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Seed size influences germination and vigor in pitanga (*Eugenia uniflora* L.) plantlets

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ABSTRACT

SUMMARY: Influence of seed size on germination and vigor in seedlings of *Eugenia uniflora* L., Surinam cherry, was studied. An experiment was conducted under 50% shade and at room temperature in a nursery located on the campus of the Federal University of Semi-Arid, Mossoró, Brazil. The experiment had a randomized complete block design with 4 treatments and 5 replications. The treatments consisted of the following classes of seeds, defined according to the size and mass of 50 seeds: small, medium, large, and extra large. Ninety days after sowing, evaluations were performed for the number of leaves per plant, length of shoots and roots, stem diameter, shoot dry mass, root dry mass, and total dry mass. The emergence percentage and emergence rate index of seedlings were also evaluated. The data were analyzed using analysis of variance, and the mean values were compared using a Tukey test at 5% probability. Analyses were performed using the SISVAR system. Seed mass and size influenced the process of emergence, and seeds with larger masses had higher averages for all the parameters assessed, justifying the use of size classes for growing seedlings. The selection and classification of seeds should be recommended in Surinam cherry tree seedling production.

Key words: exotic fruit, plant propagation, Surinam cherry, tissue reserves.

Influências do tamanho da semente na germinação e vigor em plântulas de pitanga (Eugenia uniflora L.)

RESUMO

Foi estudada a influência do tamanho da semente na germinação e vigor de mudas de *Eugenia uniflora* L., (pitanga),. O experimento foi conduzido em 50% de sombra e à temperatura ambiente em um viveiro localizado no campus da Universidade Federal do Semi-Árido, Mossoró, Brasil. O delineamento experimental foi de blocos casualizados, com quatro tratamentos e cinco repetições. Os tratamentos constaram das seguintes classes de sementes, definidos de acordo com o tamanho e a massa de 50 sementes: pequeno, médio, grande e extra grande. Noventa dias após a semeadura, as avaliações foram realizadas para o número de folhas por planta, comprimento da parte aérea e raízes, diâmetro do caule, massa seca, massa seca da raiz e massa seca total. Também foram avaliados a porcentagem de emergência e índice de velocidade de emergência das plântulas. Os dados foram analisados por meio de análise de variância, e as médias foram comparadas utilizando o teste de Tukey a 5% de probabilidade. As análises foram realizadas utilizando o sistema SISVAR. Massa e tamanho das sementes influenciou o processo de emergência, e as sementes com maior massa tiveram médias mais altas para todos os parâmetros avaliados, justificando o uso de classes de tamanho para produção de mudas. A seleção e classificação de sementes deve ser recomendado no Suriname cerejeira produção de mudas.

Palavras-Chaves: frutas exóticas, propagação de plantas, pitanga, as reservas de tecido.

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INTRODUCTION

Pitanga, also known as "Brazilian cherry" or "Surinam cherry," is one of the best-known species in the genus Eugenia, and it is found in various countries because of its ability to adapt to different soil and climate conditions. In Spain it is called "cereza de cayena"; in Venezuela, "pedanga"; in El Salvador "guinda"; in Argentina, "ñanga-piré"; and in Colombia, "cereza quadrada" (Morton, 1987).

Eugenia uniflora L., a native Myrtaceae from South Brazil, shows potential for economic exploitation. The indigenous name "pi´tãg," meaning red for the predominant color of its fruit, is derived from the Tupi language (Donadio et al., 2002). Fruits can be consumed fresh or as jams, candies, juice, sweet beverages, ice cream, and even in mixtures with other fruit juices and milky beverages. Trees are also recommended for heterogeneous reforestation for the recovery of degraded areas for permanent preservation, in order to provide food for the birds of the region.

The state of Pernambuco is one of the main producers in Brazil, harvesting more than 1,700 tons of fruits per year. According to laboratory analysis, 100 g of pulp has 38 kcal, 0.3g protein, 10mg calcium, 20mg phosphorus, 2.3mg iron, 0.03mg vitamin B2, and 14mg vitamin C.

