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Size, imbibition, and viability of seeds of two creole melon (*Cucumis melo* L.) from the state of Guerrero, Mexico

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ABSTRACT

Objective: To determine the physical and physiological characteristics of Creole melon seeds from the Costa Chica of Guerrero.

Design/Methodology/Approach: It was established under a completely randomized design, and Student's t-test (α=0.05) and correlation with Pearson's test were performed. Viability and imbibition were carried out using the methodologies described by the International Seed Testing Association (ISTA).

Results: Creole seeds of the two varieties presented a significant statistical difference (95% confidence level) in the physical quality variables. The imbibition ended at 18 and 32 h in V_2 and V_1, respectively, after being submerged in water. The humidity percentage was higher in V_1 (7.19); while, V_2 presented a higher percentage of germination and viability (96 and 90%, respectively). There is a positive association between the humidity and the physical dimensions of the seed and the germination and viability (r^2=0.954) that is highly significant (P=0.003).

Study Limitations/Implications: Morphological and taxonomic classification studies of the Creole genotypes of the Costa Chica region of Guerrero are required.

Findings/Conclusions: There was a positive correlation between the physical and physiological quality of the Creole melon seeds.

Keywords: Correlation, Germination, Seeds, Tetrazolium.

INTRODUCTION

Melon (*Cucumis melo* L.) is a species in the Cucurbitaceae family and is originally from Asia and Africa (Mehra *et al.*, 2015). This family includes more than 900 species, among which 10 genera are of economic importance, mainly including cucumbers and melons (*Cucumis* sp), squashes and zucchinis (*Cucurbita* sp.), and watermelons (*Citrullus lanatus* L.) (Paris *et al.*, 2017). Melon is considered one of the most diverse

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species within the *Cucumis* genus due to its extensive genotypic and phenotypic variation (Farcuh *et al*., 2019). The 16 recognized melon groups have merged, five of them being divided into the subspecies *agrestis* and 11 into the subspecies melo, including the *cantalupensis* (cantaloupe), *reticulatus* (muskmelon) and *inodorus* (honeydew) groups (Assis-Dantas *et al*., 2015).

Commercially, melon is one of the most important horticultural crops in México due to the surface area farmed and production volume (Monge-Pérez, 2013). In Guerrero, melon farming is economically important: in 2019, 99,862 t were produced with a yield of 29.66 Ton ha^{-1} (SIAP, 2019). Additionally, traditional production systems subsist in the state where native varieties are conserved *in situ* and gathered species are also used (Vera-Sánchez *et al*., 2016).

The main method of melon propagation is by seed, which generates high demand for quality seeds that should have different attributes including genetic, physiological, physical, and sanitary quality (Basra, 1995; Copeland and McDonald, 1995). For this purpose, characteristics such as fidelity to the variety, germination percentage, purity, vigor, appearance, and absence of disease are considered, since they contribute to a higher productive varietal efficiency and the ability to sprout quickly and uniformly under different environmental conditions (Finch-Savage and Bassel, 2016). Germination and viability tests have been widely used in seed evaluation (Villa *et al*., 2019). Therefore, the objective of the present study was to collect seeds in the municipality of Florencio Villareal in the state of Guerrero to determine the physical and physiological characteristics of Creole melon (*Cucumis melo* L.) seeds.

MATERIALS AND METHODS

The collection site was in the region of Costa Chica, in the municipality of Florencio Villareal, located in the southern part of the state of Guerrero (16° 43' 26'' LN y 99° 07' 24'' LW). The varieties were classified according to the fruit's shape; V$_1$ were round, corrugated or segmented fruits, and V$_2$ were elongated and smooth fruits, commonly known as cucumber-melons. After collecting, the seeds were washed to eliminate pulp residues and placed on drying paper to eliminate water excess. Later, they were sent in hermetically sealed, labeled containers to the Multidisciplinary Laboratory of the Faculty of Biological and Agricultural Sciences at the University of Colima, located in the municipality of Tecomán, Colima.

