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Physiological and phytosanitary potential of cucumber seeds

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Abstract: Cucumber is a very important crop produced and consumed in Brazil. Its seeds have high commercial value and deserve special attention due to their physiological and sanitary quality. Therefore, studies aiming at determining seed vigor should be intensified because the germination test used for assessing the quality of seeds often shows no correlation with field emergence. The present study was carried out to evaluate the physiological and sanitary qualities of cucumber seed lots. The study was conducted at the Laboratory of Seed Analysis and at the greenhouse of the Department of Plant Science, at the Universidade Federal de Pelotas (UFPel). Four lots of cucumber seeds, cultivar 'Aoidai', were tested for the following features: germination, first count germination, accelerated aging, electrical conductivity, cold test, sanitary condition, and seedling emergence. The experimental design was completely randomized with four replications. Means were compared by Tukey test at 5% probability. It was concluded that the accelerated aging test showed efficiency to evaluate the physiological quality of cucumber seeds. However, the cold test, with evaluations at 3 and 5 days, proved to be the most efficient for distinguishing differences in vigor levels between the seed lots.The incidence of fungi associated with seeds of cucumber do not affect the physiological quality of the lots.

Keywords: *Cucumis sativus* L., Vigor, Vegetable**.**

Potencial fisiológico e fitossanitário de sementes de pepino

Resumo: O pepino é uma cultura muito importante produzida e consumida no Brasil. Suas sementes têm alto valor comercial e merecem atenção especial devido à sua qualidade fisiológica e sanitária. Portanto, estudos com o objetivo de determinar o vigor das sementes devem ser intensificados porque o teste de germinação usado para avaliar a qualidade das sementes, geralmente, não mostra correlação com o emergência no campo. O presente estudo foi realizado para avaliar a qualidade fisiológica e sanitária de lotes de sementes de pepino. O estudo foi conduzido no Laboratório de Análise de Sementes e na estufa do Departamento de Ciência das Plantas, na Universidade Federal de Pelotas (UFPel). Quatro lotes de sementes de pepino, cultivar 'Aoidai' foram testadas para as seguintes características: germinação, primeira contagem de germinação, envelhecimento acelerado, condutividade elétrica, teste de frio, sanidade e emergência de plântulas. O delineamento experimental foi inteiramente casualizado com quatro repetições. Os dados foram comparados pelo teste de Tukey com probabilidade de 5%. Conclui-se que o teste de envelhecimento acelerado mostra eficiência para avaliar a qualidade fisiológica das sementes de pepino. No entanto, o teste frio, com avaliações em 3 e 5 dias, mostra-se mais eficiente para distinguir as diferenças nos níveis de vigor entre os lotes de sementes. A incidência de fungos associados a sementes de pepino não afetam negativamente a qualidade fisiológica dos lotes.

Palavras chave: *Cucumis sativus* L., Vigor**,** hortaliças.

Introduction

Seed vigor tests are necessary tools for determining seed physiological potential and subsequent seed quality assurance intended for commercialization (Marcos, 1999, 2015). Therefore, rapid and uniform emergence of vigorous seedlings of the cultivar is key events to ensure high plant performance that affects uniformity of plant development, yield and quality of the harvested product. These factors emphasize the importance of selecting high quality seed lots that are available in sufficient quantities to meet demand of the farmers (Marcos, 2015, Nascimento, Melo, 2015, Peske et al., 2015 & Peske et al., 2016).

The quality of information generated by vigor test depends on the proper method choice and depending on the intended goals. For example, the use of only one test may provide incomplete information. Thus, the predominant trend is the combination of results of several tests and these results taking into account the main objectives to be attained and the restrictions of each test (Marcos, Novembre, 2009 & Chiquito et al., 2012).