Most existing orchards use undefined cultivars generally derived from plants propagated by seeds, resulting in high variability caused by genetic recombination arising from the use of such methods in the formation of the orchards. Efforts to characterize and define improved cultivars have been limited to the efforts of research groups in northeastern Brazil. Bezerra et al. (1995, 1999) evaluated 122 pitanga accessions in the North Forest Zone of Pernambuco under rain-fed conditions, pointing out10 genotypes with high yield potential and good agronomic traits. After clonal evaluation, access IPA-2.2 was released as the first Brazilian commercial cultivar, under the name of 'Tropicana', which has as its main advantages the high production of fruits, estimated at20.8 kg/year (average over 10 years); average fruit weight of 3 to 4.5g; a reddish pulp; and a TSS/acidity ratio of 4.1.

However, the species has also drawn research attention for the therapeutic properties of its leaves. According to Auricchio and Bacchi (2003), the first studies on this plant focused on the elucidation of its essential oil composition and antimicrobial action, variation in oil composition as a function of different factors, and the behavior of the displayed antimicrobial activity in this context of variation. There are suggestions of pharmacological activity related to acute toxicity, inhibitory action against xanthine oxidase, anti-inflammatory effects, decreased intestinal propulsion, and decreased blood pressure. However, the most recent findings indicate that it inhibits the enzymes β-glucosidase, sucrase, and maltase, revealing a possible use in the treatment of diabetes and further justifying interest in the use of the leaves of *E. uniflora*.

According to Machado and Parente (1986), *E. uniflora* is mainly propagated through seeds. The species shows variation in the number of seeds per fruit that, among other factors, is the cause of variation in seed size. However, to develop the economic use of the species, knowledge of seed germination is the first step to obtain plantlets with vigor and quality. Physiological seed quality has been characterized on the basis of germination and vigor. Seed vigor is the sum of attributes that give a seed the potential to germinate and emerge quickly, resulting in normal seedlings under a wide range of environmental conditions (HÖFS et al., 2004).

Nevertheless, there is a lack of research on the influence of seed mass on the germination and subsequent development of seedlings. A seed's size can be an indication of its physiological quality (POPINIGIS, 1985). Generally, bigger seeds can receive larger amounts of reserve substances during the development phase, which will provide better formed embryos; thus, it is thought that they are the most vigorous seeds (CARVALHO and NAKAGAWA, 2000) for the establishment, growth, and survival of seedlings (MICHAELS, 1988).

Therefore, the classification of seeds by size or weight can be used to standardize seedling emergence and the yield of seedlings of similar size and high vigor. Larger or denser seeds of the same species are potentially more vigorous, yielding seedlings that are much better developed (CARVALHO and NAKAGAWA, 2000).

Given this knowledge, in the present study we aimed to evaluate the effect of seed mass on the emergence and early development of pitanga (*E. uniflora*) seedlings.

MATERIALS AND METHODS

The experiment was conducted in the seedling production nursery of the Universidade Federal Rural do Semiárido, located in the municipality of Mossoró, Brazil, between November 2010 and March 2011. It is located at 5°11'S and 37°20'W and at 18-m altitude, with a mean annual temperature of around 27.5°C, 68.9% relative humidity, and average annual rainfall of 673.9mm. Mossoró is located in the semiarid region of Northeast Brazil (CARMO FILHO and OLIVEIRA, 1995), whose climate, according to the Köppen classification, is hot and dry.

Brazilian or Surinam cherry seeds were obtained from the mature fruits of plants grafted with the cultivar 'Tropicana', selected by IPA in Pernambuco, and planted in the experimental area of the State University of North Fluminense - UENF, in Campos, RJ. After pulping the fruit, the seeds were washed in running water to remove the leftover pulp. They were placed to dry in the shade at room temperature for 48 h and then sown. Before sowing, the seeds were sorted by size into small, medium, large, and extralarge categories. This classification was made on the basis of the mass, placing 50 seeds in each size class with subsequent measurement of the average length and width.

One seed was sown per 1-L capacity container. Each experimental unit consisted of 10 polyethylene bags, totaling 10 seeds per experimental unit and 50 seeds per treatment. The substrate was composed of soil (75% by volume) and cattle manure (25% by volume). Irrigation was carried out by spraying 3 times a day, and weeds were eliminated manually. The phytosanitary control of seedlings consisted of applying 25 ml of the fungicide Cercobin (1.3 g per5L of water) per container every 25 days.

Nitrogen top dressing was applied every 7 days, with the addition of20mL of N (7.6 g urea diluted in5 L of water) containing 45% nitrogen. Three applications were performed during the experiment.

