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Classification of West Indian gherkin seeds vigor by respiratory activity[1]

Classificação do vigor de sementes de maxixe por meio da atividade respiratória

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ABSTRACT - Seed companies increasingly need to obtain rapid and reliable results on seed quality. In this context, this study aimed at verifying the efficiency of CO_2 measurement for classifying West Indian gherkin (*Cucumis anguria* L.) seed vigor, using three seed lots of cv. "Do Norte" and three of cv. "Nordestino". In order to assess seed physiological quality, the following tests were performed: germination, seedling emergence, emergence speed index, shoot length, total dry matter, electrical conductivity and respiratory activity. The experimental design was completely randomized, with four replicates, and the means were compared by Tukey test at 5% probability level. Pearson's simple correlation was performed between the traditional vigor tests and the respiratory activity. The results of the germination and vigor tests allowed the West Indian gherkin seed lots to be classified into different levels of physiological quality. CO_2 measurement is a promising method to identify vigor differences among West Indian gherkin seed lots.

Key words: *Cucumis anguria* L.. CO_2 measurement. Physiological quality.

RESUMO - As empresas produtoras de sementes necessitam cada vez mais da obtenção de resultados rápidos e confiáveis quanto a qualidade das sementes. Nesse sentido, objetivou-se verificar a eficiência da medição do CO_2 na classificação do vigor de sementes de maxixe (*Cucumis anguria* L.). Para isso, utilizaram-se três lotes para cada uma das cultivares "Do Norte" e "Nordestino". Para a avaliação da qualidade fisiológica das sementes utilizou-se os testes de germinação, emergência, índice de velocidade de emergência, comprimento da parte aérea, matéria seca total, condutividade elétrica e atividade respiratória. O delineamento experimental foi o inteiramente casualizado, com quatro repetições, sendo as médias comparadas pelo teste de Tukey a 5% de probabilidade. Efetuou-se a correlação simples de Pearson entre os testes de vigor tradicionais e a atividade respiratória. Os resultados dos testes de germinação e vigor permitiram classificar os lotes de sementes de maxixe em diferentes níveis de qualidade fisiológica. A medição do CO_2 constitui-se em um método promissor para identificar diferenças de vigor entre lotes de sementes de maxixe.

Palavras-chave: *Cucumis anguria* L.. Medição de CO_2. Qualidade fisiológica.

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INTRODUCTION

West Indian gherkin (*Cucumis anguria* L.), Cucurbitaceae family, is a vegetable widely used in the traditional cooking of the North, Northeast and Mid-West regions of Brazil (OLIVEIRA *et al*., 2017). Besides Brazil, it is cultivated in countries such as Cuba, India, United States and massively in the African continent (YOON; CHUNG; THIRUVENGADAM, 2015). Although it is a species with high production potential and well adapted to adverse conditions, studies allowing its expansion are still scarce, especially related to the handling of its seeds (GUIMARÃES *et al*., 2008).

The germination test is the official method to determine physiological quality of seed lots. However, it is not always correlated with the result in the field, and its conduction may be time-consuming, thus creating the need to develop quick tests which are consistently correlated with the emergence of seedlings in the field. For *C. anguria* seeds, the germination test lasts 8 days (BRASIL, 2009), a relatively long period for decision-taking.

Seed vigor reflects the evaluation of several attributes, which determine the potential for fast and even emergence, resulting in a uniform stand of seedlings in the field under variable environmental conditions (ASSOCIATION OF OFFICIAL SEED ANALYSTS, 2009). Various tests have been developed aiming at fast and reliable results on seed quality. In this context, those that are highly correlated with field emergence stand out, such as electrical conductivity and respiratory activity tests, which are low-cost and easily replicated.

