e as avaliações iniciais. O teste do pH do exsudato é promissor na separação de lotes de semente de crambe, podendo ser utilizadas as combinações 25ºC/30 minutos ou 30ºC/45 minutos.

Palavras-chave: ***Crambe abyssinica*** *Hochst, viabilidade, vigor.*

INTRODUCTION

Crambe (***Crambe abyssinica*** Hochst) is a native rapeseed of the Mediterranean that is cultivated in some tropical and subtropical regions (CARNEIRO et al., 2009). It is considered a rustic plant that, until recently, was only used as forage in crop rotation and soil cover (VARISCO & SIMONETTI, 2012). However, with the discovery of its high potential for vegetable oil production, research ended up directing its use as raw material for biodiesel (TRZECIAK et al., 2008), as the seeds present about 40% of oil (SOUZA et al., 2009). In addition, its cultivation is fully mechanized, using the same implements used in the cultivation of soybeans and corn, with only minor modifications (FERREIRA & SILVA, 2011).

Researches involving crambe, especially regarding the quality control of seeds, are critical to the use of the culture and are justified by the potential of the species and lack of information regarding the physiological quality with which its seeds are

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produced, especially in the state of Mato Grosso do Sul, where the production accounts for most of the national production (MASETTO et al., 2009). Only the germination test, most of the times, is not sufficient to detect differences in physiological potential of seeds, and the use of vigor testing becomes an important tool in seed analysis laboratories.

Until the moment, there is no universally accepted test to evaluate the vigor of seed of a determined species or a group of species. Some vigor tests are described in the literature for the crambe, such as first count (BRASIL, 2009), accelerated aging (CRUZ et al., 2013; AMARO et al., 2014) and controlled deterioration (SANTOS & ROSSETTO, 2013); others, however, still need to be studied. The pH of exudate test is a vigor test considered promising in several species, enabling the determination of seed quality, being based on the permeability of membranes and solute leaching. When the seed imbibes water the release of sugars, organic acids and ions, including H^+, takes place, which contributes to the acidification of the medium, resulting in a decrease in the pH of exudate, in which the most deteriorated seeds have a higher leaching and hence, exudates with more buffering capacity (PESKE & AMARAL, 1984).

Many indicators solutions can be used to measure the pH, one is phenolphthalein. The pH of the exudate with phenolphthalein test was used to determine the viability of soybean seeds by AMARAL & PESKE (1984). These authors considered as non-viable, seeds whose exudates presented weak pink color or colorless and concluded that 30 minutes of imbibition is the most efficient to estimate the germination of seeds.

Separating lots of soybean seeds, BARROS & MARCOS FILHO (1990) reported that test to find the pH of exudate is effective and in the determination of feasibility; on top of being fast, presents a high reliability. The same was observed in corn seeds (SANTANA et al., 1998; CABRERA & PESKE, 2002) and wheat (AMARAL & PESKE, 2000).

Based on the efficiency of the pH of exudate test in some species, this study aims to adjust its methodology to crambe seeds, allowing to safely and quickly identify the vigor levels of lots.

MATERIALS AND METHODS

The experiment was conducted at the Seed Laboratório de Tecnologia da Universidade Federal de Mato Grosso do Sul, Campus of Chapadão do Sul using five lots of crambe seeds, 'FMS Brilhante' cultivar, subject to the determination of water content, germination and vigor for the initial lots characterization.

Determination of water content was performed using the oven method at 105±3°C for 24h, using two replicates with approximately 4g per lot (BRASIL, 2009). For the germination test, four replicates of 100 seeds distributed in gerboxes on two sheets of germitest paper moistened with distilled water equivalent to 2.5 times the mass of the non-hydrated substrate. Boxes were kept in a germinator set at 20-30°C and the evaluations were made in the fourth and seventh days, used as primary root issue with 2mm in length. Together with germination it was also performed the first germination count test, which consisted in recording the percentage of normal seedlings obtained on the fourth day after the test installation (BRASIL, 2009).

In emergence test, the seeds were sown in polystyrene trays with individual cells, containing the commercial substrate Plantmax®, kept in a greenhouse with an automatic irrigation system. For each treatment, four replicates of 25 seeds were used, and the percentage of normal seedlings on the tenth day after planting was recorded.