The evaluation of the seed physiological potential is an important component of seed quality control programs to ensure satisfactory performance of the seeds and there is a close relationship between this potential and seed sanitary quality. For some species, there are standard tests to evaluate the effect of seed vigor, for example, the accelerated aging for soybean and pea electric conductivity. However, further studies are needed on the use of vigor tests to assess the physiological and sanity of vegetable seeds. The commercial value of vegetable seeds is high (Pullano, 2013); especially hybrid seeds, in this sense, seed quality is particularly critical when new cultivars or hybrids are used, and high unit cost of the seed determines the need for the use of efficient technologies to maximize germination and seedling emergence (Nascimento & Melo, 2015).

Besides vigor tests, the sanity test performed by accredited laboratories can detect different pathogens associated with seeds and becomes important to decide the type of treatment and the product to be used (Nascimento & Melo, 2015).In addition, pathogens transmitted or not by seeds can also affect seed vigor in the field, and this effect will be pronounced when organisms colonize the internal tissues of the seeds (Carvalho & Nakagawa, 2000).

Cucumber crop is produced in significant quantities in Brazil and in many countries and the seeds have great commercial value, which reinforces the importance of assessing the physiological and sanitary quality. For some vegetable seeds of economic importance such as tomato, lettuce, cabbage and cucumber some vigor and sanitary seed studies are becoming available; however, more information on the vigor and sanitary conditions still needs additional research works. Therefore, the purpose of this study was to evaluate different vigor tests to determine physiological quality and evaluate the sanitary quality of cucumber seeds.

Material and methods

The work was conducted at the Seed Analysis Laboratory and in the greenhouse of the Federal University of Pelotas, located in Pelotas, Rio Grande do Sul. Four lots (number 34226/2013, valid for one and a half year) of cucumber seeds cultivar Aodai were subjected to following tests and assessments described below:

Determination of moisture content - Initial moisture content of the lots (IMC), moisture content after accelerated aging (MCA)**:** was conducted in an oven with forced air circulation at 105 ± 3 ºC for 24 h, in accordance to the Rules for Seed Analysis - RAS (Brasil, 2009), using two samples of 4 g seed for each lot. Results were expressed as mean percentage weight loss per lot. The moisture content of the seed was assessed before and after the accelerated aging test.

Germination test: was conducted at 20 °C with 200 seeds per seed lot (four replicates of 50 seeds). The seeds were distributed on two sheets of paper, previously moistened with distilled water in an amount equivalent to 2.5 times the mass of paper. Evaluations were conducted at four and eight days after sowing and the results expressed in percentage of normal seedlings (Brasil, 2009).

First count of germination: was made in conjunction with the germination test, by computing the percentage of normal seedlings on the fourth day after sowing (Brasil, 2009). Normal seedlings were the seedlings which possess all the essential structures such as the radicle (embryonic root), the hypocotyl (embryonic shoot),

and the cotyledons (seed leaves).

Electrical conductivity: was assessed with four replications of 50 seeds per seed lot. The seeds were soaked in 200 mL plastic cups containing 50 mL of deionized water. The readings were taken at 2, 4, 6, 8 and 24 hours. The conductivity value provided by the device was expressed in mS $cm^{-1}g^{-1}$.

Accelerated aging with NaCl solution: a total of 200 seeds (four replicates of 50 seeds) per seed lot were distributed over an aluminum screen placed inside a plastic box (gerbox) containing 40 mL of NaCl solution, maintained at 41 °C for 48 hours. This solution was prepared by adding 40g of NaCl in 100 mL of water, establishing an environment with relative humidity of 76%, according to the procedure proposed by Jianhua and Mcdonald (1997). After this period, the seeds were put to germinate following the methodology used in the germination test described above. The percentage of normal seedlings was assessed on the fourth day after sowing.

Seedling emergence in substrate: a total of 200 seeds (four replicates of 50 seeds) per seed lot were distributed in polystyrene trays with 200 cells filled with commercial substrate Plantmax®. Evaluations were performed at 13 days after sowing, computing the normal seedlings and the results expressed as percentage of normal seedlings emerged.

Seed health testing: A seed health testing was performed to determine the seed sanitary quality. A blotter test for detection of the fungi was used. Twelve sub samples of 25 seeds per seed lot were placed in plastic boxes containing three filter papers moistened with -0.5 MPa of osmotic potential of polyethylene glycol PEG 6000 (Villela et al., 1991). The seeds were incubated at 25 °C for seven days under a photoperiod of 12 hours light and 12 hours of dark.

