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Controlled deterioration to evaluate okra seed vigor

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ABSTRACT

Germination and vigor tests are essential components of seed quality control programs adopted by seed industries. We studied different procedures to perform the controlled deterioration test to identify differences in vigor among okra seed lots. Four seed lots of Colhe Bem cultivar and five seed lots of Santa Cruz 47 cultivar were submitted to the following tests: germination, seedling emergence, controlled deterioration (seeds with moisture contents of 18, 21 and 24% at 45°C for 24 and 48 hours) and moisture content. The controlled deterioration test is efficient to evaluate the physiological potential of okra seeds, and the combination of 24% water, 45°C during 24 hours is recommended.

Keywords: *Abelmoschus esculentus*, physiological potential, vigor, germination.

RESUMO

Deterioração controlada para avaliação do vigor de sementes de quiabo

Os testes de germinação e de vigor são componentes essenciais do processo de controle de qualidade das empresas produtoras de sementes. Objetivou-se com essa pesquisa estudar a metodologia do teste de deterioração controlada, visando a identificação de diferentes níveis de vigor de lotes de sementes de quiabo. Utilizaram-se as cultivares Colhe Bem e Santa Cruz 47, representadas por quatro e cinco lotes de sementes, respectivamente. As nove amostras de sementes foram submetidas às avaliações de germinação, emergência de plântulas, deterioração controlada (sementes com umidade de 18, 21 e 24%; a 45ºC durante 24 e 48 horas) e grau de umidade no início dos testes e durante o monitoramento para o teste de deterioração controlada. O teste de deterioração controlada conduzido a 45ºC, com grau de umidade das sementes de 24% e período de exposição de 24 horas é eficiente para a avaliação do potencial fisiológico de sementes de quiabo.

Palavras-chave: *Abelmoschus esculentus*, potencial fisiológico, vigor, germinação.

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Okra (*Abelmoschus esculentus*), a vegetable fruit adapted to tropical and subtropical conditions, is widely cultivated in Brazil, especially by small farmers, which most grown cultivars are Colhe Bem and Santa Cruz 47 (Purquerio *et al*., 2010).

It is an important crop in the socioeconomic aspects and it is very appropriate to family farming, especially, for the large number of services spent on labor for harvesting, grading and packing, besides the early production and a relatively long period of harvest (Filgueira, 2008). It is an important species, considering the economic perspective; however, studies on okra are rare, mainly in seeds.

The use of high quality seeds provides the basis for increasing agricultural productivity, and the physiological features of the seed quality, which is the aim of a number of studies on other cultivated species, is poorly researched regarding to oleraceous (Tunes *et al*., 2011). Accordingly, Kikuti & Marcos Filho (2012) emphasize that vigor tests are important tools to complement the information obtained in the germination test, showing closer relationship with the performance of the seeds during storage and field conditions.

Among the vigor tests, the controlled deterioration test was initially developed to evaluate the vigor of the small seeds lots, like carrot, onion, lettuce and brassica (Powell & Matthews, 1981). The results of this test have shown excellent relationship with seedling emergence in field, for a large number of vegetable species such as onion, lettuce, turnip, Brussels sprout and carrot (Matthews, 1980).

Controlled deterioration is a technique based on aging test, incorporating better control of seed water content and temperature, during the period of aging (Krzyzanowski & Vieira, 1999). In this test, the initial seed water content is adjusted to the same content in all samples before beginning the controlled deterioration period, at relatively high temperatures (Hampton & TeKrony, 1995). According to Powell (1995), this test is more suitable for species with small seeds as vegetables and ornamentals, allowing the maintenance of seeds with uniform moisture content during all the artificial aging process.

The use of the controlled deterioration test in oleraceous was performed successfully in broccoli seeds (Mendonça *et al*., 2003), melon (Bhering *et al*., 2004), gherkin (Torres, 2005), sweet pepper (Basak *et al.,* 2006), radish (Marcos Filho & Kikuti, 2006), rocket (Goulart & Tillmann, 2007), beet (Silva & Vieira, 2010), cucumber (Lima

& Marcos Filho, 2011) and coriander (Torres *et al*., 2012).

However, although this test is directed to evaluate the physiological potential of vegetable seeds, no record in literature for its use for okra seeds is verified, thus indicating more study on the subject is recommended.

