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Morphological Aspects of Seeds, Emergence and Growth of Seedlings of Surinam Cherry Trees Sown at Different Depths

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Abstract: It was intended to obtain the correlations between the biometric characteristics and the influence of the seed size at different depths upon the initial vigor of seedlings of *Eugenia uniflora*. The work was conducted at Embrapa Roraima, in the Seed Analysis Laboratory and greenhouse. The experimental design (CRD), with the treatments in factorial scheme 2x3, with five replications, was utilized. The separation of the intact seeds and classified into three classes of mass: small (0.6 to 0.79g), medium (0.8 to 0.99g) and large (1.0 to 1.19g) was performed as well as determinate the length, breadth, and thickness. Seeds (small, medium and large) were sown in sand bed at the depth of 2 and 4 cm and maintained for 90 days in nursery with 50% of shading. At 90 days after sowing (DAS), measurement of the shoot height, main root length was carried out and, number of leaves, dry and fresh mass of the shoot and root system and total of the seedlings was determined. As conclusion, the biometric characteristics length, thickness and width of seeds can estimate the seed mass of *Eugenia uniflora*, due to the strong correlation among these characteristics. Medium and large-sized *E. uniflora* seeds sown 4cm in depth in medium textured sand constitute a promising option aiming the production of vigorous and better quality seedlings.

Keywords: *Eugenia uniflora* L., Viability, Seed Vigor

1. Introduction

In Brazil, there is a great biodiversity of native fruit-bearing trees, among them the surinam cherry tree (*Eugenia uniflora* L.; Myrtaceae) [1]. Due to its adaptability, it was disseminated and it is at present found in the most different regions of Brazil and of the world [2] and its fruits are appreciated by the wild fauna [3]. The wide geographic distribution, from Bahia to Rio Grande do Sul, presents broad expansion possibilities and economic potential specially due to some unique characteristics of its fruits, such as exotic taste, high contents of A vitamin, total carotenoids and good acceptability among consumers [4, 5].

It is estimated that Brazil is the greatest world producer of surinam cherry fruit, although the commercial growing is restricted to the states of Pernambuco and Bahia [4]. In the state of Pernambuco, about 300 ha are cultivated with Surinam cherry trees and produce between 1.300 and 1.700 t. year^{-1}, which means a yield of around 5 t ha^{-1} [6].

Most of the existing Surinam cherry tree orchards is formed from seedlings resulting from propagation through seeds, being relatively easier and less expensive, compared with the clonal propagation methods, by cutting and *in vitro*. But, scarcity of research related is found to production of rootstock for Surinam cherry seedling production [7], which could contribute towards the obtainment of more productive and profitable orchards [8, 9].

In this context, it becomes necessary to develop strategies to add quality to the production chain of tropical fruit-bearing trees, like *E. uniflora.* The obtainment of seedlings with high standard is essential to warrant the competitivity of the nursery and the return of investment made in the business establishment, besides warranting customer´s meeting of his needs and to the seedling producer good reputation and stability of the business over time.

[10] studying the quality of surinam cherry seeds in relation to fruit mass and seed size in their physiological maturity, found that small seeds were the most viable, but medium and large seeds gave rise to more vigorous seedlings.

During ripening, seeds grow in size till they reach the characteristic value for the species [11], but within the same species there are individual variations due to environmental influence during seed development and due to genetic variability among the parent plants [12, 13]. In this way, seed size can range both between and within parent trees. According to [14] the morphometric traits of seeds as well as the mass present a direct relationship with the physiological quality which can be reflected into better percent of germination, emergence and vigor of seedlings.

On the other hand, the adequate sowing depth of seed provides homogeneity at germination and uniformity of seedlings [15]. Studies conducted by [16] evaluating the effects of different positions and sowing depths at emergence and initial growth of seedlings of *Talisia esculenta,* belonging to the family of Sapindaceae, found that sowing depth affected the emergence and initial growth of seedlings so that sowing should be at depth between 2.5 and 3.0 cm.

However, emergence of seedlings of *Alternanthera tenella* Colla of the family Amaranthaceae was significantly reduced from depth of 4.0 cm on, becoming null when the seeds were positioned at 10 cm [17]. For the species *Oenocarppus minor* Mart. (Palmae Jussieu) emergence percentage reduced with increasing sowing depth [18] while for *Zizyphus joazeiro* Mart. (Rhamnaceae) emergence percentage of seedlings increased as sowing depth increased, reaching the maximum of 88% at 1.6 cm, since from that depth on, marked reduction at emergence percentage of seedlings was found, reaching values close to 40% at the depth of 5 cm [19].

