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ARTICLE

Viability of melon seeds using the pH testing of exudate

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ABSTRACT: Melon is one of the most consumed cucurbits and commercially significant cucurbits in the world. It is planted predominantly through seedlings, and seed quality is essential in this process. Among the rapid tests used in seed analysis, the pH testing of exudate has shown promise for several cultivated species. Thus, this study aimed to verify the viability of melon (*Cucumis melo* L.) seeds through the pH testing of exudate. For this, seeds of the hybrids Premier and Supreme (three lots each) were subjected to germination and vigor tests (first germination count, tetrazolium, accelerated aging, emergence speed index, and emergence). The pH testing of exudate was evaluated using phenolphthalein and sodium carbonate solutions. The results indicated the possibility of classifying the lots into three quality levels for both hybrids, similar to most tests used to assess initial quality. Therefore, the pH testing of exudate was effective in determining the viability of lots of melon seeds and can be conducted with a soaking period of 120 minutes at 25 or 30 °C.

Index terms: *Cucumis melo* L., Cucurbitaceae, rapid test, seed analysis.

RESUMO: O meloeiro é uma das cucurbitáceas mais consumidas e de maior interesse comercial do mundo. Seu plantio é realizado predominantemente por meio de mudas, sendo a qualidade das sementes fundamental neste processo. Dentre os testes rápidos utilizados na análise de sementes, o pH do exsudato tem se mostrado promissor para várias espécies cultivadas. Com isso, objetivou-se verificar a viabilidade de sementes de melão (*Cucumis melo* L.) por meio do teste de pH do exsudato. Para isso, sementes dos híbridos Premier e Supreme (três lotes de cada) foram submetidas aos testes de germinação e vigor (primeira contagem de germinação, tetrazólio, envelhecimento acelerado, índice de velocidade de emergência e emergência). O teste de pH de exsudato foi avaliado com a utilização de soluções de fenolftaleína e carbonato de sódio. Os resultados indicaram que para ambos os híbridos foi possível classificar os lotes em três níveis de qualidade, semelhantes a maioria dos testes utilizados na avaliação da qualidade inicial. Com isso, o teste de pH do exsudato mostrou-se eficaz para determinar a viabilidade de lotes de sementes de melão, podendo ser conduzido com período de embebição de 120 minutos a 25 ou 30 °C.

Termos para indexação: *Cucumis melo* L., Cucurbitaceae, teste rápido, análise de sementes.

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INTRODUCTION

Melon (*Cucumis melo* L.), family Cucurbitaceae, stands out among vegetables due to its appreciation and growing popularity among consumers (Nunes et al., 2018). Brazil produced 613,933 thousand tons of melon in 2020, ranking ninth in the world production ranking (IBGE, 2020). In this context, the Northeast region was responsible for approximately 96% of national production, with the state of Rio Grande do Norte standing out with 375,574 t (Anuário Hortifruti Brasil, 2022).

Melon planting is conducted using seedlings (indirect sowing). Therefore, the seeds need to present high quality to obtain vigorous seedlings and productive plants in the field. Furthermore, the use of high-quality seeds allows the reduction of cultivation practices, such as replanting and thinning, resulting in higher uniformity of seedlings in the field and, consequently, uniform maturation of fruits (Escobar-Álvarez et al., 2020).

Melon seed viability is officially determined through a germination test lasting eight days to obtain results (Brasil, 2009). However, in addition to the relatively long period, this test also has some limitations, as it is unable to differentiate lots under controlled environmental conditions. As a result, the discrepancy in seed emergence results under field conditions is influenced by the variation in environmental factors, which are difficult to control, and reduced under laboratory conditions (Marcos-Filho, 2015; Araújo et al., 2017). Therefore, sensitive, reliable, and rapid methods to determine seed viability for sowing, storage, or marketing purposes are necessary. In this sense, the pH testing of exudate stands out, as it is a rapid method of assessing viability, providing results in a few hours. Thus, this method can be used as an alternative, presenting ease of execution, low cost, and speed in obtaining results and avoiding the storage of unviable lots (Amaral and Peske, 2000).