In this sense, the work aimed to study the methodology of controlled deterioration test, seeking to verify its efficiency in identifying different levels of vigor lots of okra seeds.

MATERIAL AND METHODS

The research was carried out in the laboratory of the Universidade Rural do Semi-Árido, in Mossoró, Rio Grande do Norte state, Brazil, from August 2011 to June 2012, using four okra seed lots of Colhe Bem cultivar and five lots of Santa Cruz 47 cultivar, from national companies, produced in crop year 2010/2011.

The samples of nine seed lots were homogenized, packed in multifoliated paper bags and stored in a controlled environment (15^{0}C and 45% relative humidity) during the experiment.

The lots were evaluated using the following tests and/or determination:

Germination – In aluminum trays (41x28x3 cm), four subsamples of 50 seeds of each lot were distributed in washed and sterilized sand, initially moistened with water equivalent to 60% of field capacity and incubated at 20-30^{0}C. The evaluations were performed at four and 21 days after sowing, with results expressed as percentage of germination (Brasil, 2009).

Seedling emergence – In the field, using four subsamples of 50 seeds per lot were distributed manually in seedbeds at 2 cm depth. The evaluation was carried out at 21 days after sowing; by counting the emerged seedlings, according to the criteria used for the aboveground part of seedlings in a germination test (Brasil, 2009). The results were expressed in average percentage of emergence for each lot.

Controlled deterioration – Initially, the seed moisture content was adjusted to different levels, 18%, 21% and 24%, by the humid atmosphere method (Rossetto *et al*., 1995), conducted in transparent plastic boxes (11x11x3.5 cm), using samples of 4 g of seeds, placed upon internal screen through the distribution of a single layer. These boxes, capped and containing 40 mL of water, were kept in the incubator, at 20^{0}C. During the artificial damping, the seed moisture content was monitored, through successive weighings, to obtain the desired values; on this occasion, each sample was placed in aluminum foil container, sealed, staying for five days in cold storage (8-10^{0}C) to reach hygroscopic balance. Then, the seeds were kept in a water bath, at 45^{0}C, during 24 and 48 hours. Later, the seed moisture content was determined using the greenhouse method at 105±3^{0}C (Brasil, 2009) and the germination test was installed, as previously described. The evaluation was performed four days after sowing, computing the average percentage of normal seedlings for each lot.

Seed water content (GU) – Determined by the greenhouse method (Brasil, 2009), using two replications of approximately 2 g of each lot, at 105±3ºC for 24 hours, with results expressed in percentage (wet basis), before and after the adjustment of GU for the controlled deterioration test.

The experimental design was completely randomized, with four replications of 50 seeds, separately for each test and cultivar. The data were subjected to the analysis of variance and the average were compared by the Tukey test ($p \leq 0.05$) (Banzatto & Kronka, 2008). The moisture content data were analyzed statistically. The simple correlation coefficients of Pearson (r) between the controlled deterioration and the seedling emergence in the field tests were also calculated, and the significance of r values were determined by the t test at 1 and 5% of probability.

RESULTS AND DISCUSSION

The germination of the lots of both cultivars was higher than the minimum established (≥80%) for marketing of okra seed. The differences in the physiological potential of seed of each cultivar were narrow and were not detected by the germination test (Table 1). According to Marcos Filho (1999), it is important to compare the seed lots with similar germination and, according to Powell (1986), situated preferably at Phase I of the viability loss curve. In this study, all the lots showed high germination, ranging between 87 and 96%, being situated at Phase I of the viability loss curve, characterized as

Table 1. Germination (G), germination first count (PC) and field seedling emergence of lots of okra (*Abelmoschus esculentus*) seeds, cv Colhe Bem and Santa Cruz 47 (germinação (G), primeira contagem de germinação (PC) e emergência de plântulas em campo de lotes de sementes de quiabo (*Abelmoschus esculentus*), cultivares Colhe Bem e Santa Cruz 47). Mossoró, UFERSA, 2012.

Cultivar	Lot	G (%)	PC (%)	E (%)
Colhe Bem	1	88 a[1]	9 b	72 b
	2	91 a	12 ab	72 b
	3	87 a	6 b	70 b
	4	91 a	17 a	80 a
CV (%)		6.82	20.23	6.79
Santa Cruz 47	5	93 a	35 a	87 a
	6	95 a	27 ab	75 b
	7	92 a	14 c	78 b
	8	96 a	28 ab	88 a
	9	96 a	13 c	76 b
CV (%)		4.83	9.7	7.35

[1]Means followed by same letter in the colums do not differ by the Tukey's test, p>0,05 (médias seguidas pela mesma letra na coluna não diferem entre si, pelo teste de Tukey p>0,05).