Seed respiratory activity results from oxidation of organic substances in the cell system with release of energy and CO_2. The main substrates of the respiratory process are carbohydrates such as starch and glucose, lipids, organic acids and proteins (MARENCO; LOPES, 2007). As they come into contact with water, seeds begin the soaking process, increasing respiratory activity and producing ATP. Hence, the quantity of CO_2 released from seeds is measured and then associated with vigor. Thus, high respiration rates are related to vigorous seeds, due to the large amount of carbohydrates and lipids stored in their cells, whereas less vigorous seeds exhibit reduced respiratory activity, due to the natural deterioration process, responsible for the oxidation of their reserves (MARCOS-FILHO, 2015).

Respiratory activity by the Pettenkofer method consists in measuring the amount of CO_2 released from the seeds in the respiration process, which is associated with seed vigor. It is an economic, quick and easily conducted test, as reported by Dode *et al*. (2012) in *Helianthus annuus*; Dode *et al*. (2013) in *Glycine max*; Dode *et al*. (2016) in *Triticum aestivum*; Oliveira *et al*. (2015) in *Citrulus vulgaris*; Torres *et al*. (2016) in *Eruca sativa* and Dutra *et al*. (2016) in *Capsicum chinense*, besides being highly correlated with traditional vigor tests conducted for these cultivated species.

Thus, adjusting the respiratory activity test for *C. anguria* seeds is of great importance to the crop, allowing lots with higher physiological quality to be selected and a fast decision-taking with respect to the utilization of the seeds.

Based on the above, this study aimed to verify the efficiency of CO_2 measurement on the classification of vigor in lots of *C. anguria* seeds.

MATERIAL AND METHODS

The experiment was conducted at the Laboratory of Seed Analysis and in a greenhouse at the Center of Agrarian Sciences of the Federal Rural University of the Semi-Arid Region (UFERSA). Six lots of *C. anguria* seeds were used, three of cv. "Do Norte" and three of cv. "Nordestino", which were purchased from national seed-producing companies in the 2014/2015 agricultural year. Along the experiments, seeds remained stored in a cold chamber (10 °C and 50% RH). Then, seeds from each lot were subjected to physiological quality evaluations, as described below.

Moisture content: conducted by the oven method, at 105 ± 3 °C for 24 hours, using two subsamples for each lot, with results expressed in percentage, wet basis (BRASIL, 2009).

Germination test (G): four replicates of 50 seeds per lot were sown on three sheets of Germitest® paper, initially moistened with a water volume equivalent to 2.5 times its dry weight, and germinated in Biochemical Oxygen Demand (B.O.D.) incubation chamber, at 20-30 °C under 8 h of light at the highest temperature. Evaluations were carried out 8 days after sowing, by counting normal seedlings (BRASIL, 2009).

Seedling emergence (E) and Emergence speed index (ESI): conducted in a greenhouse, with four replicates of 50 seeds per lot, which were sown on polystyrene trays with 200 cells, using coconut fiber as substrate (pH: 6.0; EC: 0.5 mS/cm). Seedling emergence was daily counted until 12 days, according to Maguire (1962). After this period, the percentage of normal seedlings was determined.

Seedling shoot length (SL) and total dry matter (TDM): after the counting to determine emergence, the shoots of seedlings within the useful area of each plot were measured using a ruler graduated in millimeters, and

then the seedlings were placed in paper bags and dried in a forced air circulation oven at 60 °C until constant weight, to obtain the total dry matter by weighing on a precision analytical scale (0.001 g). The results obtained for SL were expressed in cm and those for TDM in mg.

Electrical conductivity (EC): determined using four replicates of 50 seeds, which were weighed and soaked in plastic cups containing 50 mL of distilled water, maintained in B.O.D.-type incubation chamber at 25 ºC for four hours (TORRES *et al*., 1998). Reading in the soaking water was taken using a conductivity meter and the results were expressed in μS cm^{-1} g^{-1} of seeds.