After the incubation period the seeds were examined individually for fungal growth. Structures of the fungi present in surface of the seeds were observed using a stereoscopic microscope with 40X magnification at and when necessary, slides were mounted, then observed under an optical microscope. In addition, the identification of the genera of fungi was done based on characters referring to the Illustrated genera of Imperfect fungi (Barnett & Hunter 1998), Button (1980) and Dematiaceous Hyphomycetes (Ellis, 1971) and the incidence of pathogenic fungi was expressed as percentage seeds infected.

Cold test: a total of 200 seeds were used (four replicates of 50 seeds). The seeds were distributed on two sheets of paper, previously moistened with distilled water in an amount equivalent to 2.5 times the mass of paper. The germination rolls were placed in plastic bags and incubated at 10 °C, for seven days, after this period, the seeds were put to germinate at 20 ºC. The percentage of normal seedlings was assessed on the fourth, fifth and seventh days after sowing.

The data expressed in percentages were subjected to normality tests that indicated they did not need any transformation. The experimental design was completely randomized layout with four repetitions. In the statistical procedure, analysis of variance was performed separately for each test and the means of the lots were compared by Tukey test at 5% probability. The conductivity test was analyzed in a factorial of 4 lots x 5 seed imbibition periods

Results and discussion

The moisture content of seeds was very similar and it ranged from 8.9 to 9.3 before the execution of the tests to evaluate the seed physiological quality (Table 1). The low difference of moisture content between lots is important to guarantee the vigor test efficiency and according to Marcos (1999) the maximum range of two percentage points accepted for guarantee credibility of the tests.

Initially, the lots had similar germination rates and the first count of germination also showed the same result (Table 1). However, when the physiological quality of the cucumber seeds was evaluated by the accelerated aging, it was observed that only the lot 4 was found to be statistically inferior to the other lots. Thereby, seed lots with low vigor show higher decrease in its viability after exposure to artificial aging stress (Marcos, 1999), so that there is possibility of establishing differences in the physiological potential of different seed lots (Panobianco & Marcos, 2001).

Table 1 - Percentages for germination test (G), first count of germination (FCG), Initial moisture content of the lots (IMC), moisture content after accelerated aging (MCA), accelerated aging with NaCl-76% (AA), emergence of seedlings in substrate (ESS) of the different lots of cucumber seeds, cultivar Aodai.

Lots	IMC*	MCA*	G	FCG	AA	ESS
				----------%----------		
1	8.9	9.8	89 a	79 a	92 a	71 a
2	8.9	10.4	88 a	78 a	92 a	66 b
3	9.3	10.6	88 a	79 a	93 a	70 a
4	9.2	10.2	86 a	78 a	87 b	65 b
CV(%)			4.29	2.11	4.62	4.60

*Means followed by the same letter in a column do not differ in level of 5% probability by the Tukey test. According to Brasil (2009).

The accelerated aging test grouped the lots into two categories: physiological high (lots 1, 2 and 3) and lower quality (lot 4). Bhering et al. (2000) also using different tests to evaluate the effect of vigor in cucumber seeds obtained that accelerated aging test only separated the lower vigor seed from the others. However, for lettuce seed lots, this test proved to be the most sensitive to the stratification of the lots (Nascimento & Pereira, 2007). The accelerated aging test was more sensitive than germination to detect differences in seed vigor in rocket seeds (Vieira et al., 2015). Importantly, each test has its peculiarity in the differentiation of physiological quality of seeds, so it is necessary to use several tests to be able to detect small differences in vigor between lots.

According to Delouche and Baskin (1973), and Marcos (1999), the process of deterioration is progressive and it starts with the degeneration of membrane and concluding with loss germination. In this sense, the test most sensitive in assessing deterioration is one that identifies differences after seed physiological maturity. The damage caused to membrane through deterioration that provides increase in the leakage of solutes to the environment has been one of the main causes of the decline in the physiological quality of seeds. The electrical conductivity test is considered an important tool to evaluate the seed vigor, since it indirectly assesses the cell membrane degradation degree by determining the amount of electrolytes released in the seed soaking solution (Panobianco & Vieira, 2007).