Physical Characterization

The physical quality of the seeds was determined by their size (Pérez-Mendoza *et al*., 2016). The variable studied was the weight of 1000 seeds (WTS), which was estimated by weighing 100 seeds in eight repetitions and calculating the average, typical deviation, and variation coefficient that resulted in ≥3.1%, then the average was multiplied by 10 (ISTA, 1996). The seed length (SL), width (SW), and thickness (ST) were measured with a digital caliper (Truper®), and the ratio between seed length and width (LWR) was calculated. The humidity content was determined with the stove method (ISTA, 2014) at 70 °C for 72 hours, and 25 complete seeds were used with three repetitions. The weight was recorded in an analytical balance (Sartorius®, BP221S), and calculations were made based on the fresh weight (Poulsen, 2000; Lezcano *et al*., 2007). The humidity content (H_C) was calculated with Eq. 1 and expressed in percentage, were H_C = humidity content, W_1 = weight of the container expressed in g, W_2 = initial container and seed weight (g) and W_3 = final container and seed weight (g).

$$H_C = (W_1 - W_3) * \frac{100}{(W_1 - W_2)} \qquad \text{Eq. 1}$$

Imbibition Kinetics

Using an analytical balance (Sartorius®, BP221S), 25 g of seeds of each variety were weighed. Later, they were submerged in distilled water for 36 h at room temperature (23±2 °C). The increase in weight was registered every two h and the amount of water absorbed was expressed through Eq. 2, where W_{ad} = water adsorbed, W_i = initial weight, W_f = final weight, and H_i = humidity content (Domínguez-Domínguez *et al*., 2007).

$$W_{ad} = \frac{W_f - W_i}{W_i\left(1 - \frac{H_i}{100}\right)} \qquad \text{Eq. 2}$$

Physiological Quality in the Laboratory

The standard germination test (SGT) was carried out according to the ISTA rules (2014) with modifications. Fifty seeds were distributed in five rows in a transparent polyethylene clamshell with a layer of cotton previously moistened with distilled water. These were then placed inside a germination chamber at temperature of 25±2 °C. Counting started from the first day and germination was calculated using Eq. 3 and expressed as a percentage, where *GP* = germination percentage, *ni* = total germinated seeds, and *N* = total seeds sampled.

$$GP(\%) = \frac{ni}{N} * 100 \qquad \text{Eq. 3}$$

The germination speed index (GSI) was obtained through the methodology proposed by Maguire (1962) and Martínez-Solís *et al.* (2010). Germinated seeds were counted daily, seeds with sprouted radicles were considered, and Eq. 4 was applied, where *GSI* = germination speed index, *Ti* = time in hours passed between the test start and the end of the interval, and *Ni* = number of germinated seeds within consecutive time intervals.

$$GSI = \sum \frac{Ni}{Ti} \qquad \text{Eq. 4}$$

The coefficient of germination speed (CGS) is a distribution measure based on the number of germinated seeds through time and the number of germinated seeds per day (González-Zertuche and Orozco-Segovia, 1996), and was determined by Eq. 5, proposed by Kotowski (1926), where *CGS* = coefficient of speed, *n* = number of seeds germinated per day *i*, and *t* = number of days since planting.

$$CGS = \frac{\sum ni}{\sum (niTi)} * 100 \qquad \text{Eq. 5}$$

The viability analysis (V) was done with the technique involving tetrazolium chloride, described by ISTA (2014) and Maldonado-Peralta *et al.* (2016). One-hundred seeds were placed in an uncovered jar with distilled water, and this was put in a water bath at a temperature of 35 °C for 14 hours. Later, a 1% tetrazolium chloride solution was added, and the jar of seeds was put in a water bath at a temperature of 35 °C for 4 h. Finally, the seeds were rinsed with distilled water and examined under a stereoscopic microscope (LEICA, EZR®). The embryos were classified according to color intensity: 1) alive with high vigor, when they were completely dyed with an intense red color, 2) alive with low vigor, when their coloration was a pale red, and 3) not viable, when they remained colorless. This was expressed as the percentage of viable and unviable embryos.