Table 2. Mean values obtained for the controlled deterioration test, after periods of priming okra (*Abelmoschus esculentus*) seeds, cv Colhe Bem and Santa Cruz 47, with different moisture contents (valores médios obtidos no teste de deterioração controlada, após os períodos de condicionamento de sementes de quiabo (*Abelmoschus esculentus*), cultivares Colhe Bem e Santa Cruz 47, com diferentes graus de umidade). Mossoró, UFERSA, 2012.

Cultivar	Lot	24 hours			48 hours		
		18%	21%	24%	18%	21%	24%
Colhe Bem	1	70 a	52 a	42 b	53 a	51 a	41 a
	2	69 a	53 a	44 b	45 ab	40 ab	34 b
	3	52 c	49 a	34 c	36 b	34 b	30 b
	4	60 b	54 a	55 a	39 b	38 b	32 b
CV (%)		5.14	5.84	7.48	11.54	12.92	7.19
Santa Cruz 47	5	60 b	59 b	58 a	50 b	48 b	46 a
	6	80 a	70 a	54 b	59 ab	49 b	41 ab
	7	79 a	72 a	47 c	64 a	58 a	45 a
	8	78 a	62 b	60 a	49 b	45 b	43 ab
	9	76 a	58 b	52 b	56 ab	53 ab	46 a
CV (%)		3.22	7.05	4.03	6.08	6.43	7.33

Means followed by same letter in the colums do not differ by the Tukey's test, p>0,05 (médias seguidas pela mesma letra na coluna não diferem entre si, pelo teste de Tukey p>0,05).

being relatively long and with few dead seeds.

The first count of the germination test was more sensitive than the germination test, showing differences among the lots for all cultivars (Table 1). In this evaluation, lot 4 of Colhe Bem cultivar was the most vigorous, however it did not differ statistically from lot 2 and, this lot 2 did not differ from lots 1 and 3. On the other hand, lot 5 of Santa Cruz 47 cultivar showed high vigor, but did not differ from lots 6 and 8 and, lots 7 and 9 were identified as having low physiological quality. This higher sensitivity of the first count of germination test for detecting differences among the seed lots was, also, confirmed by Bhering *et al*. (2000) and Torres *et al.* (2012) when they evaluated, respectively, different cucumber and coriander seed lots. According to Nakagawa (1999), the first count of germination test, many times, expresses better the differences of speed of germination among lots than the germination speed index. According to the same author, it is an important evaluation for identifying lots with ability for faster establish and be less labor intense than the speed of germination, besides being part of the germination test, not requiring special equipment.

The seedling emergence test confirmed the superiority in vigor of lots 4, 5 and 8 pointed out by the germination test and the evaluation of the first count of germination test; lots 1, 2, 3, 6, 7 and 9 were confirmed as low physiological potential (Table 1). According to Marcos Filho (1999), the seedling emergence test is an indicator of efficiency of the tests to evaluate the physiological potential of the seed lots.

The values for the initial moisture contents of okra seeds were similar for the nine studied lots, ranging from 11.0 to 12.7% ('Colhe Bem') and 11.2 to 13.3% ('Santa Cruz 47'). This variation is relatively small, indicating that no interference of moisture contents of the seeds on the results obtained in vigor tests was noticed. Silva & Vieira (2010) emphasizes that the use of seeds with a similar initial moisture degree is important in soaking procedure which must be performed before the controlled deterioration test, because it allows all lots to reach pre-set values in next period, making the beginning of phase of a water bath faster, besides being a fundamental prerequisite for obtaining reliable results. As for seed moisture content after the period of a water bath, the seed lots remained practically with the same moisture content degree, reached after the desired adjustments of 18, 21 and 24%, which variation was of 17.5 to 18.4%, 20.7 to 21.4% and 23.6 to 24.0%, respectively. Thus, the technique used for controlled deterioration was efficient, to maintain constant seed moisture content during the test and very close to those planned. According to TeKrony (2003), a percentage point of difference in moisture content among the seed lots may cause a great impact in germination after the controlled deterioration, mainly for lots of medium and low vigor.