The pH testing of exudate is a biochemical method based on the chemical reactions that occur in the deterioration process, determining a decrease in seed viability (Santos et al., 2011). Metabolites such as sugars, organic acids, and hydrogen ions (H^+) are released during the soaking of seeds in water, acidifying the medium and causing a decrease in the pH of the seed exudate (Rech et al., 1999; Carvalho et al., 2002). Therefore, seeds with high physiological quality have low solute leaching and do not cause major changes in the pH of the medium, while deteriorated seeds release a greater quantity of ions, resulting in lower pH values (Matos et al., 2009).

Research using the pH testing of exudate has already been carried out for several agricultural species, with promising results in soybean (Amaral and Peske, 1984), pea (Rech et al., 1999), crambe (Alves et al., 2016), rice (Santos et al., 2020), and wheat (Grzybowski et al., 2022). Considering that there is no research using this test on melon seeds, this study aimed to evaluate the effectiveness of this rapid viability method on seeds of this fruit vegetable.

MATERIAL AND METHODS

The experiment was conducted at the Laboratory Seed Analysis (LAS), belonging to the Agricultural Sciences Center of the *Universidade Federal Rural do Semi-Árido* (UFERSA) at the Mossoró campus, RN, Brazil. Six lots of melon seeds, three from the hybrid Premier and three from the hybrid Supreme, were used. During the experiment, the seeds remained packed in kraft paper bags and kept in a controlled environment (17 °C and 50% relative humidity).

The seeds from each lot were initially subjected to water content and quality assessments, as described below:

Water content (WC): determined by the oven method at 105 ± 3 °C for 24 hours (Brasil, 2009), using two replications of 4 g, and the results were expressed as a percentage (wet basis).

Germination (G) and first germination count (FGC): carried out with four replications of 50 seeds per lot, distributed on two sheets of paper towel and covered with a third one. The substrate was moistened with distilled water in an amount of 2.0 times the weight of the dry paper. Then, the paper rolls were packed in transparent plastic bags to avoid water loss and placed in a germination chamber at 25 °C. The evaluations were carried out four (FGC) and eight days (G) after sowing and the results were expressed as a percentage of normal seedlings (Brasil, 2009).

Total dry mass (TDM): normal seedlings from the germination test without cotyledons were counted, packed in kraft paper bags, and placed in a forced-air circulation oven at 60 °C until constant weight, with approximately 48 hours. The total dry matter of seedlings was obtained by weighing on an analytical balance (0.0001 g) and the results were expressed in mg.seedling^{-1}.

Emergence (E): conducted in a greenhouse with four replications of 50 seeds per lot, sown in polystyrene trays filled with commercial substrate (Plantmax®). Seedling emergence was evaluated at 12 days after sowing and the results were expressed as a percentage.

Emergence speed index (ESI): carried out together with the emergence test, with daily counts of the number of emerged seedlings. ESI was determined according to the formula proposed by Maguire (1962).

Tetrazolium (TZ): conducted in four replications of 50 seeds per lot. Initially, the seeds were pre-conditioned in distilled water for 30 minutes at 40 °C to remove the seed coat manually. To do this, a small cut was made with a knife at the end of the integument opposite the embryonic axis (Barros et al., 2005). Subsequently, a new immersion was conducted in distilled water at 40 °C for 60 minutes to remove the internal membrane. After this preparation, the seeds were immersed for coloring in a 0.075% 2,3,5-triphenyl tetrazolium chloride solution at 40 °C for 60 minutes in the dark (Lima et al., 2010). The seeds were classified according to their color as viable and non-viable, and the results were expressed as a percentage.

Accelerated aging (AA): the seeds were distributed evenly on stainless steel screens and suspended inside a transparent plastic box (11 x 11 x 3 cm) containing 40 mL of distilled water on its bottom. The boxes were closed and kept in germination chambers at 41 °C ± 0.3 for 72 hours (Torres et al., 2009). After this period, the seeds were subjected to a germination test with normal seedling counts four days after sowing and determination of water content. The results were expressed as a percentage of normal seedlings for each lot.

pH of exudate: initially, phenolphthalein and sodium carbonate indicator solutions were prepared, the former using 1 g of phenolphthalein diluted in 100 mL of absolute alcohol and 100 mL of distilled and boiled water, and the latter using a concentration of 0.8 g.L^{-1} sodium carbonate dissolved in distilled and boiled water (Cabrera and Peske, 2002). Four replications of 25 seeds per lot were used. Each seed was soaked in 2 mL of distilled water inside a plastic container with an individual cell for periods of 30, 60, 90, and 120 minutes at temperatures of 25 and 30 °C. After each period, three drops of phenolphthalein and three drops of sodium carbonate were added and mixed using a glass rod, obtaining a strong pink color as a reference. The reading was carried out after the indicator solutions came into contact with the soaking solution for the tested periods. The interpretation was performed according to the color of the solution: a strong pink color was an indication of viable seeds that give rise to normal seedlings; a light pink color indicated seeds that produced abnormal seedlings; and colorless and weak (very light pink) indicated low-viability seeds (Grzybowsk et al., 2022).