Knowledge about biometric aspects of seeds and sowing depth and their influence upon germination and initial vigor of *Eugenia uniflora* seedlings, are still, scarce, above all, in relation to surinam cherry rootstocks, there need for research being needed over this subject. So, it was aimed to obtain the correlations on seed biometric characteristics and to understand the influence of the size of seeds at different sowing depths on the initial vigor of *Eugenia uniflora* seedlings.

2. Material and Methods

2.1. Description of the Study Area

The research was conducted at Embrapa Roraima, in the Seed Analysis Laboratory (LAS) at temperature of 22 ± 4°C and greenhouse, with annual average temperature of 25.5°C. The propagative material utilized consisted of seeds coming from plants of *E. uniflora*. The planting of the experimental area was accomplished in 2005, with 5.0 x 1.5 m spacing, amounting to 1.333 plants ha^{-1} in a private farmer in the municipality of Cantá in the state of Roraima (N 2°23'45.31"; W 60°58'44.34"). The altitude is of 90 m above sea level and the soil is classified as Dystrophic Yellow Latosol, medium textured, containing 1.5% of organic matter and 15.5% of clay.

2.2. Experimental Methodology

After harvest, the fruits were placed into plastic boxes and taken to the Seed Laboratory where the removal of pulp and washing of seeds in running water was performed. Seeds were subsequently dried at the LAS at temperature 24 ± 4°C, for 48 hours. In sequence, the seed water content was determined in oven (105 ± 3°C) for 24 hours [20], with four replications of 10 seeds.

At the same time, separation of seeds with intact external appearance was performed, taking as a basis the individual fresh mass of the seeds, obtained on precision balance (0.001 g). On the basis of the mass, the seeds sample presented distribution of frequency into values (0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2 g), since the seeds with masses of 0.5 and 1.2 g were discarded in order to prevent the marginal values from influencing the results. The other seeds owing to the quantitative ones necessary to the experiment were grouped into three classes of mass: small (0.6 to 0.79 g), medium (0.8 to 0.99 g) and large (1.0 to 1.19 g). With the aid of the digital precision pachymeter (0.01 mm), the length, breadth and thickness of 240 seeds water were measured for each class of mass. It was regarded as seed length the measure from the tip to the base and the breadth with thickness were taken at the median region of the seed.

The seeds characterized by the masses as small, medium and large were sown on a sand bed 2 and 4 cm deep maintained for 90 days in nursery with 50% of shading, the substrate moisture being maintained by means of scheduled sprinkling every four hours during the daytime, each watering lasting five minutes.

The experimental design was completely randomized with the treatments arranged in factorial scheme 2x3, with five replications and twenty seeds per plot. The first factor corresponded to two sowing depths (2.0 and 4.0 cm), while the second was composed by the three classes of seeds (small, medium and large) with 200 seeds, respectively. Daily, the counts of emergence from the first seedling at least 2 centimeters above surface till stabilization occurs. For evaluation of the effect of the treatments, seedling emergence and number of leaves per seedling were monitored for 90 days and emergence speed determined.

At 90 days after sowing (DAS) since the surinam cherry seeds (*E. uniflora*) can range as to the germination time from 7 to 60 days [21], added of 30 days for the seedlings to be able to develop before the evaluation. So, four samples with eight seedlings were taken, measurement of the height (cm), of the length of the root principal (cm), of number of leaves and of the dry and fresh matter mass of the shot and root system and total mass of the seedlings of *E. uniflora*. For obtaining the fresh and dry mass, each seedling was divided into root and shoot, since the roots were washed for elimination of substrate residues, later, after shade-drying, were weighted (root and shoot) and placed separated into paper bags, remaining in drying oven at 70 °C, with forced air circulation to constant mass (72 hours). After dried, they were again weighted in precision balance of 0.01 g for determination of the mass of the dry matter of the shoot (DMS), root system (DRS) and by the summation of these, the dry mass total of the seedling was calculated (DMT).

2.3. Statistical Analysis

The data obtained for the different variables were submitted to the variance analyses ADN the means were compared by the Tukey test at 5% of probability utilizing the Sisvar statistical software [22].

The variables breadth, thickness, length, seed mass, seedling emergence (%), emergence speed of seedlings, number of leaves were submitted to the Pearson analysis of correlation utilizing the [23]. The homogeneity and the normality of the sampling distribution were analyzed by Bartlett's and Shapiro-Wilk's tests, respectively.

