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Evaluation of physical and physiological characteristics of seeds and seedlings of physic nut genotypes

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The physic nut is an oleaginous plant that has distinguished itself as a drought tolerant native species, with a view to producing raw material for the biodiesel production. The purpose of the study reported here was to evaluate the quality of seeds and seedlings of five physic nut genotypes. The evaluated characteristics were: humidity content, mass of 1000 seeds, seed dimensions (using caliper gauge and sieves), germination tests, vigor tests (the first count of the germination test, electrical conductivity, accelerated ageing, seedling dry mass and length, emergence, emergence speed index and analysis of emerged seedlings). The experimental design was a completely randomized block with 4 replications. All data were submitted to analysis of variance by F test and the means were compared by the Tukey test at 5% probability. The physiological quality was not influenced by the seeds dimensions. The tests of accelerated ageing, emergence percentage, shoot dry mass, seedling length and dry mass were the bests at differentiating the genotypes. The Paraguaçu genotype presented characteristics that were superior in relation to the others in the evaluations of accelerated ageing, root length and seedling total length.

Key words: *Jatropha curcas,* germination, vigor, viability, seed size.

INTRODUCTION

The physic nut (*Jatropha curcas* L.) that belongs to the Euphorbiaceae family, is a shrub species which can reach a height of four meters, being widely distributed in tropical and subtropical areas, and offers a potential for biodiesel production (Sujatha et al., 2008).

One of the ways to enhance plant potential is the development of new technologies aimed to improve its production, to obtain qualified seeds to generate vigorous plants with high productivity. High germination capacity, vigor, appropriate humidity content and good appearance

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are indicators of good seed quality. These factors, when properly balanced, provide high plant vigor, high homogeneity and, consequently, higher quality and productivity (Lacerda, 2007).

The seed physiological quality is assessed using standardized methods, under laboratory controlled conditions, aiming to obtain its value for the sowing, maturation, and to compare the physiological quality that will contribute for the seed commercialization (Teixeira et al., 2010). Among the tests that evaluate seed quality, Souza (2007) refers to germination and vigor tests (seed viability, accelerated ageing, electrical conductivity, among others). The physical quality of seeds can be evaluated according to humidity, physical purity, mechanical damage, mass of 1000 seeds, size or volumetric weight and appearance (Peske et al., 2006). The seed size is a component of quality that has been evaluated for many species. According to Carvalho and Nakagawa (2000), larger seeds were better nourished during their development, presenting well-formed embryos with higher quantity of storage substances, being consequently more vigorous.

Given the lack of information referred to this culture, the present study aimed to evaluate the physical and physiological quality of seeds and seedlings of five physic nut genotypes, as well as to generate information for the standardization of tests as it is a under researched culture.

MATERIALS AND METHODS

The study was conducted in the Laboratory of Seed Technology, State University of Southwest of Bahia, Vitória da Conquista, Bahia, located at 923 m of altitude, of latitude 14°53' S and 40°48' West Longitude and in the Laboratory of Seed Analysis of the College of Agricultural Sciences (Faculdade de Ciências Agronômicas - FCA), of the State of São Paulo University (UNESP), in Botucatu, SP, located at 770 m of altitude, latitude of 22° 49' 31"S and 48° 25' 37" West longitude. The average daily temperature during the experimental evaluations remained between 16.87 and 20.73°C.

The seeds used in this study came from commercial planting of five physic nut genotypes collected in January 2008, in the city of Janaúba, MG. The characterization of seed physical quality was conducted using tests that evaluate the humidity content, mass of 1000 seeds and seed size.

The seed moisture content was determined by the stove method at 105±3°C, throughout 24 h (Brasil, 2009). The mass of 1000 seeds was determined using eight replications of 100 seeds (Brasil, 2009).

The dimensional evaluation of seeds was conducted using samples taken at random from 50 seeds and four replications for each genotype. A digital caliper gauge was used to measure the length, width and thickness of the seeds expressed in mm. In the seed classification with sieves, three samples of each genotype were taken. The seeds were classified by stirring per minute in manual sieves with oblong holes, dimensions of 9.52 × 19.05 mm, 8.73 × 19.05 mm, 7.94 ×19.05 mm, 7.14 ×19.05 mm, 6.35 × 19.05 mm and bottom. The seeds that were retained by the indicated sieve that had passed by the superior mesh were weighted and had their percentages calculated (Brasil, 2009).