For the electrical conductivity test (Table 2) it was found that there was no significant interaction between the periods of imbibition and cucumber seed lots and it was no possible rank of the lots using this test. With increasing time of imbibition, there was more leaching of exudates from the seeds and all lots had similar behavior during the evaluation periods. However, the electrical conductivity test was effective in the stratification of the seed lots of cauliflower, when the seeds were imbibed for 24 hours (Paiva et al., 2005). However, in this work the bulk electrical conductivity test did not seem to be suitable to evaluate the vigor of the seeds.

In the cold vigor test, the lots showed a scoring in two levels, higher and lower physiological potential (Table 3). For example, lots 2 and 4 showed lower seed vigor than lots 1 and 3, when the seeds were submitted to the exposition time for 3 days. The same result was found when seeds lots were submitted to cold stress for five days and which also verified by the emergence test. These tests proved to be the most efficient in distinguishing cucumber seed lots.

Table 2 - Electrical conductivity test with soaking 50 seeds in 50 mL of distilled water for periods of 2, 4, 6, 8 and 24 hours from four cucumber seed lots, cultivar Aodai.

Lots	Periods of imbibing (h)				
	2	4	6	8	24
1	14.71 a	18.04b	19.24bc	20.11c	24.04d
2	15.46 a	18.43b	19.37b	20.27b	24.28c
3	15.00 a	17.59b	18.78b	19.51c	23.25c
4	15.53 a	18.03b	19.48bc	20.17c	23.39d
CV(%)			10.8		

Means followed by same letter in lower case in the column do not differ by Tukey test at 5% probability.

Table 3 - Results of the cold test conducted in three periods of stress, in four lots of cucumber seeds, cultivar Aodai.

Lots	Periods of stress at 10 °C (days)		
	3	**5**	**7**
1	80 abA	82 aA	68 aB
2	67 cA	69 bA	70 aA
3	84 aA	77 aB	71 aB
4	75 bA	66 bB	60 bB
CV (%)		9.00	

Means followed by same letter in lower case in the column and capital letters on the line do not differ by Tukey test at 5% probability.

Miguel et al. (2001) concluded that use of the paper roll without soil for five days at 10 °C may be used as an additional procedure for evaluating seed cotton physiological quality. Similar results were obtained by Rodo, Tillman and Villela (1998) as the cold test was shown to be able to differentiate the quality of tomato seed lots, showing a high correlation with seedling emergence in the field. This indicates that the exposure time may vary depending on the species, it is important for the standardization of the methodology.

Abdo et al. (2005) reported that in germination test at low temperature, the germination on the fourth day after sowing was inadequate, since it was not possible to obtain normal seedlings during this period, with only primary root emission being observed. When evaluated on the eighth day, the test was not efficient for detecting lots with different levels of vigor.

The sanitary conditions of the lots, in general, occurs variable fungal species and 12 genera of fungi were identified in the four lots: *Aspergillus* spp., *Nigrospora* sp. *Rhizopus* sp., *Fusarium* spp., *Alternaria* sp., *Penicillium* sp., *Phoma* sp., *Pestalotia* sp., *Trichoderma* sp., *Curvularia* sp., *Cladosporium* sp. *Rhizoctonia* sp., and the last six genera showed low incidence (Table 4). On cucumber seeds, Nakada et al.

(2010) also observed the occurrence of *Aspergillus* sp., *Nigrospora* sp., *Fusarium* sp., *Penicillium* sp., *Phoma* sp. *Epicoccum* sp. and *Cladosporium* sp.