Data Analysis

The data obtained for each measured variable in percentages was transformed with the *Arcsine* $\sqrt{x/100}$ formula and the averages were compared in pairs using the Student's t-test (α=0.05). For all the physical and physiological characteristics evaluated in the seeds, a correlational analysis was done with the Pearson test (α=0.05), using the MINITAB 18 statistical software.

RESULTS AND DISCUSSION

Physical Characterization

The analysis demonstrated statistical differences ($P<0.0001$) in seed size among varieties, except in the length/width ratio, which was statistically equal ($P=0.427$). Concerning the weight of 1000 seeds, V_1 was statistically different ($P<0.0001$) from V_2 (Table 1). Mansouri *et al.* (2017) reported average values of 7.75, 3.50, and 1.00 mm in length, width, and thickness, respectively. The weight of 1000 seeds fluctuated between 40.66 and 49.57 g, similar to the weight of V_2 in this study.

Karayel *et al.* (2004) evaluated the uniformity of a seeder with different vacuum pressures on different seeds. The averages found in melon seeds were 11.44 mm in length, and 4.62 and 2.35 mm in width and thickness, respectively, while the weight of 1000 seeds without coats (testa) was 36.70 g. The physical attributes of seeds are important for calculating the surface area and volume of the grains to model the storage and design of separation equipment (Sologubik *et al.*, 2013; Mansouri *et al.*, 2017), as well as agricultural implements (Karayel *et al.*, 2004).

Table 1. Average values for physical quality variables of Creole melons (*Cucumis melo* L.).

Variety	SL (mm)	SW (mm)	TS (mm)	LWR	W1000s (g)
V_1	10.97 ± 0.21^a	5.06 ± 0.04^a	1.74 ± 0.05^a	2.16 ± 0.05^a	172.3 ± 13^a
V_2	6.26 ± 0.11^b	2.82 ± 0.05^b	1.38 ± 0.02^b	2.21 ± 0.08^a	41.27 ± 1.27^b
p value	<0.0001	<0.0001	<0.0001	0.427	<0.0001
C.V.	1.95	1.37	2.66	3.36	8.66

Means ± standard deviation with different literals is statistically different with a confidence level of 95% (Student's t). SL = seed length. SW = seed width. TS = thickness of seeds. LWR = length / width ratio. W1000s = weight of one thousand seeds. C.V. = coefficient of variation.

Imbibition Kinetics

Figure 1 shows the imbibition curve of Creole melon seeds, in which within the first two hours, the water adsorbed by the seed increased in weight by approximately 56% in V_1 and by 42% in V_2, which constitutes the first phase of imbibition (Suárez and Melgarejo, 2010). Domínguez-Domínguez *et al*. (2007), for their part, found the first phase in hibiscus seeds (*H. sabdariffa* L.) between the first six and 10 h. These authors explain that, since they are irregular geometric bodies, the speed of water transference to the interior cannot solely be explained by geometric variables, such as the volume to surface area ratio.

Teixeira Pinheiro *et al*. (2016) report that melon seeds induced to saline stress in different imbibition times, increase enzymatic activity and decrease germination rates. In the end, V_1 seeds stopped water absorption after 32 h, while V_2 after 18 h, at which time the biochemical activity begins and the radicle appears (Maynard, 2007).

Physiological Quality

Table 2 shows that the percentage of humidity and germination were statistically different between V_1 and V_2 ($P=0.010$ and $P=0.030$, respectively); viability, speed index and coefficient were statistically equal ($P \geq 0.065$). Barros-Torres and Marcos-Filho (2003) evaluated the humidity content in different lots of two melon hybrids and noted it fluctuated between 7.0 and 7.3%, while in this study, V_2 was reported to have a lower content (5.77%). These same authors indicate that the variation in humidity content of the seeds is probably due to their size. Casenave and Toselli (2010) reported that in 'Honeydew' melon seeds subjected to different conditions of water stress, germination was ≤94%, and they indicate that by modifying the water potential, the percentage of germination was reduced in the seeds.

