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Coffee (*Coffea arabica* L.) harvesting time and its influence on the seed quality of the Costa Rica 95 and Garnica varieties

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ABSTRACT

Objective: To determine the impact of the harvesting time of two coffee varieties on the physical quality and viability of seeds, using the tetrazolium testing.

Design/Methodology/Approach: The research was carried out using a completely randomized design; the evaluation of the embryo viability was based on a five-color pattern staining design. The Garnica and Costa Rica 95 varieties and two harvesting times were used (December and January). An analysis of variance and a Tukey's means comparison test ($p \leq 0.05$) were carried out, using the SAS 902 software.

Results: The study varieties showed significative differences in all the variables related to the physical quality of the seeds, including volumetric weight and weight of 1,000 seeds. The best seed viability was obtained during the harvest carried out in January.

Study Limitations/Implications: The results obtained are limited to the varieties in question, as well as the environmental conditions and period during which the said varieties were evaluated.

Conclusions: The harvesting time of the two varieties of coffee has an influence on the physical characteristics of the seeds and on the viability, evaluation carried out using tetrazolium.

Keywords: viability, tetrazolium, coffee, physical characteristics.

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INTRODUCTION

Genetically speaking, most coffee (*Coffea arabica* L.) varieties worldwide are very similar; however, morphologically speaking, they are very different, and their fruits have contrasting pre- and post-harvest qualities (Steiger *et al.*, 2002).

In the international market, some environmental factors are known to influence the quality of coffee, such as the altitude of the coffee plantation (Bertrand *et al.*, 2006) and the shade under which it grows (DaMatta *et al.*, 2007). Nevertheless, the chosen variety also

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plays a key role in any production system, because the quantity and quality of the harvested fruits depends on the genotype selection and its capacity to adapt to the environment (Hein and Gatzweiler, 2005; Kathurima *et al.*, 2009).

Good quality seeds are required to produce excellent plants; consequently, several studies have been carried out to establish the optimal harvesting time that results in good quality seeds. The main harvesting time of coffee in Mexico goes from January to March (ASERCA, 2010); however, in some regions, the harvesting time starts in December. Nevertheless, this harvesting time is very long and the ripening of the fruits varies widely. Consequently, the seeds produced have a highly variable physiological quality.

In order to establish coffee plant greenhouses, the best seeds from well-ripened fruits must be chosen. These fruits must be harvested from healthy and well-developed plants, whose productivity has been proved. They must be 4-7 years old and free of pathogens and diseases. Usually, the best fruits can be in the middle part of the branches, in the center of the plant (Aranda-Bezaury *et al.*, 2017). However, the fruits are usually harvested regardless of their color or ripening stage. They are taken from different parts of the plant and, sometimes, they are harvested at different times without a post-harvest classification process. Usually, this situation has an impact on the different physiological or physical quality of the coffee seeds which, on their turn, produce seedlings of different germination and vigor. Therefore, the objective of this research was to determine the importance of the harvesting time of two varieties of coffee and its relationship with the physical quality and viability of the seed, which was evaluated using the tetrazolium testing.

MATERIALS AND METHODS

Plant material and experiment location

Seeds from the Garnica and Costa Rica 95 varieties were used. They were harvested in Zacamitla, Ixhuatlán del Café, Veracruz, Mexico. The laboratory stage was carried out in the Laboratorio de Análisis de Semillas of the Postgrado en Recursos Genéticos y Productividad-Programa de Producción de Semillas, Colegio de Postgraduados - Campus Montecillo.

Harvest

Two harvests were carried out: one in mid-December and the other in mid-January. The fruits were harvested by hand when they reached an optimal ripening —*i.e.*, when the color of the fruits ranged from "Cherry" red to a dark red (Aparecida-Sagio *et al.*, 2013). Immediately, the pulping was carried out and the resulting raw material was left to ferment for 24h to obtain the seed. Subsequently, the seed was washed and dried in the shade. Once it was dry, the physical quality and viability were determined using the tetrazolium testing.