The physiological quality of seeds and seedlings were determined by the test of germination and vigor (first count of the germination test, electrical conductivity, accelerated ageing, length and dry mass of the seedlings, emergence, emergence speed index and analysis of emerged seedlings.

The germination test was conducted using four replications of 50 seeds, in dampened paper towel under the proportion of 2.5 × the subtract mass (g), at the temperature of 30°C, with counts at four and seven days after test set up. First germination counting (FGC) was performed together with germination test, which is the percentage of regular plants from four days after sowing. The mass system with four replications of 50 seeds per batch was used for electrical conductivity determination (Marcos Filho, 1987). The accelerated ageing was determined using four replications of 50 aged seeds at 40°C for 72 h (Krzyzanowski et al., 1999). After this stage, the seed moisture content (MC) and germination percentage (GER) were measured. At 9 days after sowing, the seedlings root length (R), shoot (S) and total (TOT) were determined using four replications of 10 seeds per genotype, sown on a line drawn on the upper third of the paper towel Germitest, according to recommendations of Vieira and Carvalho (1994). All seedlings from seedling length test were used to evaluate dry mass separated in two portions - root and shoot, dried in stoves at 60±5°C until they reach uniform weight. The results were expressed in grams as in Nakagawa (1999).

Percentage and speed emergence were simultaneously conducted in disposable aluminum dishes containing 2-liter substrate made of 2/3 washed sand and 1/3 soil (under field conditions) with four replications of 50 seeds each batch. The counting was conducted daily, after the emergence (EMERG) of the first seedling until 36 days after the sowing. The emergence speed index (ESI) was calculated using the formula proposed by Maguire (1962). To analyse the emergence at 36 days after sowing, seedlings were removed from aluminium dishes and number of leaves was recorded (NL), trunk diameter (TD), seedling height (SH), root length (RL), root dry mass (RDM) and shoot dry mass (SDM). The stove method was used for the dry mass analysis at 70°C±3 throughout 72 h.

The used experimental design was totally randomized with four replications for all treatments. The data were submitted to F test variance analysis and means compared by the Tukey test ($P \leq 0.05$).

RESULTS AND DISCUSSION

One thousand seed average mass values are shown in Table 1. By these values, it is possible to observe significant differences among batches, pointing out Filomena batch as the highest mass seeds and Gonçalo as the least one. Differences found in the 1000 seed evaluation would possibly be explained by the genetic characteristic differences as field conditions were similar for genotypes. Braga (2010) assessing seed quality of 5 batches of physic nut, found an initial moisture content between 6.7 and 7.7% and 1000 seed average mass varying from 680.38 and 730.29 g. Silva et al. (2008), evaluating physic nut seed physical and physiological quality, found 9.47% humidity and 468.95 g for 1000 seed mass. Considering the initial moisture content as a crucial factor for evaluation standardization, the results ensure data credibility in which there was no difference between the moisture contents of the genotypes.

The average values of seed classification, indicates Filomena and Oracília as the largest seeds and Gonçalo

Table 1. One thousand seed mass, moisture content and seed dimensions of five physic nut genotypes. Vitória da Conquista, BA, Brazil, 2008.

Genotypes	1000 seed mass (g)	Moisture content (%)	Length (m)	Dimensions (mm)	
				Width	Thickness
Bento	688.48[c*]	6.89[a]	18.0778[b]	10.7282[b]	8.5748[a]
Filomena	745.68[a]	7.40[a]	18.2082[ab]	11.1826[a]	8.7394[a]
Gonçalo	566.37[e]	7.77[a]	16.7642[e]	10.5870[b]	7.8604[b]
Oracília	722.71[b]	7.88[a]	18.6728[a]	11.2840[a]	8.6812[a]
Paraguaçu	657.19[d]	6.07[a]	18.1284[b]	10.7206[b]	8.4962[a]
CV (%)	1.85	11.57	4.93	5.27	5.56

* Averages followed by the same lower case letter in the column do not differ from each other by the Tukey test at 5% of probability (P<0.05%).

Table 2. Seed fractions (%) by sieve of five physic nut genotypes. Vitória da Conquista, BA, Brazil, 2008.