Nery *et al*. (2007) reported that in seeds of five watermelon cultivars (*C. lanatus* L.), germination values were 39% in a triploid hybrid and from 89 to 99% in diploids. Of viable seeds, the triploid hybrid presented 83% and the diploids >98%, similar values to those obtained in this study. The quality of *C. Lanatus* L. seeds could be low in hybrids (triploids compared to diploids) due to the time they take to germinate and that they present embryonic malformation problems from chromosomal alterations necessary to avoid seed formation (Souza *et al*., 1999; Grangem *et al*., 2003; Nery *et al*., 2007). The speed index, reported by these same authors, was >2.24; while in V_1 melon seeds, it was 2.24, and in V_2 it was 2.08. Concerning the speed coefficient, 44% and 48% of V_1 and V_2 seeds, respectively, germinated per day. In *Arachis hypogaea* L. genotypes, Caroca *et al*. (2016) found that GSI varies from 2.94 to 20.84 due to the effect of temperature, and they note that seeds exposed to high temperatures (>32 °C) are negatively affected in germination speed.

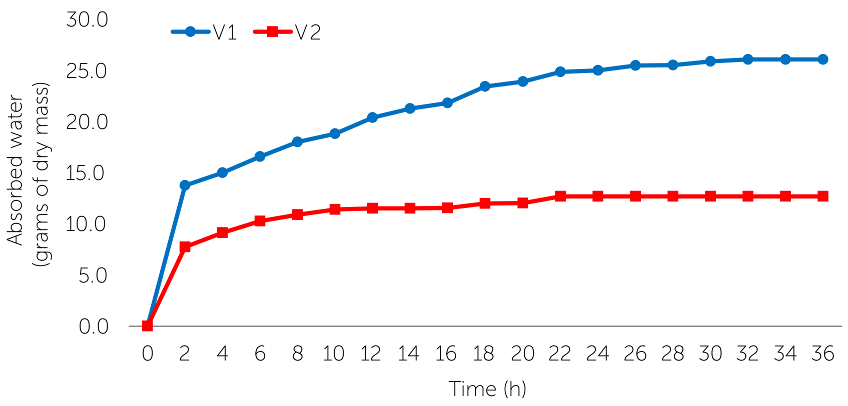

Figure 1. Imbibition curves of two varieties of Creole melon (*Cucumis melo* L.) seeds from the Costa Chica region of Guerrero, Mexico.

Regarding the viability analysis, the results obtained showed that 78% and 90% (Table 2) of V_1 and V_2 seeds, respectively,

Table 2. Average values for physical quality variables in Creole melons (*Cucumis melo* L.).

Variedades	H (%)	GP (%)	V (%)	GSI	CGS
V_1	7.19 ± 0.27^{a}	84.0 ± 6.0^{b}	78.0 ± 8.0^{a}	2.24 ± 0.18^{a}	0.44 ± 0.03^{a}
V_2	5.77 ± 0.46^{b}	96.0 ± 2.0^{a}	90.0 ± 2.0^{a}	2.08 ± 0.07^{a}	0.48 ± 0.01^{a}
p value	0.010	0.030	0.065	0.230	0.227
C.V.	5.89	4.97	6.94	5.37	5.98

Means ± standard deviation with different literals is statistically different with a confidence level of 95% (Student's t). H = humidity. GP = germination percentage. V = viability. GSI = germination speed index. CGS = coefficient of germination speed. CV = coefficient of variation.

presented complete staining of the embryo and cotyledons (Figure 2A and 2C), indicating that they were alive; while Figure 2B and 2C show the unviable seeds that were not stained by the tetrazolium.