Respiratory activity (RA): CO_2 release from seeds was determined through the Pettenkofer method by adapting the methodology described by Oliveira *et al.* (2015) for watermelon seeds, using four replicates of 5 g of *C. anguria* seeds per lot. Seeds of different lots were initially soaked in 50 mL of distilled water for 60 minutes, at room temperature of 25 °C. After this period, the seeds were placed in the Pettenkofer device for more 60 minutes and their respiration was measured (MENDES *et al*., 2009). Respiratory activity was calculated according the following equation: N x D x 22 (MÜLLER, 1964), in which: N = normality of the acid used (0.1 N HCl); D = difference between the HCl volume spent in Blank Titration and the HCl volume spent in Sample Titration; 22 = normality of CO_2, and the result was expressed in quantity of carbon dioxide released per gram of seed, per hour (μCO_2 g $seed^{-1}$ h^{-1}).

The experimental design was completely randomized with four replicates. The data were subjected to analysis of variance and means were compared by Tukey test ($P \leq 0.05$) using the program SISVAR® (FERREIRA, 2011). Pearson's simple correlation ($P \leq 0.05$) was performed between the traditional vigor tests and respiratory activity of the seeds.

RESULTS AND DISCUSSION

The moisture contents of the seed lots of the cultivars "Do Norte" and "Nordestino" were similar, ranging from 7.2 to 8.2% for the former and from 7.8 to 7.9% for the latter, respectively. Uniformization of moisture contents of different lots is an important factor to conduct the tests and is fundamental to standardize the evaluations and obtain consistent results, as emphasized by Kikuti; Marcos-Filho (2012).

The germination test demonstrated significant differences between the lots of *C. anguria* seeds, with variations from 62 to 92% for the cv. "Do Norte" and from 68 to 82% for the cv. "Nordestino", allowing them to be classified as of high and low germination. For the cv. "Do Norte", lots 2 and 3 were superior, while for "Nordestino" lots 5 and 6 showed better performance (Table 1).

Emergence and ESI tests allowed the lots of *C. anguria* seeds to be classified into different levels of vigor. For "Do Norte", lots 1 and 3 were those of lowest and highest vigor, respectively. For the cv. "Nordestino", lot 4 was considered as that of lowest vigor compared to the others. Unlike the germination test, conducted under favorable conditions of moisture, temperature and substrate, which may overestimate seed physiological quality, the emergence test evaluates the maximum potential of production of normal seedlings in the field, under often unfavorable conditions.

Table 1 - Germination (G), seedling emergence (E), emergence speed index (ESI), shoot length (SL), total dry matter (TDM), electrical conductivity (EC) and respiratory activity (RA) of lots of West Indian gherkin (*Cucumis anguria* L.) seeds for the cultivars "Do Norte" and "Nordestino"

Cultivar	Lot	G	E	ESI	SL (cm)	TDM (mg)	EC (dS m^{-1} g^{-1})	RA (μg CO_2 g seed/h^{-1})
		-------(%)-------						
	1	62 b	32 c	2.85 c	1.71 b	8.11 b	17.61 c	0.70 c
"Do Norte"	2	92 a	70 b	8.99 b	2.44 a	12.95 a	12.32 b	1.89 b
	3	92 a	90 a	14.09 a	2.52 a	13.05 a	2.58 a	3.08 a
CV (%)		3.69	2.83	11.76	6.55	7.2	9.93	13.5
	4	68 b	58 b	5.03 c	1.45 b	7.74 b	54.15 b	0.47 b
"Nordestino"	5	80 a	80 a	10.31 b	2.19 a	10.64 a	34.75 a	1.64 a
	6	82 a	80 a	12.26a	2.29 a	11.34 a	27.77 a	1.80 a
CV (%)		5.5	5.09	6.66	3.01	5.55	14.83	23.84

*Means followed by the same letter, in the column, do not differ by Tukey test ($P \leq 0.05$)

Shoot length and total dry matter showed similar results. Lot 3 of the cv. "Do Norte" was indicated as the one with best result for SL and TDM, although it did not differ statistically from lot 2. As observed for emergence and ESI, the parameters SL and TDM of the cv. "Nordestino" indicated lot 4 as the least vigorous, and lots 5 and 6 as statistically equal.