The experimental design was completely randomized. The data were subjected to analysis of variance using the F-test and the means were compared by the Tukey test at 5% probability using the program SISVAR (Ferreira, 2019). Multivariate statistics was also performed with the standardization of the variables, with the mean being 0 and the variance being 1, through principal component analysis, using the software Past4.

RESULTS AND DISCUSSION

Table 1 shows the data regarding the initial water content of lots. These data were not statistically analyzed, serving only for initial characterization. Both hybrids showed no marked variation between seed lots, being 1.2% for Premier and 0.6% for Supreme. The variation in water content with values below 2% is essential in the evaluation of lots, as well as in obtaining reliable and consistent results (Marcos-Filho, 2015). Furthermore, this determination has great importance, especially when associated with physiological quality and vigor tests, as it helps in the correct classification of lots in terms of physiological behavior during storage and, consequently, enables the planning of storage and conservation strategies for the species (Ferreira et al., 2020).

The germination test in the hybrid Premier indicated that lots 1 and 2 were superior relative to 3. Moreover, this test indicated that lot 5 of the hybrid Supreme had superior quality compared to the others. On the other hand, first count, accelerated aging, tetrazolium, emergence, and emergence speed index tests classified the lots into three quality levels for the two hybrids. In these tests, lots 1, 2, and 3 of the hybrid Premier presented superior, intermediate, and inferior quality, respectively. Lots 5, 4, and 6 of the hybrid Supreme were classified as high, medium, and low vigor, respectively (Table 1).

All seed lots showed an increase in water content after accelerated aging. The variation reached 5% for the hybrid Premier and 4.6% for the hybrid Supreme. According to Marcos-Filho (2020), samples subjected to accelerated aging can vary by up to four percentage points, as this parameter helps with reliability for comparing germination and vigor results (Table 1). Excessive increase in water in seeds can cause damage, leading to an earlier deterioration process or even favoring the development of pathogens, resulting in loss of germination power and seed vigor (Carvalho and Nakagawa, 2012).

The total dry mass of seedlings resulted in significant differences between lots of the hybrid Premier, with lot 1 standing out as the best. Lot 5 of the hybrid Supreme had superior performance compared to the others, which did not differ statistically from each other (Table 1). Vigor tests based on seedling performance show that more vigorous seeds give rise to more developed seedlings, translating the efficiency of the action of repair mechanisms, mobilization of reserves, and synthesis of new tissues during germination (Krzyzanowski et al., 2020).

The results obtained by the pH testing of exudate indicated that viable seeds were those with a dark or light pink soaking solution after contact with phenolphthalein and sodium carbonate solutions (Figures 1A and 1B). On the other hand, seeds classified as unviable were those that presented a very light pink soaking solution or remained colorless (Figures 1C and 1D). These classifications were based on the results of wheat seeds by Grzybowski et al. (2022).

Normally, sugars, organic acids, and ions, including H^+, are released during the process of water-soaking by seeds, contributing to acidification, and resulting in a decrease in the pH of the seed exudate solution. Thus, more deteriorated seeds result in higher leaching and, consequently, the exudate has greater buffering power (Tillmann et al., 2019). This fact causes a change in the color of the soaking solution of seeds subjected to the pH testing of exudate using phenolphthalein as an indicator solution.

Table 1. Average values of initial water content (WC), germination (G), first germination count (FGC), emergence (E), accelerated aging (AA), water content after accelerated aging (WCAA), tetrazolium (TZ), emergence speed index (ESI), and total seedling dry mass (TDM) of melon (*Cucumis melo* L.) seed lots of the hybrids Premier and Supreme.