3. Results and Discussion

3.1. Distribution of Frequency into Classes of Seeds

The tropical tree-like species present a great variability relative to fruit and seed size as well as in the seed mass [12, 24, 25]. The biometric description constitutes an important instrument to detect the genetic variability within populations of the same species and can provide information which allows the differentiation of species of the same genus [26, 27, 28].

The analysis of the class frequency distribution established by the mass of seeds of *E. uniflora* collected in the municipality of Cantá revealed that the seeds presented initial water content between 45 and 48% and that 90% presented between 0.6 and 1.19 g, following the normal frequency curve model (Fig. 1).

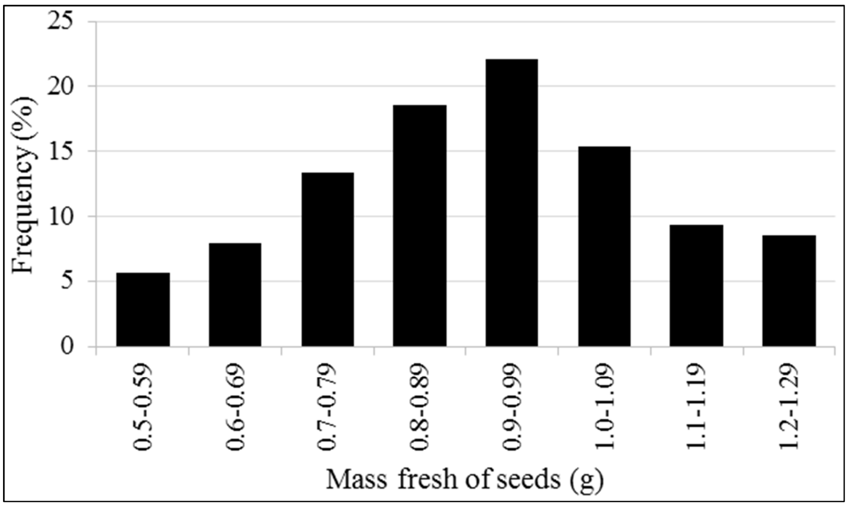

Figure 1. *Distribution of frequency into classes of fresh of seeds (%) of Eugenia uniflora obtained in the sample utilized in the study. Boa Vista, RR, 2015.*

3.2. Morphometric Characteristics of Seeds

For the thickness of the seeds of *E. uniflora*, variation between 0.7 and 1.0 cm in the classes of seed mass, but for the breadth, it was of 1.0 to 1.2 cm and the length from 1.2 to 1.5 cm. (Table 1).

Table 1. *Values of growth (cm), breadth (cm) and thickness (cm) of seeds of surinam cherry tree (Eugenia uniflora), obtained on the basis of three classes of seed mass: small (0.60 to 0.79 g), medium (0.80 to 0.99 g), large (1.0 to 1.19 g). Boa Vista- RR, 2015.*

	Size	Class	Medium	Variance	Maximum	Minimum	CV%
Length	Small	0.60-0.79	12.87±0.42	0.335	15.02	11.03	3.38
Breadth	Small	0.60-0.79	10.71±0.43	0.253	11.56	8.94	4.13
Thickness	Small	0.60-0.79	8.01±0.41	0.291	8.85	5.90	5.28
Length	Medium	0.80-0.99	13.71±0.39	0.244	15.48	11.51	2.91
Breadth	Medium	0.80-0.99	11.77±0.37	0.199	12.55	9.69	3.14
Thickness	Medium	0.80-0.99	8.89±0.36	0.224	10.91	6.54	4.07
Length	Large	1.0-1.19	14.54±0.35	0.162	15.51	12.65	2.36
Breadth	Large	1.0-1.19	12.41±0.37	0.187	13.68	10.09	2.99
Thickness	Large	1.0-1.19	9.26±0.44	0.322	11.74	8.04	4.68

CV%= coefficient of variance in percent.

3.3. Correlation Person, the Morphometric Characteristics of Seeds and Seedlings

Seeking to check the correlation among the biometric characteristics of the seeds, mass, sowing depth, size, seedling emergence (%), seedling emergence speed (index) and number of leaves, Person's correlation (r) was performed. The estimates of correlation for the quoted variables are in table 2, having as highest values the significant positive correlations of seed length with seed mass (Table 2), of seed length with seed breadth and of seed mass with emergence in sand (EA), and negative and significant correlations of VE with sowing depth and of the EA with sowing depth. According to the criterion of [29], the correlation is regarded as strong when it presents the variation coefficient of 0.8 ≤ p <1, in the present research the greatest coefficient occurred among the biometric variables of seeds and fresh mass of seeds with the values ranging from 0.80 to 0.99 classified as strong.