Greater translocation of seed reserves to embryo axis growth culminates in seedlings with greater shoot length, which are the most vigorous, as emphasized by Oliveira *et al*. (2015). Dode *et al*. (2012) point out that seedlings with higher values of length, fresh matter and dry matter, for the same species, indicate higher physiological quality of the seeds which originated them and, therefore, are considered as more vigorous, as observed for seeds from lots 2 and 3 ("Do Norte") and lots 5 and 6 ("Nordestino").

Table 2 - Pearson's correlation coefficients between respiratory activity and tests of germination (G), seedling emergence (E), emergence speed index (ESI), shoot length (SL), total dry matter (TDM) and electrical conductivity (EC) of lots of West Indian gherkin (*Cucumis anguria* L.) seeds, for the cultivars "Do Norte" and "Nordestino"

Tests	Pearson's correlation	
	"Do Norte"	"Nordestino"
G	0.83**	0.75**
E	0.94**	0.83**
ESI	0.94**	0.80**
SL	0.78**	0.88**
TDM	0.78**	0.80**
EC	-0.95**	-0.85**

**, significant at 0.01 probability level

The test of electrical conductivity obtained results similar to those of emergence in the field for lots of both cultivars. It was possible to stratify the lots of the cv. "Do Norte" into low, medium and high vigor, and lots 1 and 3 were the ones with lowest and highest vigor, respectively. Lots 5 and 6 ("Nordestino") were statistically similar and superior to lot 4, which has inferior vigor. Higher values of conductivity are related to reduction in membrane integrity, directly compromising seedling development because less vigorous seeds have poorly structured membranes and damaged cells, which is associated with the deterioration process (OLIVEIRA *et al*., 2015).

Based on respiratory activity, the lots were stratified into different levels of vigor. For lots of the cv. "Do Norte", the results were similar to those of emergence, ESI and EC, and the lots were classified as of high, medium and low vigor, confirming lots 1 and 3 as those with lowest and highest vigor, respectively. For the cv. "Nordestino", respiratory activity led to results similar to those of the other vigor tests conducted, classifying the lots as with high and low vigor, demonstrating the inferiority of lot 4 in comparison to the others. As observed in the present study for *C. anguria* seeds, Mendes *et al*. (2009), with soybean seeds, cv. 8000, found that respiratory activity measured in the Pettenkofer device is efficient in separating lots into different levels of vigor, and this method is an alternative to traditional vigor tests.

For both cultivars evaluated, respiratory activity was positively correlated with germination, emergence, ESI, SL and TDM, and was inversely proportional to the electrical conductivity test, because the higher the quantity of CO_2 released in cell respiration, the lower the quantity of leachates released into the soaking solution, which characterizes seeds of high vigor.

In lots of lower physiological quality, such as lot 1 ("Do Norte") and lot 4 ("Nordestino"), the speed of membrane reconstruction during the soaking process is reduced, resulting in lower respiratory rates due to the lower number and/or efficiency of mitochondria, leading to less vigorous seedlings, as evidenced by the correlation with the other vigor tests conducted (OLIVEIRA *et al*., 2015). Conversely, viable embryos have their mitochondria intact, whose respiratory activity increases with the beginning of the soaking process, which makes these organelles more efficient in the production of adenosine triphosphate (ATP), thus requiring greater consumption of oxygen and, consequently, increase in carbon dioxide production (BEWLEY *et al*., 2013). In this context, more vigorous lots of seeds exhibit higher respiratory rate, as evidenced for seeds from lot 3 ("Do Norte") and lots 5 and 6 ("Nordestino").

Thus, the high correlation observed between traditional vigor tests and respiratory activity test confirms the efficiency of the latter, and its use is a viable, quick and easily executed alternative to evaluate the physiological potential of West Indian gherkin seeds.

CONCLUSIONS

Respiratory activity measurement based on the CO_2 released is a promising test to identify differences in the vigor of lots of *C. anguria* seeds, cultivars "Do Norte" and "Nordestino".

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