Genotypes	Sieves					
	9.52	8.73	7.94	7.14	6.35	Bottom
Bento	4.29[Bab]*	42.01[Ac]	48.09[Aa]	5.33[Bc]	0.31[Ba]	0.00[Ba]
Filomena	7.38[Ca]	64.64[Aa]	26.12[Bc]	1.50[Dc]	0.12[Da]	0.00[Da]
Gonçalo	0.60[Db]	10.20[Cd]	36.26[Bd]	50.37[Aa]	2.43[Da]	0.07[Db]
Oracília	6.84[Ca]	54.09[Ab]	34.89[Bd]	4.17[CDc]	0.00[Da]	0.00[Da]
Paraguaçu	5.81[Cab]	37.92[Ac]	41.56[Ab]	13.97[Bb]	1.07[Ca]	0.31[Ca]
Coefficient of variation				15.97		

*Means followed by the same capital letter in line and lower case in column do not differentiate from the other by the Tukey test at 5% of probability (P<0.05%).

as the smallest one (Table 1), confirming the results found for 1000 seed evaluation in which the Filomena showed the highest mass.

The variance analysis means of sieve classification showed the significant effect of different genotypes and sieves used (Table 2).

There was a higher percentage of retained seeds in the sieve 8.73 × 19.05 mm of the Filomena genotype, representing more than 64% as observed in the mass evaluation. The Gonçalo genotype is highlighted by its small dimensions with most seeds equal or smaller than 7.14 mm of diameter (52.87%) (7.14 × 19.05 mm, 6.35 × 19.05 mm and bottom sieves).

Pádua et al. (2010) assessing soybean seeds (*Glycine max*), noticed that seeds of different sizes showed differences in physiological quality, in which bigger seeds (7.0 mm sieve) showed higher germination percentages and vigor. Martins et al. (2005) verified that heaviest papaya seeds also presented the highest germination power and vigor. Valdés-Rodríguez et al. (2013a) highlighted that both productivity and seed quality in terms of weight and size can be influenced by soil fertility, climate and agricultural management.

Through the germination assessment, it was possible to differentiate Filomena genotype from the others as it presented the lowest germination average. The data of the first germination counting, conducted in the seventh day after the sowing, presented differences on the germination percentage. It can also be verified that Filomena genotype did not present any germinated seed in the first counting (Table 3).

A possible explanation for the lower percentage and germination absence of Filomena can be due to dormancy related to environmental conditions and/or genetics itself, once these genotypes were cultivated and harvested at the same time. Joker and Jepsen (2003) asserted that the physic nut freshly harvested seeds present dormancy and need a rest period after the harvest to germinate. This is a statement that can be applied to the Filomena.

In the evaluation of the first counting, the Oracília and Gonçalo genotypes stand out from the others with better performance; however, the Gonçalo genotype was also statistically similar to the Paraguaçu genotype. Even though Carvalho and Nakagawa (2000) ensured that bigger seeds produce more vigorous seedlings, the results showed that size classification does not comply with results of germination. When evaluating physic nut seed germination at constant temperature of 30°C, Silva et al. (2008) found 77.5%. Martins et al. (2008), evaluating the germination and the first count of physic nut seeds, obtained an average of 76 and 61%, respectively.

For the accelerated ageing test, it is recommended to

Table 3. Germination percentage (GER) and first germination count (FGC) of seed five physic nut genotypes. Vitória da Conquista, BA, Brazil, 2008.

Genotypes	GER (%)	FGC (%)
Bento	86.0^{a}*	22.5^{c}
Filomena	32.5^{b}	0.0^{d}
Gonçalo	82.5^{a}	38.5^{ab}
Oracília	83.5^{a}	42.5^{a}
Paraguaçu	85.0^{a}	29.0^{bc}
CV (%)	7.4	20.7

* Averages followed by the same lower case letter in the column do not differ from each other by the Tukey test at 5% of probability (P<0.05%).

Table 4. Germination percentage (GER) and moisture content (UMI) after accelerated ageing test and electrical conductivity (EC) seed of five physic nut genotypes. Vitória da Conquista, BA, Brazil, 2008.

Genotypes	GER (%)	UMI (%)	EC (µS.cm^{-1}.g^{-1})
Bento	69.0^{c}	24.02^{a}	0.1123^{a}*
Filomena	73.0^{bc}	23.88^{a}	0.1121^{a}
Gonçalo	80.0^{b}	23.64^{a}	0.1046^{a}
Oracília	67.0^{c}	23.95^{a}	0.1071^{a}
Paraguaçu	91.0^{a}	23.49^{a}	0.1059^{a}
CV (%)	5.77	2.86	5.17

* Averages followed by the same lower case letter in the column do not differ from each other by the Tukey test at 5% of probability (P<0.05%).