Table 2. *Matrix of Pearson's correlation coefficient of the characteristics sowing depth (SD), seed size (SS), emergence speed (EV, index), emergence of seedling in sand (ES), number of leaves (NL), fresh mass of seeds (MassS, g), length (mm), breadth (mm), thickness (mm) of seeds of Eugenia uniflora.*

Correlations	SD	SS	EV	ES	NL	MassS	Length	Breadth	Thickness
SD	1	-	-	-	-	-	-	-	-
SS	0.00	1	-	-	-	-	-	-	-
EV	-0.68	0.60*	1	-	-	-	-	-	-
ES	-0.50	0.74*	0.74*	1	-	-	-	-	-
NL	-0.29	0.48	0.49	0.77*	1	-	-	-	-
MassS	-0.10	0.98*	0.71*	0.79*	0.50	1	-	-	-
Length	0.10	0.96*	0.60*	0.68*	0.43	0.97*	1	-	-
Breadth	0.00	0.97*	0.67*	0.75*	0.55*	0.99*	0.99*	1	-
Thickness	-0.29	0.72*	0.71*	0.91*	0.77*	0.82*	0.80*	0.81*	1

*= significant at 5%.

The knowledge of the correlations existing between biometric characteristics and seed mass allows knowing the behavior of a variable by means of the analysis of another. In this case, with the measurements of breadth and thickness and mainly of the seed mass, one can estimate the amount in grams of seeds collected and the demand space for sowing. In addition, in a few species, the larger the seeds were, greater the germination rates and seed vigor [30].

In this way, the distinction and classification of seeds per mass and size can be an effective manner of improving the quality of lots of seeds in relation to emergence uniformity and seedling vigor [31], warranting increased values of the commercialized lots.

3.4. Morphometric Characterization of Seedlings

As to the classes of mass of *E. uniflora* seeds, the seeds characterized as small submitted to the depth 2.0 cm interfered significantly in the average values of the variables analyzed, with the exception of the number of leaves (Table 3).

Table 3. *Average values of seedling emergence (%), emergence speed of seedlings (index) and number of leaves per seedling of surinam cherry tree (Eugenia uniflora L.), obtained based on three classes of seed mass: small (0.6 to 0.7 g), medium (0.8 to 0.9g), large (1.0 a 1.1 g), submitted to different sowing depths. Boa Vista- RR, 2015.*

Class	Depths					
	2 cm		4 cm		mean	
	Seedlings emergence (%)					
Small	87.5	b	85.0	a	86.3	b
Medium	96.3	a	87.5	a	91.9	a
Large	97.5	a	88.7	a	93.1	a
mean	93.7	A	87.1	B		
dms	6.895		6.895		4.875	
	Seedlings emergence speed					
Small	0.64	c	0.62	a	0.65	b
Medium	0.95	b	0.63	a	0.79	b
Large	1.68	a	0.61	a	1.14	a
mean	1.09	A	0.63	B		
dms	0.239		0.239		0.169	
	Number of leaves per seedling					
Small	9.65	a	9.51	a	9.58	a
Medium	9.68	a	9.52	a	9.60	a
Large	9.93	a	9.23	a	9.58	a
mean	9.75	A	9.42	A		
dms	0.779		0.779		0.551	

*Means followed by distinct letters, small in the column and capital in the row, differ from one another at 5% of probability by the Tukey test. dms= significant minimum deviation.

For the variable emergence of seedlings (%) submitted to the depth of 2.0 cm provided increment of 6.6% when compared with sowing at 4.0 cm. studies carried out by [19] reported that there was reduction of 12.4% for percentage of emergence of *Zizyphus joazeiro* seedlings, at each centimeter of increase at sowing depth, a fact that likely, with increase at sowing depth, the seedlings consumed more energy during the germination process, which provided slower emergence.

In the current research, seedling emergence occurred in every class of seed mass and depth, nevertheless, it was observed that the depth of 4.0 cm was decisive to the reduction both of the speed and in the emergence of the *E. uniflora* seedlings. At excessive depths, according to [32], especially in seeds with less mass, impediment to seedling emergence occurs due to absence of enough energy for that.

The seeds, characterized as medium and large, sown at the depth of 2.0 cm were significantly superior to the small ones by 8.8 and 10.0% of seedlings emerged (Table 3). In the case of açaí (*Euterpe oleraceae*) the most superficial depths contributed to increase the emergence speed index [18]. For carnauba wax seeds (*Copernicia prunifera*) with mass 1.82 g arranged at 2.0 cm presented a significant increase of 61% at the emergence when compared with sowing at 4.0 cm.