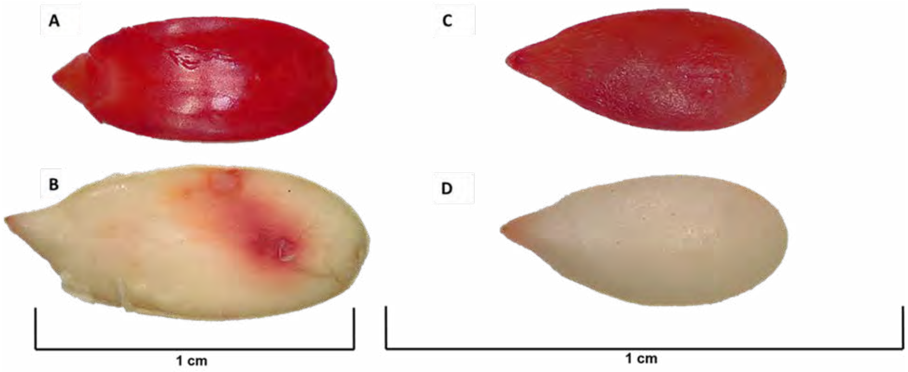

Figure 2. Viability trial in Creole *Cucumis melo* L. seeds. A and C) Viable seeds of V_1 and V_2. B and C) Unviable seeds of V_1 and V_2.

Barone *et al*. (2016) report that the greatest difficulty to ensure good interpretation of results is finding an adapted protocol; although it has been used in different species, there are limited reports on melon seeds. However, Inácio-Barros *et al*. (2005) showed that the tetrazolium test is efficient to determine the viability of squash seeds, finding between 59 and 100% of viable seeds among different batches. Another factor that influences viability is the age of the seed. Enríque-Peña *et al*. (2004) reported that seeds of *Taxodium mucronatum* (Ten.) presented an average reduced viability of 63% after 21 months in storage.

Correlation Analysis

A high positive correlation (r^2>0.859) was found between humidity and length, width, and thickness, and P1000s (Table 3). Concerning the germination percentage, it presented r^2=0.954 with high significance (P=0.003), where a trend or increase was observed between germination and viability, and with these results, the yield potential of the studied varieties can be predicted (Panwar *et al*., 2018). Likewise, the physical characteristics of the seeds showed positive correlations, indicating that weight is influenced by the seed dimensions (Table 3). Nuraini *et al*. (2018) suggested that associations in seed quality, specifically seed weight, are useful for improving the existing varieties and developing new genotypes.

A negative correlation (r^2=−0.868) was found between humidity and germination, where it was observed that the higher the humidity content, the lower the germination percentage. This could be explained with Table 1, where

Table 3. Correlational analysis of physical and physiological variables in Creole melon (*Cucumis melo* L.) seeds.

	H (%)	GP (%)	GSI	CGS	V (%)	w1000s (g)	SL (mm)	AS (mm)	TS (mm)
PG (%)	−0.868*								
	0.025**								
GSI	0.591	-							
	0.217	-							
CGS	-0.574	-	−0.998						
	0.234	-	0.001						
V (%)	-	0.954	-	-					
	-	0.003	-	-					
W1000s (g)	0.895	−0.865	0.516	−0.517	-				
	0.016	0.026	0.294	0.293	-				
LS (mm)	0.903	−0.828	0.581	−0.586	-	0.987			
	0.014	0.042	0.227	0.221	-	0.001			
AS (mm)	0.921	−0.858	0.554	−0.555	-	0.996	0.996		
	0.009	0.029	0.254	0.253	-	0.001	0.001		
TS (mm)	0.859	−0.743	0.512	−0.525	-	0.969	0.990	0.979	
	0.028	0.091	0.299	0.285	-	0.001	0.001	0.001	

* Pearson's correlation coefficient (α=0.05). ** p values with a confidence level of 95%. GP = germination percentage. V = viability. GSI = germination speed index. CGS = coefficient of germination speed. SL = seed length. SW = seed width. TS = thickness of seeds. LWR = length / width ratio. W1000s = weight of one thousand seeds. C.V. = coefficient of variation.

seeds of V_1 showed a greater humidity percentage and a lower germination percentage compared to V_2, which was the inverse. The physical characteristics showed a significant negative association with the germination percentage. The speed index and coefficient presented $r^2 = -0.998$ with $P = 0.001$ as an inverse value; that is, the higher the germination average per day, the lower the value obtained for the Kotowski index (speed coefficient) (González-Zertuche and Orozco-Segovia, 1996).

CONCLUSIONS

The physical and physiological characteristics among varieties showed differences in seed dimensions and weight. Concerning the physiological variables, V_2 presented >90% in germination and viability, which are tools used for genetic improvement and increased yield in the field.

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