Treatments and experimental design

A 2×2 factorial experiment was conducted, using the following four treatments which were the result of combining two varieties and two harvesting times: Treatment 1: Costa Rica variety, harvested in December (CR-D); Treatment 2: Costa Rica variety, harvested in

January (CR-J); Treatment 3: Garnica variety, harvested in December (GAR-D Treatment 4: Garnica variety, harvested in January (GAR-J).

A completely random design was used, and the experimental units were made up of Sanitas towels with 100 seeds. Each treatment had four repetitions.

When the seed had reached the appropriate humidity, the physical quality variables were determined. Subsequently, the embryo viability was established. All the quality evaluations of the seeds were carried out according to the international standards of the ISTA (ISTA, 2015).

Seed quality characteristics

Humidity Content (**HC**). In order to determine the humidity content, the stove dried method was used (Central Scientific Division of CENCO). The seeds were dried at 103 °C for 20 h. Two repetitions were carried out to determine humidity content, using 5-cm diameter aluminum boxes with tops. Subsequently, 10 g of pure coffee seeds were placed in the boxes. Once the box and the seeds had been weighted, the boxes were placed over the tops and put in the stove. After the dry procedure, the boxes were taken out of the stove and they were immediately weighted. A ≤0.2% difference was recorded and therefore humidity determination was considered appropriate (ISTA, 2009; MAPA, 2009).

The humidity content was calculated using the following formula:

$$\%Humidity = M_2 - M_3 \times \frac{100}{M_2 - M_1}$$

Volumetric Weight (**VW**). The volumetric weight was determined from a 100 g pure coffee seed sample, which was poured into a 250-mL test tube. Subsequently, the value was determined based on the volume it occupied. The data were obtained from each of the four repetitions per treatment. The volumetric weight was calculated as follows:

$$VW = \left(\frac{100\,g\ seed\ weight}{Volume\ occupied\ by\ the\ 100\,g\,(ml)} \right) \times 100$$

Weight of 1,000 seeds (**WTS**). The weight of 1,000 seeds was determined counting and weighting eight repetitions of 100 coffee seeds. Based on the data obtained, the mean, variance, standard deviation, and variation coefficient were calculated. When the coefficient of variation was <4%, we considered that the data was correct. The weight of a thousand seeds was obtained multiplying tenfold the arithmetic mean of the eight repetitions; the result was expressed in grams (ISTA, 2015).

Shape of the seed. The different shapes of the seeds that prevail in coffee varieties are part of their physical quality. They are frequently considered as characteristics that are sometimes correlated with the germination behaviour, viability, and vigor of the seeds. Based on their shape, coffee seeds are classified as: flat coffee seed, peaberry coffee seed,

triangular coffee seed, and black Ivory coffee seed. Therefore, a physical characterization of the seeds of all treatments —resulting from the combination of the two study varieties and the two harvesting dates— was carried out. Subsequently, the percentage of composition was determined based on the different shapes of the coffee seeds.

Digital analysis of the coffee seed images. As part of the characterization of the physical quality, coffee seed images of the four evaluated treatments were processed. The said images were taken with an Epson scanner and were processed in a HP laptop, using the version 1.46r of the ImageJ software (Ferreira and Rasband, 2012). Four-hundred coffee seeds —divided up among four repetitions of 100 seeds— were used per variety. The following variables were recorded: area, perimeter, length, and width of the coffee seeds (Linskens and Jackson, 1992).

TZ viability (TV). In order to extract the embryos, a 100-coffee seeds sample was taken from each of the treatments —obtained from the combination of the varieties and the harvesting times— following the Dias and Silva (1998) methodology, with some adaptations (Figure 1).

The parchment of the coffee seed was extracted from 100 seeds. Subsequently, they were placed into distilled water for 24 h, at room temperature. Afterwards, lengthwise sections and cross sections were made to allow the water to enter and to soften the seeds.

After the sections were made, the water of the seeds was changed, and they were submerged again in distilled water for 24 h to perform the extraction. Later, the embryos were extracted and placed in distilled water. Figure 2 shows the modified extraction sequence.