Tetrazolium test was carried out with two replicates of 50 seeds preconditioned in germitest paper moistened with distilled water for 16h at temperature of 25°C; and after this period, the seeds were immersed in a 0,075% solution of 2,3,5 triphenyl tetrazolium chloride for 8h at 41°C in the dark. Subsequently, they were washed in running water and assessed individually and internally, verifying the intensity of color, and classifying it as viable (red color) and non-viable (colorless), according to the recommendation for ***Brassica*** spp. (BRASIL, 2009), with the result expressed as a percentage.

To conduct the test to find the pH of exudate two temperatures were tested (25 and 30ºC), and three periods of seed imbibition (15, 30 and 45 minutes). Seeds were individually distributed in plastic trays and divided into unit cells submerged in 2mL of distilled water, and subsequently subjected to the respective soaking periods and temperatures. Then, it was added one drop of phenolphthalein solution (1g of phenolphthalein dissolved in 100mL of absolute ethanol and added to 100mL of distilled and boiled water) and a drop of a sodium carbonate solution (0,8g of sodium carbonate in 1000mL of distilled and boiled water) in each cell and the trays were agitated to promote mixing. The color change were observed in which pink color indicated the viable seed, while no color change indicated the no-viable seed being expressed in percentage. The experiment was

conducted in a completely randomized manner with four replicates and the mean values were compared by Tukey test at 5% probability; Pearson correlation was also made between the initial assessments and the pH of exudate test.

RESULTS AND DISCUSSION

Seed water content was studied for both groups, with a maximum change of 0,6 percentage points (Table 1). Standardization of the seed moisture content is essential for the standardization of evaluations of vigor and achieving consistent results, since influences the occurrence of different metabolic processes that the seeds may be submitted (MARCOS FILHO, 2005).

In the germination test (Table 1) there was no significant difference in the percentage of normal seedlings, emphasizing that all lots were with germination higher than 60%, the minimum value for the marketing crambe seed in Brazil (BRASIL, 2013). This is important since vigor tests should compare the performance of lots which follow the requirements established for commercialization and should have similar germination values (POWELL, 1986).

The tests of first count and tetrazolium ranked lots 1 and 2 as more vigorous and lots 3, 4 and 5 as less vigorous. Since the emergence test was more sensitive, lot 2 was identified as the more vigorous, lot 1 as intermediate vigor lots 3 and 4, and lot 5 as the less vigorous (Table 1). The vigor tests identify lots that are likely to have lower or higher performance; however, they did not indicate that the lower performance may also not present adequate development in the field. If the environmental conditions are favorable, all lots will have opportunity to present good results, providing the establishment of the appropriate population on the field (SILVA & VIEIRA, 2012).

The highest percentage of normal seedlings in the germination test when compared to the emergence test may be related to the greater availability of water, light and ideal temperature in the germinator during the germination test, ensuring a greater number of normal seedlings. When environmental conditions after sowing in the field deviate from the ideal conditions, it is expected that the percentage of seedling emergence is less than the germination determined in the laboratory.

The results obtained in the test for the pH of exudate found that, for all combinations of time and temperature tested, there was a separation of lots at different levels of vigor (Table 2). Overall, lot 2 showed the highest values in all combinations of pH of exudate testing (Table 2), except for 15'/25°C and 45'/25°C, standing out as the most vigorous; and lot 5 as the least vigorous. All other lots behaved differently depending on the time and temperature used in the test.

In temperature of 25ºC, for the period of 15 minutes, lot 1 was rated as the most vigorous along with lot 2; at 30 minutes there was a distinction between lots 1 and 2, and lot 1 was considered as intermediate vigor, matching statistically lots 3 and 4; with 45 minutes of soaking, it was not possible to separate lots 2, 3 and 4 in different levels of vigor (Table 2).

Similarly, at 30°C in the 15 minutes period, there was no difference of vigor between lots 1 and 2, or among batches 3, 4 and 5; already at 30 minutes, it was possible to separate lots 1 and 2 as the most vigorous, lots 3 and 4 as medium vigorous, and lot 5 as less vigorous; and, after 45 minutes of soaking, there was a classification of the lots into four levels of vigor, again being lot 2 considered as more vigorous and lot 5 as less vigorous (Table 2).

