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Adequacy of tests to evaluate the physiological quality of seeds of different gherkin varieties

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ABSTRACT

Gherkin is a vegetable widely consumed in the North, Northeast and Midwest of Brazil. Recent studies highlight the crucial role of seed physiological characteristics not only in the initial establishment of the crop, but also throughout its production cycle. Given the lack of research on appropriate methodologies to evaluate the vigor of gherkin seeds, this study proposed to investigate different techniques for evaluating the physiological quality of Liso gherkin and northern gherkin seeds. To establish the appropriate tetrazolium test, varying times of soaking and concentration. Germination, accelerated aging, germination speed index and exudate pH were also evaluated. The results indicated that, in general, a concentration of 1% was satisfactory for identifying high vigor seeds through the tetrazolium test, with soaking times not having much influence. Additionally, the accelerated aging test proved to be efficient in identifying the Liso gherkin variety as more tolerant to adverse conditions, while in ideal situations, the northern gherkin stood out. The pH of the exudate was not efficient in separating the vigor of the varieties in the concentrations and times used.

Keywords: *Cucumis anguris L.* Tetrazolium. Accelerated aging.

Adequação de testes para avaliação da qualidade fisiológica de sementes de diferentes variedades de maxixe

RESUMO

O maxixe é uma hortaliça amplamente consumida nas regiões Norte, Nordeste e Centro-Oeste do Brasil. Estudos recentes destacam o papel crucial das características fisiológicas das sementes não apenas no estabelecimento inicial da cultura, mas também ao longo do seu ciclo produtivo. Diante da escassez de pesquisas sobre metodologias adequadas para avaliar o vigor das sementes de maxixe, este estudo se propôs a investigar diferentes técnicas de avaliação de qualidade fisiológica de sementes de maxixe liso e de maxixe do Norte. Para se estabelecer o teste de tetrazólio adequado, foram testados variados tempos de embebição e concentração. Avaliou-se também a germinação, o envelhecimento acelerado, índice de velocidade de germinação e o pH do exsudato. Os resultados indicaram que, no geral, concentração de 1% foi satisfatória para a identificação de sementes de alto vigor através do teste de tetrazólio, sendo que os tempos de embebição tiveram influência relevante. Adicionalmente, o teste de envelhecimento acelerado mostrou-se eficiente em identificar a variedade maxixe liso como mais tolerante a condições adversas, enquanto em situações ideais, o maxixe do norte se destacou. O pH do exsudato não foi eficiente em separar o vigor das variedades nas concentrações e tempos utilizados.

Palavras-chave: *Cucumis anguris L.* Tetrazólio. Envelhecimento acelerado.

INTRODUCTION

Originating from Africa, gherkin is a rustic plant, easy to grow, and resistant to many pests and diseases. Due to its prolonged fruiting period, it allows for staggered harvesting, which facilitates its large-scale consumption in the North and Northeast regions of Brazil, thus increasing its economic interest. Recent studies, such as the one by Da Silva et al. (2023), highlight the antioxidant properties of gherkin in combating free radicals, in addition to its lack of toxicity for animal organisms and its hypoglycemic and antimicrobial activities, enhancing its nutritional and medicinal value.

The growing demand for gherkin seeds in the market highlights the need for high-quality seeds for the effective establishment of this crop. In this context, Santos et al. (2023) emphasize the importance of obtaining seeds that meet these quality standards. Thus, it becomes essential to conduct quick tests that can assess the physiological potential of the seeds, aiming at their sowing, storage, and commercialization (COSTA et al., 2023; MARCOS-FILHO, 2015b; MARTINS et al., 2014).

Despite the importance of vegetables in general, little emphasis has been placed on evaluating the physiological quality of gherkin seeds. Research focused on comparing the efficiency of vigor tests in gherkin seeds is practically nonexistent (ALMEIDA et al., 2019), making it extremely important to study and define more appropriate methodologies and to verify if there is variation in the behavior of different varieties in tests of this segment.