The experimental design was a randomized complete block design with 4 treatments and 5 replications. The treatments consisted of 4 classes of seed sizes: small, medium, large, and extralarge. Ninety days after sowing, evaluations were made of the number of leaves per plant, length of the shoot and root system, stem diameter, dry mass of shoots and roots, total dry mass, germination, and emergence rate index.

The length of shoots and roots was measured with a ruler graduated in millimeters. The length of the stem above the soil level was obtained by measuring the distance between the neck and the apex of the seedling, whereas the root length was obtained by measuring the distance from the neck to the apex of the root. The stem diameter was measured used a digital caliper with values expressed in millimeters. Evaluation of the dry mass of shoots and roots began with the separation of the shoots and roots using pruning shears properly washed in running water. Thereafter, we prepared samples separately in previously labeled paper bags, which were placed on a stove with forced air circulation at a temperature of 65° C for 48 h. After that, the material was weighed on an analytical balance, with data expressed in grams, to measure the dry mass of shoots and roots. The total dry mass was obtained as the sum of the dry mass of shoots and roots, with data expressed in grams. The emergence percentage was calculated according to Labouriauand Valadares (1976), using the formula:

$$E = (N/A) * 100$$

where E = emergence, N = total number of seedlings, and A = total number of seeds placed to germinate.

The velocity of emergence index (VEI) was determined daily by recording the number of germinated seeds from the 27th to the 67th day, and emergence was defined as when seedlings had completely free and normal cotyledons. The VEI was calculated using the formula proposed by Maguire (1962):

$$VEI = E1/N1 + E2/N2 + ... + En/Nn$$

where VEI=velocity of emergence index; E1, E2 … En = number of normal seedlings computed in the first, second, and last count; and N1, N2 … Nn = number of days after sowing.

Data were subjected to analysis of variance, and mean values were compared using a Tukey test at 5% probability (GOMES, 2000). Analyses were performed using the computer program System Analysis of Variance - SISVAR (FERREIRA, 2000).

RESULTS AND DISCUSSION

The number of leaves was correlated with seed size, and the extra large seed class had a greater number of leaves (18.04 leaves·seedling-1) and differed statistically from the other sizes. Small, medium, and large seeds did not differ according to the results of the Tukey test at 5% probability (Table 2).Similar resultswere found bySilvaetal. (2010) who studied the influence of seed size on the germination and seedling vigor of jackfruit (*Artocarpusheterophyllus* Lamk.) and found that the number of leaves was influenced by seed size. The extra large seeds had a greater number of leaves and differed statistically from the other sizes, while small, medium, and large seeds did not differ among each other. Klein et al.(2007) found higher numbers of leaves in Surinam cherry seedlings germinated from seeds with higher mass, because of the seed's greater amount of accumulated reserves.

Shoot length was significantly different among the size classes of seeds. The extra large seeds yielded the longest shoots (14.79 cm), which was significantly different from the other classes of seeds; the small and medium seeds did not significantly differ from each other and were the shortest at 6.39 and 8.43cm, respectively. On the other hand, the large seeds differed significantly from the other treatments, as shown in Table2. Similar results were found by Favarin et al. (2003), who studied the characteristics of seeds in relation to their physiological potential and seedling quality in coffee (*Coffea arabica* L.); they found that plant height was significantly different depending on the size of the seeds, and seeds with larger masses yielded larger plants. The same was observed by Silva et al. (2010), who found that jackfruit seedlings originating from extra large seeds had longer shoots.

According to Carvalho and Nakagawa (2000), the largest seeds have well-developed embryos with a greater amount of reserve substances, and are therefore,the most vigorous.

Seedlings from small seeds had shorter root systems; however, they were not statistically different from the other classes (Table 2), according to a Tukey test($p < 0.05$). This result is corroborated by Kleinet al.(2007), who studied the development of Surinam cherry seedlings from seeds of different masses. Ferreira and Torres (2000) found that the average length of primary roots and seedlings of *Acacia senegal* (L.) Willd. increased with seed size. Silva et al. (2010) found that the smallest jackfruit seeds had smaller root systems than those from larger seeds; their data confirm the findings of our study.

Extra large seeds yielded significantly higher mean stem diameters (1.60cm) than the seeds of other sizes (Table 2). Small seeds produced a significantly lower mean diameter (1.05 cm) than the other seeds. The medium and large seeds yielded diameters of 1.25cm and 1.34cm, respectively, which, while not statistically different from each other, were different from the seeds of other sizes. Seedlings from extra large seeds of jackfruit showed greater stem diameters, according to Silva et al. (2010). The same was observed by Klein et al. (2007) for Brazilian cherry, where seeds with a higher mass showed higher stem diameter.