A 1.0%-concentration tetrazolium solution (TZ, 2,3,5-tryphenil tetrazolium chloride) was prepared, diluting one gram of tetrazolium salt in 100 mL of distilled water, with a 7.0 pH.

The embryos were placed in the solution and then moved to a light-less chamber for 24h. Subsequently, the TZ excess was eliminated, and the stained embryos were observed. Their coloring varied from pink to red, indicating that the embryos were alive. Meanwhile, unstained embryos were considered dead, because they did not show any reaction (França-Neto *et al*., 1998).

Evaluation of the embryos. The embryos were placed in a previously soaked Sanita paper towel, to avoid dehydration. Subsequently, they were put under an Olympus SZX7 stereoscopic microscope using tweezers and the staining pattern was determined. The number of viable embryos was determined according to the staining pattern obtained, using the Munsell color chart (Munsell Color Charts, 1977) and they were classified into several categories: 2.5 R 4/10 dark red (viable embryo); 2.5 R 7/8 soft red (viable embryo);

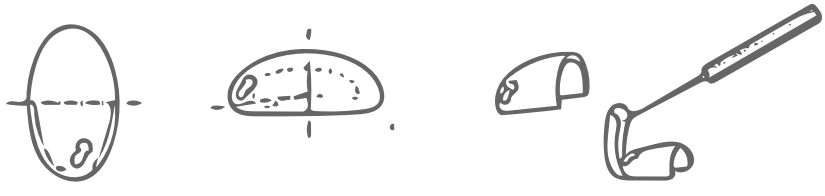

Figure 1. Extraction of a coffee seed embryo (Días and Silva, 1998).

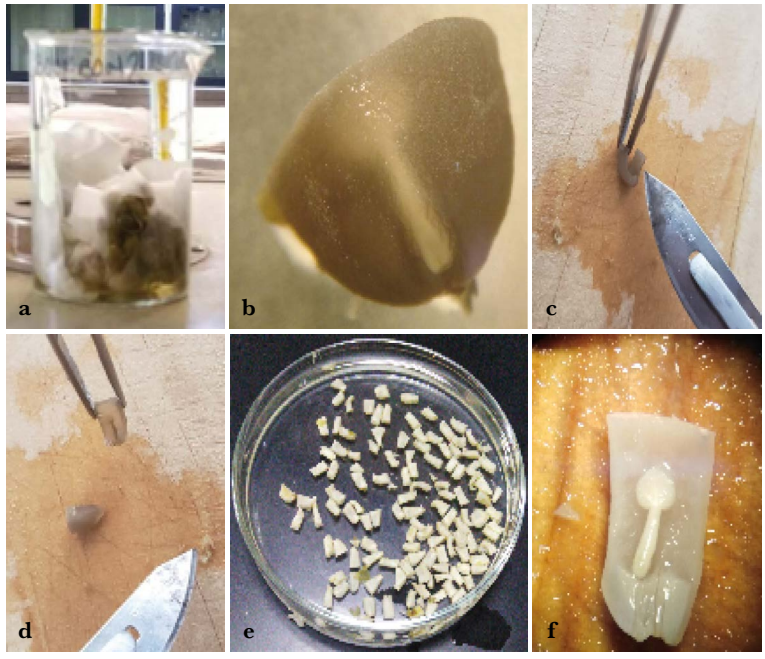

Figure 2. Embryo extraction (adapted methodology). a) First 24h of imbibition. b) Embryo localization. c) Separation of the endosperm. d) Endosperm sheet with an embryo. e) Sheet with an embryo placed in water for 24h. f) Embryo without endosperm. The viability percentage was calculated using the following formula:

$$\%\ viability = \frac{mean\ value\ of\ viable\ embryos}{Number\ of\ coffee\ beans\ used\ per\ repetition}.$$

2.5 R 7/4 dark pink (viable embryo); 1.5 R 8/4 soft pink (viable embryo); and 2.5 R 8/2 white (non-viable embryo) (Figure 3).