Hybrid	Lot	WC	G	FGC	E	AA	WCAA	TZ	ESI	TDM ($mg.plant^{-1}$)
		...... %								
Premier	1	8.3	96 a	96 a	96 a	99 a	32.7	91 a	5.24 a	0.555 a
	2	7.1	86 a	76 b	76 b	89 b	29.0	65 b	4.23 b	0.500 b
	3	8.1	70 b	59 c	59 c	59 c	27.7	41 c	2.84 c	0.415 c
	CV (%)		7.9	10.1	10.1	4.8	-	7.1	1.22	5.49
Supreme	4	6.8	69 b	59 b	59 b	76 b	28.7	69 b	3.61 b	0.437 b
	5	6.4	97 a	90 a	90 a	95 a	27.4	91 a	5.01 a	0.637 a
	6	7.4	59 b	42 c	42 c	60 c	24.1	51 c	2.49 c	0.467 b
	CV (%)		9.57	10.42	10.42	5.68	-	6.84	6.48	9.58

Means within each column followed by the same letter do not differ from each other using the Tukey test ($p<0.05$).

The results obtained in the viability assessment of melon seeds using the pH testing of exudate indicated statistical differences between lots, but the results varied between hybrids (Table 2). Seeds from Premier lots evaluated for periods of 30 and 60 minutes at 25 °C showed no differences regarding physiological quality. However, during 90 minutes of soaking, the lots could be stratified similarly to the results obtained for the first count, emergence, emergence speed index, accelerated aging, and tetrazolium tests, with lots 1, 2, and 3 being classified as high, medium, and low performance, respectively. On the contrary, this test was efficient in classifying rice lots when the seeds were soaked for 60 minutes at 25 °C (Santos et al., 2020).

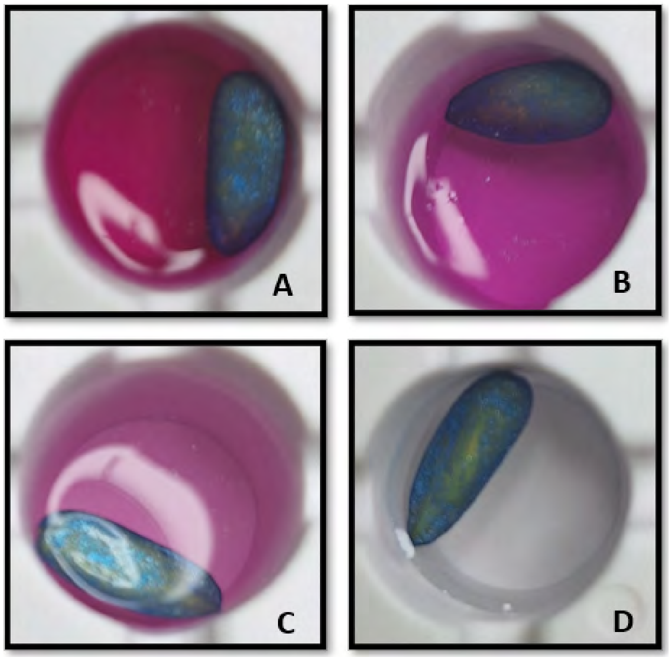

Figure 1. Exudate color obtained from melon seeds (*Cucumis melo* L.) immediately after contact with phenolphthalein and sodium carbonate solutions. Viable seeds: (A) dark pink and (B) light pink. Unviable seeds: (C) very light pink and (D) colorless.

Table 2. Means of viable seeds by the pH testing of exudate from melon (*Cucumis melo* L.) seed lots of the hybrids Premier and Supreme subjected to four periods of soaking (30, 60, 90, and 120 minutes) and two temperatures (25 and 30 °C).

		Viable seeds (%)							
Hybrid	Lot	25 °C				30 °C			
		30′	60′	90′	120′	30′	60′	90′	120′
Premier	1	90 a	71 a	93 a	87 a	93 a	94 a	86 a	92 a
	2	75 a	62 a	83 b	75 ab	53 b	51 b	68 b	81 b
	3	75 a	60 a	60 c	65 b	60 b	59 b	72 b	71 c
CV (%)		10.07	18.04	5.96	8.13	9.66	12.21	8.11	11.62
Supreme	4	83 ab	82 a	84 a	72 b	67 b	73 a	44 b	64 b
	5	90 a	92 a	93 a	88 a	92 a	87 a	85 a	83 a
	6	71 b	66 b	31 b	45 c	46 c	43 b	57 b	53 b
CV (%)		7.78	7.07	7.57	9.71	8.78	10.43	10.70	10.82

Means within each column followed by the same letter do not differ from each other using the Tukey test ($p<0.05$).