Based on the scarcity of research on appropriate methodologies for gherkin seeds (*Cucumis anguria* L.), and the importance of understanding the physiological state of the seeds for plant development, this study aimed to analyze methodologies that evaluate the physiological quality of the seed, including tetrazolium test, accelerated aging, germination speed index, and exudate pH in two varieties of gherkin.

MATERIALS AND METHODS

The experiment was conducted in the laboratory using two varieties of gherkin, Liso and northern. The tests performed were: tetrazolium test, accelerated aging, relative humidity, germination, germination speed index (GSI), dry matter content, and exudate pH.

To adapt the methodology of the tetrazolium test, a 4x2 factorial analysis was used, where the first factor was considered a combination of the 1st and 2nd soaking times

for seed coat removal, and the second factor, different concentrations of the tetrazolium solution. The soaking times were: direct immersion in water for 30 or 60 minutes for seed coat removal and another 30 or 60 minutes for internal membrane removal, both at 40°C, generating the following time combinations: 30-30, 30-60, 60-30, and 60-60. For embryo staining, tetrazolium solution at 0.075% and 1% was used for 60 minutes at 40°C. The seeds were categorized into five classes based on vigor and viability criteria, following the parameters established by Bhering et al. (2005) in their study on watermelon, where: class 1 - high vigor viable seeds, class 2 - low vigor viable seeds, class 3 - not-vigorous viable seeds, class 4 – not viable seeds, and class 5 - dead seeds. Four repetitions of twenty-five seeds were evaluated.

For the accelerated aging test, gherkin seeds were exposed to a temperature of 42°C in Gerbox® containing 40 mL of water, in four repetitions of sixty seeds. The evaluation times were at 0, 48, and 96 hours. Subsequently, a portion of the seeds (ten) were used for determining the relative humidity, while another portion was used for performing the germination test, germination speed index (GSI), and dry matter. The calculation of relative humidity was carried out according to the rules for seed analysis (Brazil 2009), as well as the germination test, which was conducted in a controlled BOD environment at 25°C, with the final count made at eight days. After the germination test, the normal seedlings from each replication were used to determine the dry matter content.

The germination speed index (GSI) was established together with the germination test. Evaluations were carried out daily and, at the end of the test, the calculation of the germination speed index was performed as indicated by Maguire (1962).

For the pH of the exudate test, two indicator solutions were formulated: phenolphthalein (composed of 1g of phenolphthalein dissolved in 100 mL of absolute alcohol, with the addition of 100 mL of distilled and boiled water) and sodium carbonate (composed of 8.5 g/L of distilled and boiled water). The soaking time of 30 and 60 minutes was used in a constant temperature chamber at 25°C, generating a 2x2 factorial for each variety, with four repetitions of ten seeds. Pink coloration was considered viable seeds, and transparent coloration was considered not viable seeds.

Statistical analyses were performed using the R software (R Core Team 2024), where the data were subjected to analysis of variance, and the means were compared by Duncan's test at a 5% probability level.

RESULTS AND DISCUSSION

The tetrazolium test is a biochemical test used to determine seed viability. According to França-Neto & Krzyzanowski (2018), in addition to assessing the viability and vigor of seed lots, it provides a diagnosis of the possible causes responsible for the reduction in their quality. For Liso gherkin, according to the F-test, the concentration of the solution was significant for classes 1, 2, 4, and 5, meaning high vigor viable seeds, low vigor viable seeds, not viable seeds, and dead seeds, respectively (Table 1). The soaking time was significant only for seed classes 4 and 5. Only in class 2 was there an interaction between time and concentration. For the northern gherkin variety, the concentration was significant for classes 1, 4, and 5, and time was significant for class 4. No interaction between time and concentration was observed for this variety.

Table 1 - Analysis in variance of classes in viability in seeds C1 (class 1), C2 (class 2), C3 (class 3), C4 (class 4) and C5 (class 5), under different solution concentrations of tetrazolium and soaking time, in Liso and northern gherkin.