Statistical analysis

The normality assumption, the homogeneity of variances, and the multi-collinearity were verified before the statistical analysis was carried out. The non-normal data were transformed using the $\sqrt{X/100}$ arcsine function. An analysis of variance and a Tukey's

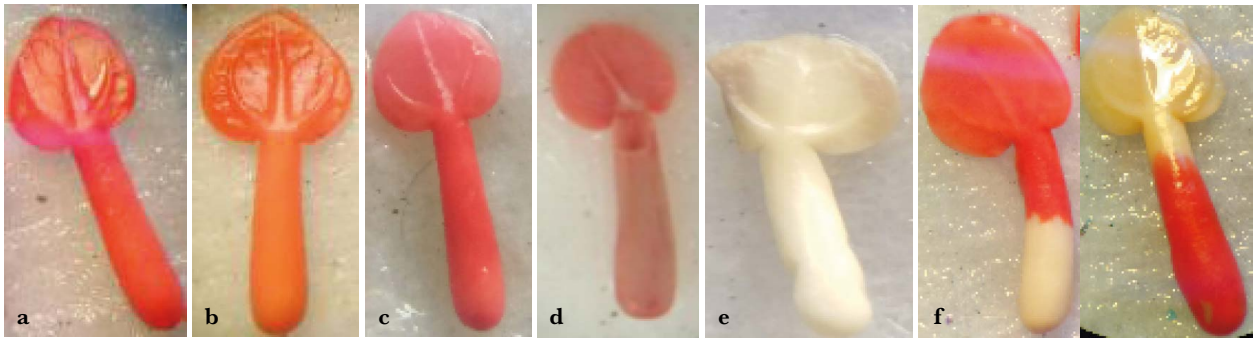

Figure 3. Staining pattern of coffee embryos. a) 2.5 R 4/10 dark red (viable embryo), b) 2.5 R 7/8 soft red (viable embryo), c) 2.5 R 7/4 dark pink (viable embryo), d) 1.5 R 8/4 soft pink (viable embryo), e) 2.5 R 8/2 white (non-viable embryo), and f) Some essential parts of an embryo without staining (non-viable embryos).

mean comparison test ($p \leq 0.05$) were carried out for each of the response variables, using the SAS statistical package software (SAS Institute, 2002).

RESULTS AND DISCUSSION

Table 1 shows the behavior of the quality variables of the coffee seeds.

There were significant differences ($p \leq 0.001$) in the physical quality variables, volumetric weight, and weight of 1,000 seeds of coffee. There were significative differences, both in the independent effects and the interaction (V×E) variables. The differences found in these physical quality variables of coffee seeds indicate a good agronomic management in the field. The environmental conditions during the crop development have a high impact on management. Ultimately, these environmental conditions have an impact on the seeds, although the genetic component should not be forgotten. Additionally, the significative effect during the harvesting times indicates that the quality of the seeds is determined in part by the moment in which the harvest is carried out.

In crops such as maize, the genetic component has greater influence in attributes such as volumetric weight and weight of a thousand seeds than the environment itself (Flint-García *et al.*, 2009; Torres *et al.*, 2010).

Table 1 also shows the results of the viability evaluated using the TZ testing, according to the harvesting time of both varieties. There were significative differences in the variables ($p \leq 0.01$) and in the harvesting times ($p \leq 0.001$), while the interaction was not significative. The germination potential of a seed is frequently evaluated using the TZ testing which has a high correlation with the behavior of the seeds, when they are subject to a direct evaluation in a standard germination evaluation. In the case of the variables evaluated in this work, Costa Rica 95 had a different viability behavior. Perhaps the genetic factor is involved in this behavior. This situation can be related to the non-significative results of the interaction, which indicates that, in both varieties, TZ-evaluated viability can also be determined by the genetic fact and the harvesting time. However, the resulting significance indicates that harvesting during different periods has an influence on the differential quality of coffee seeds. If we relate the obtained viability results with the harvesting time, it is not surprise that the coffee fruits selected for the production of high-quality coffee seeds are chosen from intermediate and late harvests and rarely from early harvests. These fruits are taken from the middle of the plant and from the middle of the branches: the areas where fruits of even color and size can be located. This practice is always reflected in better quality coffee seeds (Aparecida-Sagio

Table 1. Mean squares of the quality variables of coffee seeds from two varieties of coffee in two harvesting times.