As to the results correspondent to the root system length, fresh mass of the shoot and of the root system, dry mass of the shoot and of the root system, fresh and total mass of seedlings of *E. uniflora* did not differ from one another for classes of seed mass and for levels of sowing depth (Table 4).

Table 4. *Values of mean square of residue of the variance analysis for seedling height (SH), root system length (RSL), fresh mass of the shoot (FMS), fresh mass of the root system (FMR), dry mass of the shoot (DMS), dry mass of the root system (MRS), total fresh mass (TF), total fry mass (TFM) obtained from Eugenia uniflora L seedlings at 90 days after sowing on the basis of classes of seeds and sowing depth. Boa Vista- RR, 2015.*

		QMR							
	GL	SH	RSL	FMS	FMR	DMS	MRS	TF	TFM
Depths	1	30.60^{**}	1.65^{ns}	0.077^{*}	0.120^{**}	0.013^{ns}	0.038^{**}	0.400^{**}	0.096^{**}
Class	2	2.10^{ns}	4.08^{ns}	0.009^{ns}	0.015^{ns}	0.001^{ns}	0.023^{*}	0.011^{ns}	0.013^{ns}
D x C	2	10.82^{ns}	115.99^{**}	0.019^{ns}	0.014^{**}	0.009^{ns}	0.015^{ns}	0.182^{*}	0.036^{*}
error	18	2.93	4.37	0.012	0.011	0.003	0.004	0.033	0.009
Total	23								
CV%		14.12	14.37	15.48	17.64	14.7	17.5	14.04	12.8

^{ns,*,**}= non-significant, significant at 5% and 1%, respectively.

In research work with *Pyrus* ssp., [33] observed that the greater the total dry mass of the *seedlings,* the better the quality of the seedlings produced for considering the balance of the distribution of the biomass of the *seedling* was.

Analyzing Table 5, it is found that the seeds classified as large, medium and small at the depth of 4.0 cm presented no significant differences for average height of seedlings, the results suggested that the seed size of this species do not influence the proportion of seedlings formed and thee depth of 4.0 cm presented greatest potential of initial growth. However, [34] checking the quality of the surinam cherry seed in relation to the fruit size and to the size of the seeds at its physiological maturity, it is suggested that for the obtaining of greatest percent of vigorous seedlings, the medium-sized seeds should be selected.

Table 5. *Means of seedling height (SH, cm), root system length (RSL, cm), fresh mass of the shoot (FMS, g), fresh mass of the root system (MRS, g), dry mass of the shoot (DMS, g), dry mass of the root system (DMR, g), total fresh mass (TFM, g), total dry mass (TDM, g) obtained on seedlings of Eugenia uniflora L, at 90 days after sowing on the basis of classes of seed mass and sowing depths. Boa Vista- RR, 2015.*

Depth	Class	SH		RSL		FMS		MRS		DMS		DMR		TFM		TDM	
2.0	Small	12.43	a	11.38	b	0.73	a	0.40	b	0.40	a	0.26	b	1.13	a	0.66	a
2.0	Medium	9.23	b	14.45	b	0.57	a	0.53	ab	0.32	a	0.35	ab	1.11	a	0.66	a
2.0	Large	11.35	ab	18.65	a	0.68	a	0.63	a	0.39	a	0.44	a	1.31	a	0.83	a
4.0	Small	12.40	a	16.98	a	0.78	a	0.77	a	0.42	a	0.40	a	1.55	a	0.81	a
4.0	Medium	13.85	a	16.33	a	0.80	a	0.75	a	0.44	a	0.47	a	1.55	a	0.91	a
4.0	Large	13.53	a	9.60	b	0.75	a	0.71	a	0.39	a	0.42	a	1.22	a	0.81	a

*In the column, means followed by distinct letters differ from one another at 5% of probability by the Tukey test.

So, the intensification of the seed germination studies and growth and initial development of the species of this genus can contribute to increased knowledge and broadening of orchards of *E. uniflora*.

4. Conclusion

As conclusion, the biometric characteristics length, thickness and width of seeds can estimate the seed mass of *Eugenia uniflora*, due to the strong correlation among these characteristics. Medium and large-sized *E. uniflora* seeds sown 4 cm in depth in medium textured sand constitute a promising option aiming the production of vigorous and better quality seedlings.

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