		Liso gherkin					**Northern gherkin**				
SV	**DF**	**C1**	**C2**	**C3**	**C4**	**C5**	**C1**	**C2**	**C3**	**C4**	**C5**
Time	3	16.7^{ns}	32.6^{*}	4.5^{ns}	18.5^{*}	27.7^{*}	8.8^{ns}	3.4^{ns}	0.9^{ns}	8.3^{*}	32.4^{ns}
Conc.	1	578^{*}	26.3^{*}	3.8^{ns}	176^{*}	57.8^{*}	351^{*}	1.1^{ns}	2^{ns}	15.1^{*}	480^{*}
TxC	3	6.1^{ns}	29.2^{*}	3.1^{ns}	10.0^{ns}	4.7^{ns}	6.0^{ns}	1.7^{ns}	1.1^{ns}	4.7^{ns}	11.1^{ns}
Error	24	8.1	4.9	2.2	4.4	4.9	7.6	4.8	2.7	2.7	27.2
Total	31	608.9	93.0	13.7	208.7	95.2	373.5	11.0	6.7	30.7	551.2

* Significant ($p < 0.05$) by F test; ^{ns} Not significant ($p < 0.05$) by F test.

In both, Liso gherkin and northern gherkin, the 1% concentration was more effective in identifying high vigor viable seeds (Table 2), contradicting the results obtained by Paiva et al. (2017), who reported that lower concentrations of the tetrazolium solution were more suitable for assessing the viability of *Cucumis anguria L*. seeds, as well as Barros (2002), who also suggested that a 0.075% concentration is more suitable for testing pumpkin and zucchini seeds. In class 5, higher values were observed at the 0.075% concentration, suggesting that this concentration is more effective in identifying seed damage. This result is consistent with the study by Paraíso et al. (2019), who, when

analyzing 0.1% and 0.5% tetrazolium concentrations in chickpea seeds, found that the lower concentration is more effective for visualizing damage.

Table 2 - Mean test for viability classes of the tetrazolium test in Liso and northern gherkin seeds at different solution concentrations.

	Liso gherkin			Northern gherkin		
Concentration	**Class 1**	**Class 4**	**Class 5**	**Class 1**	**Class 4**	**Class 5**
1%	11.56 a	3.69 b	3.50 b	9.31 a	3.31 a	7.69 b
0.075%	3.06 b	8.38 a	6.19 a	2.69 b	1.94 b	15.44 a

Means followed by the same letters vertically do not differ statistically from each other according to Duncan's test at a 5% probability level.

Within the 1% concentration, in class 2, the different soaking times exhibited similar behavior (Table 3). In contrast, at the 0.075% concentration, the 60-30 time was more efficient in identifying low vigor viable seeds. Low vigor seeds tend to show poor seedling emergence under adverse conditions, which can result in significant field losses. Although not significant in other classes, in practical terms, the 60-30 time was observed to be the most effective in facilitating the removal of the seed coats and internal membranes.

Table 3 - Mean test of the interaction between soaking time and tetrazolium solution concentration for viability class 2 of Liso gherkin seeds.

Liso gherkin – Class 2		
Time	**1%**	**0.075%**
30-30	5.00 Aa	2.75 Ab
30-60	3.00 Aa	5.50 Ab
60-30	4.00 Ba	10.75 Aa
60-60	2.50 Aa	2.75 Ab

Means followed by the same uppercase letters horizontally and lowercase letters vertically do not differ statistically from each other according to Duncan's test at a 5% probability level.

Given that soaking times for seed coat removal were not significant in distinguishing between viable and dead seeds (class 1 and 5, respectively), we sought to understand if the varieties respond differently to the tetrazolium solution concentration in identifying seeds of these classes. In this case, the two factors did not interact for class 1, but they did interact for class 5 (Table 4), indicating that the 1% concentration was more efficient in identifying high vigor viable seeds for both varieties. For class 5, northern gherkin had a higher number of dead seeds at both concentrations, and the 0.075% concentration better identified this seed class.