Variable	Sources of Variation			Coefficient of variation	R^2
	Varieties (V)	Harvest time (H)	V×H		
Volumetric weight	16.81***	23.04***	27.04***	0.82	0.97
weight of 1000 seeds	27.73***	41.84***	44.16***	5.84	0.63
Viability with TZ (%)	64.00**	196***	ns	2.87	0.75

, * Significative with $\alpha = 0.01$ and 0.001, respectively; ns=not significative.

et al., 2013). Both the coefficients of variation and the coefficients of determination were acceptable, which shows the goodness of fit of the model regarding the importance of the variables considered by the said model.

Means comparison

Table 2 shows the average behavior of the varieties (Tukey, 0.05) regarding the physical quality and viability evaluated with TZ testing, according to the harvesting times. Regarding the effect of the harvesting time, the best behavior for all the variables took place when the seeds were harvested in January, although Garnica had the best behavior.

The Garnica variety that had the highest number of stained embryos (96.5%); this percentage belonged to the January harvest. This could be the result of the smaller size of the seed of the Costa Rica 95 variety (Aguilar-Vega, 1995), which suffers less damage and consequently has an enhanced viability. In all the cases, when comparing the physical characteristics of the coffee seeds —for example, those obtained subjecting the seeds to image digitalization, such as area, perimeter, length, and width (data not shown)—, differences were found between the two evaluated varieties. In all cases, the best quality of coffee seeds —according to the abovementioned physical parameters— was obtained by Garnica. This matches the results obtained.

Meanwhile, in this study, the physical characterization data was recorded according to the prevailing shape or type of coffee seeds in both coffee varieties (data not shown). Samples were gathered both from the January and December harvests. The quantity of the flat coffee seed —which is the biggest and more even-shaped seed and the one that prevailed— was quantified as the number of coffee seeds/kilogram. As a result, Garnica (679.9) obtained less flat coffee seeds than Costa Rica 95 (908.8). However, these data were remarkably similar in both harvesting times, matching the results of Castillo-Zapata and Moreno-Ruiz (1988), regarding the shape and types of coffee seeds (peaberry. "monster," and triangular coffee seeds, etc.), which are determined by genetic factors and meiotic irregularities rather than environmental or management factors. Further research is required on this matter. This research must include a detailed follow-up of field data and a combination of study factors —such as position of the fruits in the plant and the branches,

Table 2. Quality variables of coffee seeds from two coffee varieties in two harvesting times.

Source of variation	**Variable**		
Harvest time	**Weight of 1000 seeds**	**Volumetric weight (g mL^{-1})**	**Viability with TZ (%)**
January	27.46 a	51.83 a	98.00 a
December	25.17 b	49.43 b	91.00 b
Varieties			
Garnica	27.24 a	51.65 a	96.50 a
Costa Rica 95	25.38 b	49.60 b	92.50 b
MSD	1.12	0.45	2.95

MSD=minimal significant difference. Mean with the same letter inside the columns are not significatively different (Tukey, α=0.05).

ripening level of the fruit, harvesting time, size and shape of the coffee seed, etc.—, in order to determine their influence on the quality of the coffee seed.

CONCLUSIONS

January was the best harvesting time, which occurred simultaneously with a better physical quality of the coffee seeds, recording the highest volumetric weight, the weight of 1,000 coffee seeds, and viability. Garnica was the variety that behaved the best in all the variables (both physical quality and viability of the coffee seed evaluated with TZ testing). The viability percentage was higher in the coffee seeds from both varieties harvested in January.

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