This study showed that, generally, different combinations of water content and exposure period in water bath, at 45^{0}C, caused separation of the lots at different vigor levels (Table 2). Overall, the ranking of lots suffered variation

Table 3. Correlation between treatments of controlled deterioration (DC) and seedling emergence test of okra seeds (*Abelmoschus esculentus*), cv. Colhe Bem and Santa Cruz 47 (correlações entre os tratamentos de deterioração controlada (DC) e o teste de emergência de plântulas de sementes de quiabo (*Abelmoschus esculentus*), cultivares Colhe Bem e Santa Cruz 47). Mossoró, UFERSA, 2012.

Treatment		Seedling emergence	
Relative humidity	Time	Colhe Bem	Santa Cruz 47
18%	24 h	0.74*	0.79*
18%	48 h	0.65	0.78*
21%	24 h	0.87*	0.84*
21%	48 h	0.56	0.69*
24%	24 h	0.99**	0.99**
24%	48 h	0.71*	0.76*

*, **significant at 5 and 1% of probability, respectively, by test-t (*, **significativo a 5 e 1% de probabilidade, respectivamente, pelo teste t).

according to seed moisture content and the exposure period.

The combination with adjustment of moisture content for 24% for 24 hours in a water bath, at 45^{0}C, showed higher efficiency in the separation of the seed lots in different vigor levels by controlled deterioration test (Table 2). In this sense, lots 4, 5 and 8 were classified as high vigor and lots 3 and 7 as low physiological quality; lots 1, 2, 6 and 9 showed intermediate quality. For this combination, the ordering of lots was more consistent with the results of the tests used in the initial characterization of the lots for both cultivars (Table 1). As Mattheus (1980), the initial proposal for the controlled deterioration test suggests that the adjustment in moisture content is such that the 24 hours period allows the separation of small seed lots. In this sense, similar results, were also found by Basak *et al.* (2006), with sweet pepper and Silva & Vieira (2010), with beet, when using the combination 22%/24 h/45ºC, involving, thereby, 24-hour period. The same way, Panobianco & Marcos Filho (1998) with sweet pepper, Torres (2005) with gherkin and Torres *et al.* (2012) with coriander, obtained favorable results using controlled deterioration, with seed moisture content adjusted at 24%, at 45^{0}C for, also, 24 hours.

Generally, the 48 hours period of controlled deterioration provided little difference for the lots, as well as, caused a greater decrease in germination performance in the seed lots when compared to 24 hours controlled deterioration of the seed lots. This greater seed deterioration occurred, probably, due to greater exposure of the seeds to high temperature and high moisture content, causing, probably, changes which influence the synthesis of the proteins, nucleic acids and DNA metabolism (Vásquez *et al*., 1991). According to Basajavarajappa *et al*. (1991), changes in the respiratory process and in the functionality of the membranes can also occur, which the main cause is lipid peroxidation, with interference in germination.

For the correlation analysis (Table 3), the treatment which used the seed water content adjusted in 24% for 24 hours of deterioration under temperature at 45^{0}C was the one which showed higher simple correlation coefficients, for both cultivars, when compared to the results of the field seedling emergence test. This occurrence is in accordance with Matthews (1980) to inform that the controlled deterioration test shows excellent correlation with seedling emergence in field, for a great number of vegetable species. As Marcos Filho *et al*. (1984) point out, this similar variation trend between both variables does not mean that a corresponding quality of estimation accuracy of the lots is observed, according to the authors, the results of this analysis should not be interpreted separately. In this sense, Marcos Filho (1999) informs that the relationship between the results of the tests to evaluate the seed vigor in laboratory and field performance can often be incompatible due, probably, seedling emergence tests are not always appropriate to detect differences between the physiological potential of seed lots and, also, because of favorable environmental conditions during this test.

Through the analysis of the results, it was possible to identify that the controlled deterioration test, associated with the seedling emergence can be used with relative safety in the evaluation of okra seed vigor; however, the results can vary in case the environmental conditions are different from the ones used in this study. In this sense, it can be concluded that the controlled deterioration using the 24-hour period in a water bath, at 45^{0}C, with adjustment of seed moisture content for 24% demonstrates efficiency, and it should be considered in quality control programs of okra seeds.

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