Table 4 - Mean test of tetrazolium solution concentration for viability class 1 and mean test of the interaction between tetrazolium solution concentration and gherkin varieties for viability class 5.

Class 1		**Class 5**		
Concentration	**Mean**	**Concentration**	**Liso gherkin**	**Northern gherkin**
1%	10.44 a	1%	3.5 Bb	7.69 Ab
0.075%	2.88 b	0.075%	6.19 Ba	15.44 Aa

Means followed by the same uppercase letters horizontally and lowercase letters vertically do not differ statistically from each other according to Duncan's test at a 5% probability level.

The accelerated aging test evaluates the behavior of seeds subjected to high temperature and relative humidity, based on the fact that the rate of seed deterioration increases considerably under such conditions, allowing the simulation of adverse conditions that seeds may face during storage or in the field (Marcos-Filho 2015a). To assess the aging test to which the seeds were subjected, germination tests, GSI, relative humidity, and dry matter content were performed.

A significant statistical difference in germination was observed between the two varieties for the categories of normal seedlings and hard seeds (Table 5). The abnormal seedlings category showed no significant difference, and dead seeds showed an interaction between accelerated aging times and variety. Liso gherkin showed a lower number of normal seedlings compared to northern gherkin, regardless of aging time (Table 6), corroborating the results obtained by Leite et al. (2019), which indicated superior performance of northern gherkin seeds in terms of germination values compared to the northeastern variety.

Table 5 - Analysis of variance for normal seedlings (NS), abnormal seedlings (AS), hard seeds (HS), dead seeds (DS), relative humidity (RH), germination speed index (GSI), and dry matter content (DMC) for gherkin varieties subjected to different accelerated aging times.

SV	DF	NS	AS	HS	DS	RH	GSI	DMC
Time	2	10.5^{ns}	2.4^{ns}	4.7^{ns}	2.8*	1010.5*	1.6^{ns}	0.00026*
Variety	1	337.5*	117.0^{ns}	96*	5.0*	94.3*	60.4*	0.00065*
TxV	2	213.5^{ns}	109.3^{ns}	8^{ns}	2.8*	34.5*	12.7*	0.00024*
Error	18	72.5	39.1	8.6	0.5	2.5	1.5	0.00004
Total	23	633.9	267.8	17.2	11.2	1141.8	76.2	0.00119

* Significant ($p < 0.05$) by F test; ^{ns} Not significant ($p < 0.05$) by F test.

Table 6 - Mean test for germination of normal seedlings and hard seeds in Liso and northern gherkin varieties.

Varieties	Normal seedlings	Hard seeds
Liso gherkin	34.25 b	4.17 a
Northern gherkin	41.75 a	0.17 b

Means followed by the same letters vertically do not differ statistically from each other by Duncan's test at a 5% probability level.

Consistent with observations, Liso gherkin produced a higher number of hard seeds, which is unfavorable as the presence of hard seeds can lead to significant losses in crop establishment, necessitating reseeding. According to the tetrazolium test, both varieties were significantly similar in terms of viable seeds with high vigor, diverging from the results of the germination test where northern gherkin showed a higher number of normal seedlings. For dead seeds, it was observed that over the 96-hour period, northern gherkin seeds were more affected, indicating lower tolerance of this variety to adverse conditions (Table 7).

Campos & Tillmann (1996) highlight that relative humidity is one of the most important factors for seed conservation, essential for adequate quality control. Moreover, as pointed out by the authors, seed vigor can be significantly impaired when relative humidity is not appropriate. Evaluating the relative humidity of gherkin seeds before and

after aging, it was found that seeds from Liso and northern varieties exhibited initial relative humidity of 8.27% and 7.57%, respectively, values close to those observed in seeds of the Liso gibão variety, which are approximately 8.9%, as reported in the study by Da Silva et al., (2021). The varieties differed between the 96-hour and 48-hour times, with higher values obtained for northern gherkin, indicating that this variety absorbed more humidity than Liso gherkin (Table 7). Liso gherkin absorbed more humidity when exposed to 96 hours of aging, while for northern gherkin, the 48 and 96-hour periods promoted similar humidity absorption. Despite generally higher humidity absorption throughout the test, it was noted that these values did not interfere with the presence of normal seedlings, as aging time did not influence this variable.