Table 4 - Incidence of fungi *Aspergillus* spp. (Asp), *Penicillium* sp. (Pen), *Rhizopus* sp. (Riz), *Nigrospora* sp. (Nig), *Fusarium* spp. (Fus), *Phoma* sp. (Pho), *Alternaria* sp. (Alt), *Pestalotia* sp. (Pes), *Cladosporium* sp. (Cla), *Curvularia* sp. (Cur), *Trichoderma* sp. (Tri), *Rhizoctonia* sp. (Rhiz) in four lots of cucumber seeds, cultivar Aodai.

Lots	Incidence of microorganisms (%)											
	Asp[1]	**Pen[1]**	**Riz[1]**	**Nig**	**Fus**	**Pho**	**Alt**	**Pes**	**Cla**	**Cur**	**Tri**	**Rhiz**
1	54 b	13 b	23 b	74 b	26 b	10b	1 b	6 b	0 a	0 a	0 a	0 a
2	5 a	16 b	52 c	0 a	26 b	2 a	4 c	0 a	3 b	13 b	6 b	0 a
3	99 c	0 a	2 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	2 b
4	47 b	43 c	3 a	0 a	0 a	5 ab	0 a	0 a	0 a	0 a	2 b	0 a

Means followed by the same letter in the column do not differ in level of 5% probability by the Tukey test. [1]Storage fungi.

Farrag and Moharam (2012) also found the incidence of many fungi in cucumber seeds tested such as *Alternaria alternata* (Fr.) Keissler*, Fusarium oxysporum* Schltdl.*, F. solani* (Mart.) Sacc.*, R. solani* J.G. Kühn*, Bipolaris oryzae* (Breda de Haan) Shoemaker. All these fungi reduced seeds variably and only *F. oxysporum,* caused a highly reduction in seed germination. *F. oxysporum,* and *F. solani*, are also transmitted from seeds to seedlings and caused pre- and post- emergence death. However, in the present study the fungi have been identified only to genera.

In order to increase the production of cucumber it is important that the seeds must be tested before sowing in the field. Further, it necessitates seed health and free from seed-borne diseases are constantly desired with the eradication of seed-borne inoculum through several seed treatments and seed health testing for presence of these seed-borne pathogens is an important step in the management of crop diseases (Forrag & Moharam, 2012).

Aspergillus spp., *Rhizopus* sp. and *Penicillium* sp. species were found associated with seeds of all lots. *Aspergillus* spp. and *Penicillium* sp. are the most fungi found in the seeds during storage, which may favor seeds deterioration (Freitas et al., 2000). The results showed a predominance of *Aspergillus* spp. in lot 1 (54%) and lot 3 (99%), To emphasize that these fungi do not compromise the physiological quality of seed lots 1 and 3 , the high rate of occurrence of *Aspergillus* spp. apparently did not affect seed germination and vigor of these lots since the lots were as the most vigorous.

The incidences of fungi associated with seeds of cucumber do not affect the physiological quality of the seed lots. However, the seed health testing was performed about 2 to 3 months after harvest, if they were evaluated after some period of storage, probably, the results could be different.

Although no harmful effects have been observed in the incidence of fungi on the physiological quality of cucumber seeds, it is important to highlight that certain fungi can be transmitted by seeds and it can be a source of inoculum for spreading diseases in the field. Therefore, seed treatment with fungicides can be an alternative advisable and necessary.

The results of the cold test, with assessment at 3 and 5 days were similar with those obtained for seedling emergence in a greenhouse. Thus, differences in seed quality lots were confirmed in both tests, which exhibit the best physiological quality, obtaining the highest percentages of emerged seedlings. The relationship between those laboratory tests and seedling emergence for classification of seed lots

in the vigor levels is important when working with seed vigor.

It is possible to suggest that stress during the period of three to five days as the most suitable for the separation of cucumber seed lots in vigor levels, with a similar percentage of germination. Moreover, the period of three days has the advantage that less time spent under stress, allowing faster quality evaluation and allowing assist in making decisions. At 7 days was observed that only the lot 4 was found to be inferior to the other lots.

Conclusion

The accelerated aging test showed efficiency in the separation of lots of cucumber seeds in vigor levels.

The cold test with assessment at 3 and 5 days proved to be the most efficient for distinguishing differences in vigor levels between the seed lots.

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