Seeds from lots of the hybrid Premier showed different performance at the temperature of 30 °C than those verified at 25 °C for periods of 30, 60, and 90 minutes. Among them, only lot 1 was statistically superior compared to the others, while lots 2 and 3 did not differ from each other. However, the lots could be classified into three quality levels in the period of 120 minutes, similar to most tests used to assess initial quality (Table 2).

The speed at which the seed soaking process occurs influences the results obtained through the pH testing of exudate. Seed morphological characteristics, water content, shape, reserve content, and seed coat thickness are some of the factors that directly influence the flow of solutes into the soaking solution (Ferreira et al., 2020).

Viable seeds from lots of the hybrid Supreme showed a different behavior from those obtained with the hybrid Premier regarding combinations of temperatures and soaking periods. The combinations of 25 °C/ 120 minutes and 30 °C/ 30 minutes allowed a ranking similar to those obtained for most initial quality tests, and lots 5, 4, and 6 were classified as superior, medium, and inferior qualities, respectively (Table 2). However, the results of the combination of 30 °C/ 120 minutes were similar to those found for germination.

The two principal components in the principal component analysis (PCA) (Table 3) explained more than 90% of the total data variability, with values of 80.62% and 9.68% for the first and second components, respectively. Thus, PCA achieved representativeness of the viability tests through the germination, tetrazolium, and vigor tests and the applied methodologies of pH testing of exudate.

All correlation values equal to or higher than 0.60 for each of the principal components were considered relevant with discriminatory power (Rencher and Christensen, 2012). Therefore, all variables presented discriminatory power with at least one component when considering this criterion. The exception was found for the pH of exudate at 25 °C for 60 minutes, which had no discriminatory power for the first component, and 90 minutes at 25 °C, which correlated with the second component.

Table 3. Correlation matrix and eigenvectors of the principal components by the tests of germination, tetrazolium (viability), vigor, and pH of exudate from melon (*Cucumis melo* L.) seed lots of the hybrids Premier and Supreme.

Variable		Principal component 1	Principal component 2
Germination		0.96	-0.23
First germination count		0.98	-0.21
Tetrazolium test		0.93	0.24
Seedling emergence		098	-0.21
Emergence speed index		0.98	-0.06
Accelerated aging		0.94	-0.06
Total dry mass		0.83	0.06
25 °C	30 minutes	0.92	0,33
	60 minutes	0.57	0,77
	90 minutes	0.88	0,61
	120 minutes	0.96	-0,02
30 °C	30 minutes	0.92	0,20
	60 minutes	0.89	0,27
	90 minutes	0.72	-0,49
	120 minutes	0.90	-0,42
Eigenvalues		12.09	1.45
Accumulated variance (%)		80.62	9.68
Total variance (%)		90.30	