The germination speed index (GSI) provides essential parameters on the uniformity and germination rate of seeds, particularly useful for assessing seed lot quality and performance. It was found that northern gherkin showed a lower index at 96 hours, corroborating previous results in this study where this variety was found to be more sensitive to adverse conditions. The opposite was observed for Liso gherkin, which did not undergo changes in its index at different times, including at 0 hours, suggesting that this variety is resistant to potential damage caused by this type of stress. Conversely, under less extreme conditions, northern gherkin showed greater vigor, with higher indices at 0 and 48 hours compared to Liso gherkin.

In general, seeds reach maximum quality close to physiological maturity, when there is peak accumulation of dry matter, promoting complete formation of biochemical, structural, and morphological systems. Therefore, higher values of dry and fresh matter in seedlings are considered more vigorous samples of the seed lot (Nakagawa et al. 1999). Analyzing different times, it was found that northern gherkin exhibited higher dry matter content at shorter times (0 and 48 hours) compared to Liso gherkin (Table 7). According to Haeberlin et al. (2020), in their study with canola seeds, it was observed that seeds stored at higher temperatures showed a linear reduction in germination content, results that can be explained by the observed loss of dry matter under these conditions. A similar phenomenon was observed in northern gherkin, which, when exposed to higher temperatures for longer periods, showed higher dry matter content at 0 and 48 hours.

Table 7 - Mean test for germination of dead seeds, relative humidity, germination speed index (GSI), and dry matter at different accelerated aging times in Liso and northern gherkin varieties.

			Liso gherkin	**Northern gherkin**
Dead seeds	Time	96h	0.00 Ba	2.25 Aa
		48h	0.00 Aa	0.00 Ab
		0h	0.00 Aa	0.50 Ab
Relative humidity	Time	96h	29.10 Ba	34.86 Aa
		48h	24.03 Bb	32.70 Aa
		0h	8.27 Ac	7.57 Ab
GSI	Time	96h	11.91 Aa	12.20 Ab
		48h	10.81 Ba	15.09 Aa
		0h	10.09 Ba	15.04 Aa
Dry matter	Time	96h	0.02 Aa	0.02 Ab
		48h	0.02 Ba	0.04 Aa
		0h	0.02 Ba	0.03 Aa

Means followed by the same uppercase letters horizontally and lowercase letters vertically do not differ statistically from each other by Duncan's test at a 5% probability level.

The exudate pH test is a biochemical assay characterized by its rapid results and low cost, aimed at assessing seed membrane integrity (Carvalho et al. 2018), demonstrating efficiency in determining seed viability in advanced stages of deterioration. In this study, regardless of soaking time or solution used, the exudate pH test showed 100% seed viability, contrasting with the results obtained by the tetrazolium

test. This disparity between tests may be attributed to the combined assessment of seeds in the pH test, potentially causing interference among individual seed results.

CONCLUSION

The concentration of 1% tetrazolium is the most efficient in identifying seeds of class 1, viable seeds of high vigor, both in Liso and northern gherkin, regardless of soaking time. However, a soaking time of 60-30 time is more suitable for providing better handling, as it reduces damage to the seed during tegument and internal membrane removal. The 1% tetrazolium concentration at the 60-30 time is most suitable for class 2 of Liso gherkin. Under ideal conditions, northern gherkin is more vigorous than Liso gherkin. In contrast, Liso gherkin appears to better resist environmental stress conditions, showing higher vigor in tests conducted at 96 hours of accelerated aging. The 96-hour accelerated aging time was adequate for separate the physiological quality of different varieties, concerning GSI, relative humidity, and dry matter. However, aging times did not influence the germination of normal seedlings. Soaking time and solutions did not influence exudate pH evaluation.

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