In table 3 it can be seen the Pearson correlation coefficients between the initial

Table 1 - Mean values for water content (WC), germination (G), first germination count (PCG), tetrazolium (TZ) and emergence (E) in five lots of crambe seeds.

Lots	WC	G	FGC	TZ	E
	----------%----------				
1	8.9	89 a*	83 a	96 a	67 b
2	8.8	90 a	85 a	94 a	87 a
3	8.5	84 a	75 b	92 b	69 b
4	9.1	85 a	70 b	90 b	74 b
5	8.7	86 a	68 b	90 b	48 c
CV (%)	-	6,6	7,0	5,1	8,3

*mean values followed by the same letter in the column do not differ from one another according to the Tukey test at 5% probability.

Table 2 - Mean values (%) obtained from the pH of exudate test in five lots of crambe seeds in the soaking periods of 15, 30 and 45 minutes at 25 and 30°C.

Lots	----------25ºC----------			----------30ºC----------		
	15'	30'	45'	15'	30'	45'
1	91 a*	61 b	65 b	69 ab	78 a	63 b
2	84 a	93 a	78 ab	81 a	88 a	81 a
3	49 bc	68 b	84 a	56 bc	55 b	57 bc
4	63 b	76 b	78 ab	58 bc	49 b	42 c
5	43 c	42 c	38 c	44 c	9 c	18 d
CV (%)	10.26	10.79	11.64	13.25	14.25	15.10

*mean values followed by the same letter in the column do not differ from one another according to Tukey test at 5% probability.

assessments of the lots and the soaking period and temperature combinations in the pH of exudate test. To be deemed efficient, a vigor test shall provide a classification of lots at different levels, in proportion to the seedling emergence, as this is considered a parameter to indicate the effectiveness of tests to evaluate the physiological potential of seeds. Thus, the combinations that showed a significant correlation with emergence were 30 minutes at 25°C and 45 minutes at 30ºC. There was also a correlation of pH of exudate test with germination in all periods of soaking tested at 30°C.

When evaluating rapid tests for ***Brassica juncea*** (L.) Coss. seeds, PUNIA et al. (2006) observed a significant correlation of the pH of exudate test with the emergence and germination when the seeds were subjected to the combination of a 25°C/30 minute soak, allowing to estimate quickly the viability of seeds. Similar results were found by PESKE & AMARAL (1984) and BARROS & MARCOS FILHO (1990) in soybean seeds and showed that the pH of exudate test, using the combination of 25°C/30 minutes was good in distinguishing viable from non-viable seeds.

In soybean and ***Guazuma ulmifolia*** Lam. seeds, SANTOS et al. (2011) and BARBOZA et al. (2014) reported that the pH of exudate test, at 25°C/30 minutes of soaking, was not effective in the separation of lots, differently from that observed by SANTANA et al. (1998) for maize and AMARAL & PESKE (1984) for soybean. The correlation between the emergence test and the pH of exudate test shows a factor of considerable importance, since the seedling emergence are exposed to culture conditions which will find in the field, suffering direct interference environment, differently from the lab tests than in certain cases may mask the results as they are made in conditions that are controlled and appropriate for the seeds.

CONCLUSION

The pH exudates test is promising in the separation by vigor of crambe seeds lots, 'FMS

Table 3 - Pearson correlation coefficient (r) between the first count of germination (FCG), germination (G), emergence (E) and tetrazolium (TZ) and the pH of exudate test in soaking periods of 15, 30 and 45 minutes at 25 and 30°C, in five lots of crambe seeds.

	----------25ºC----------			----------30ºC----------		
	15'	30'	45'	15'	30'	45'
	----------r----------					
FCG	0.84^{ns}	0.36^{ns}	-0.07^{ns}	0.78^{ns}	0.64^{ns}	0.60^{ns}
G	0.86^{ns}	0.58^{ns}	0.41^{ns}	0.92^{*}	0.92^{*}	0.92^{*}
E	0.62^{ns}	0.99^{*}	0.83^{ns}	0.86^{ns}	0.85^{ns}	0.88^{*}
TZ	0.85^{ns}	0.29^{ns}	0.24^{ns}	0.75^{ns}	0.81^{ns}	0.76^{ns}

*significant at the 5% probability; ns not significant.

Brilhante' cultivar, and the following combinations 25°C/30 min or 30°C/45 minutes can be used.

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