Extra large seeds showed a higher shoot dry mass (3.63 g), which differed significantly from the other sizes. Small, medium, and large seeds had the lowest values: 0.07, 0.48, and 1.54g, respectively. Small, medium, and large seeds yielded root dry masses of 0.04g, 0.08g, and 0.10g, respectively; these values were not significantly different from each other, but only from that of extra large seeds, which yielded a root dry mass of 1.20g. This indicated that the extra large seeds produced a greater amount of secondary roots, since the length did not differ statistically. The same results were observed for total dry mass, where the seedlings from extra large seeds showed a higher mass (4.82g) than the other seed sizes (Table 2). These results were also confirmed by the findings of Silva et al. (2010), who observed that the extra large seeds of jackfruit yielded higher shoot, root, and total dry masses.

Seed size influenced the emergence percentage of Surinam cherry. Small seeds had 28% emergence, differing from the other treatments. Medium and large seeds had 42% and 62% emergence, respectively, which were not significantly different. Extra large seeds had the highest emergence percentage, 82% (Table 3). These results are similar to those obtained by Hoffmann (2000), who evaluated the Brazilian cerrado species and found a positive correlation between seed size and seedling survival and also that larger seeds emerged in greater proportions than smaller seeds in natural and controlled environments. Silva et al. (2010) and Klein et al. (2007) obtained similar results when studying the emergence of Brazilian cherry and jackfruit, respectively, in relation to the size of the seed. Costa et al. (2006) studied the emergence of wax jambu seeds, another species from the Myrtaceae family, and found that heavy seeds had a higher emergence percentage.

The index of emergence velocity was not affected by seed size. Ferreira and Torres (2000), Silva et al. (2010), and Klein et al. (2007), who studied the influence of seed size of *Acacia senegal* (L.), jackfruit, and Brazilian cherry, respectively, on germination and vigor, also found that the index of emergence velocity was not affected by seed size.

CONCLUSIONS

The mass and seed size influenced the process of seedling emergence in pitanga (Brazilian cherry).

Seeds with larger masses had higher mean scores for all the characteristics evaluated, justifying the adoption of selection based on seed size classes for seedling production.

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Tables and Figures

Table 1. Classification of pitanga seeds by weight, length, and width– average of 50 seeds. Mossoró, 2011.

Seed size	Weight	Length	Width
	(g)	(mm)	(mm)
Small	0.16	6.7	5.07
Medium	0.30	8.1	5.97
Large	0.40	9.1	6.97
Extralarge	0.56	12.2	7.40

Table 2. Mean values for the number of leaves, shoot length, root length, stem diameter, shoot dry mass, root dry mass, and total dry mass from seedlings of Surinam cherry (*Eugenia uniflora* L.) as a function of seed classes.

Seed sizes	NL	CPA	CSR	DC	MSPA	MSSR	MST
		(cm)	(cm)	(mm)	(g)	(g)	(g)
Small	6.60 b	6.39 c	15.79 a	1.05 c	0.07 b	0.04 b	0.11 b
Medium	10.81 b	8.43 c	18.27 a	1.25 b	0.48 b	0.08 b	0.56 b
Large	11.17 b	11.05 b	18.36 a	1.34 b	1.54 b	0.10 b	1.64 b
Extralarge	18.04 a	14.79 a	20.17 a	1.60 a	3.63 a	1.20 a	4.82 a
C.V. (%)	31.07	11.63	16.75	7.41	36.65	53.18	37.63

NL: number of leaves, CPA: shoot length, CSR: root length, DC: stem diameter, MSPA: shoot dry mass, MSSR: root dry mass,MST: total dry mass

Means within columns followed by same letter do not differ based on a Tukey test at the 5% probability level.

Table 3. Mean values of the percentage of emergence (%E) and velocity of emergence index (VEI) of Surinam cherry (*Eugenia uniflora* L.) seedlings as a function of seed class.

Seed sizes	%E	VEI
Small	28 c	0.08 b
Medium	42 bc	0.12 b
Large	62 ab	0.18 a
Extralarge	82 a	0.25 a
C.V.(%)	21.85	21.22

%E: percentage of emergence, VEI: velocity of emergence index