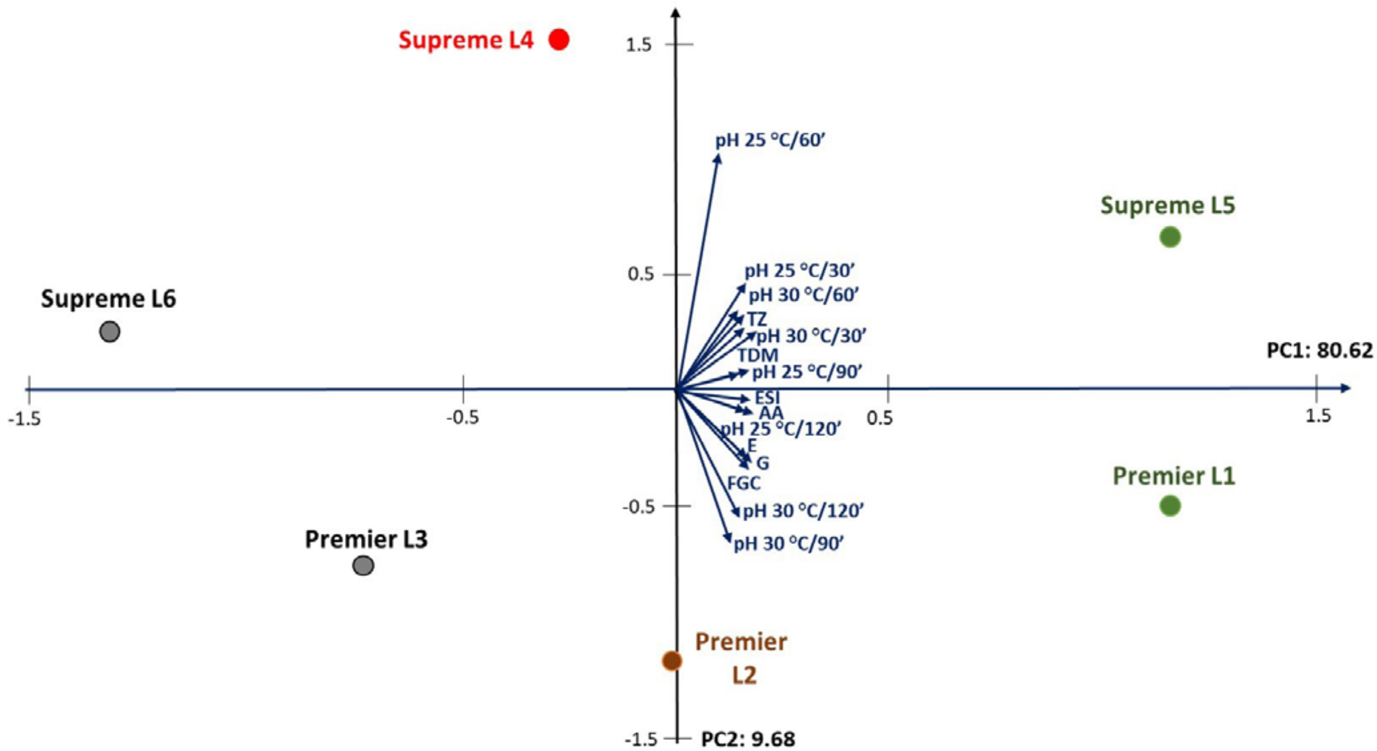

Figure 2. Scattering of eigenvectors along a circular plane and formation of groups obtained by principal component analysis according to germination (G), first germination count (FGC), tetrazolium test (TZ), seedling emergence (E), emergence speed index (ESI), accelerated aging (AA), total dry mass (TDM), and pH of exudate at times of 30, 60, 90, and 120 minutes at 25 °C (pH 25 °C/30', pH 25°C/60', pH 25 °C/90', and pH 25 °C/120') and 30 °C (pH 30 °C/30', pH 30 °C/60', pH 30 °C/90', and pH 30 °C/120') of lots of melon (*Cucumis melo* L.) seeds of the hybrids Premier and Supreme.

The biplot of the principal components analysis (Figure 2) shows the distribution of the lots into three quality classes. Seeds from lots 1 and 5 (hybrids Premier and Supreme, respectively) were classified as having better quality. They are in the same quadrants as the variables of germination, vigor, and pH of exudate, showing a positive correlation.

An intermediate quality class was constituted by lot 2 of the hybrid Premier and 4 of the hybrid Supreme, being considered intermediate due to the variables that have a high correlation with component 1, as they are vertically close to the origin. However, the pH of exudate at 25 °C for 60 and 90 minutes, which presented discriminatory power for component 2, differed from each other, as they are in opposite quadrants on the graph, thus indicating a negative correlation.

Finally, seeds from lot 3 of the hybrid Premier and 6 of the hybrid Supreme were classified as having inferior quality, located in the opposite quadrant for the variables of germination, tetrazolium (viability), vigor, and pH of exudate.

In summary, the pH testing of exudate at 25 °C for 60 and 90 minutes was not efficient in ranking lots according to quality levels. The other times at both temperatures had the potential for ranking the quality of lots of melon seeds, standing out the pH of exudate at 25 °C for 120 minutes, as it presented a higher contribution with component 1 (0.96) (Table 3). Furthermore, it is in the same quadrant as the germination and vigor variables, showing the smallest acute angles and a high correlation with them.

CONCLUSIONS

The pH testing of exudate is effective in classifying lots of melon seeds and can be conducted with a soaking period of 120 minutes at 25 or 30 °C.

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