Table 5. Root length (R), shoot (S), total (TOT) and seedling dry mass (SDM) of five physic nut genotypes, at 9 days after sowing. Botucatu, SP, Brazil, 2008.

Genótipos	R (mm)	S (mm)	TOT (mm)	SDM (mg)
Bento	76.9^{c}*	54.1^{b}	130.9^{c}	0.662^{b}
Filomena	91.0^{bc}	54.9^{b}	145.9^{bc}	0.731^{b}
Gonçalo	97.3^{b}	63.2^{b}	160.5^{bc}	0.839^{ab}
Oracília	103.0^{b}	68.0^{ab}	171.1^{b}	0.950^{ab}
Paraguaçu	121.9^{a}	80.0^{a}	204.9^{a}	1.286^{a}
CV (%)	8.43	11.91	8.87	26.17

* Averages followed by the same lower case letter in the column do not differ from each other by the Tukey test at 5% of probability (P<0.05%).

compare seed samples with alterations higher than 2% in the initial moisture content, not undermining the results because of moistening speed variations and seed deterioration during the test (Marcos Filho, 2000). By observing the humidity means of seeds after ageing, the differences in moisture content among samples were lower than 2%, not decreasing result credibility (Table 4). According to Hampton and TeKrony (1995), the recommended humidity for soybean seeds after ageing conditions to have reliable results in the germination test ranges from 27 to 30%. In the present study, after the ageing conditions the seeds presented humidity varying from 23.9 to 24.02%. This characteristic might be justified by the difference in oil content, since the physic nut present higher oil content compared to soybean.

The results of germination and accelerated ageing presented in Tables 3 and 4, respectively, showed that Gonçalo and Paraguaçu genotypes presented similar performances, also showing a performance increase of Paraguaçu after the ageing. The genotypes Bento and Oracília had a loss of viability after the accelerated ageing test, while Filomena genotype presented better performance after accelerated ageing study. The conditions provided to the seed ageing might have favored dormancy break of Filomena seeds, since there was a test improvement compared to the germination one.

According to Mello and Tillmann (1987), there are result variations of standardization by the use of accelerated ageing test, with the major causes being the initial moisture content, the species, the cultivar, the exposition period and temperature.

In the results of the electrical conductivity test, there was no difference between the evaluated genotypes, as this test was not efficient to differentiate the studied genotypes (Table 4).

Vanzolini and Nakagawa (1998) studying the electrical conductivity in peanut seeds, concluded that the comparison of peanut genotypes by electrical conductivity tests suffers interference by the different seed sizes. However, despite these differences (Tables 1 and 2), it was not possible to differentiate seeds evaluated by the electrical conductivity test, once the conductivity value is directly related to the cellular membrane integrity (Hampton and TeKrony, 1995).

The seed moisture content is extremely important for the electrical conductivity test standardization. In general, it has been observed that very low moisture contents (<10%) or very high (>17%), present significant influence on the results. A greater effect has been observed when the seed moisture content is very low (<10%), causing a significant increase in the test results for many species (Hampton et al., 1992). By observing the moisture content in the studied batches (Table 1), it is verified that the contents were below 10%, a factor that might have contributed to the non-occurrence of significant differences between the genotypes in the electrical conductivity evaluation.

The means obtained from the data of root system and aerial part length, total length and dry mass of the seedlings from the seeds of different physic nut genotypes were statistically different (Table 5).

The Paraguaçu genotype presented higher means in

Table 6. Emergence speed index (ESI), percentage emergence (EMERG), number of leaves (NL), trunk diameter (TD), seedling height (SH), root length (RL), root dry mass (RDM) and shoot dry mass (SDM) of five physic nut genotypes, at 36 days after sowing. Vitória da Conquista, BA, Brazil, 2008.

Genotypes	ESI (%)	EMERG (%)	NL	TD (mm)	SH (mm)	RL (mm)	RDM (g)	SDM (g)
Bento	19.5[a]*	84.5[ab]	2.6[a]	53.0[a]	49.0[a]	109.0[a]	6.2[a]	15.7[ab]
Filomena	19.5[a]	85.5[ab]	2.8[a]	53.0[a]	45.0[a]	111.0[a]	5.7[a]	16.5[a]
Gonçalo	20.0[a]	78.0[b]	2.9[a]	53.0[a]	42.0[a]	124.0[a]	7.0[a]	13.0[b]
Oracília	20.0[a]	83.0[ab]	2.7[a]	52.0[a]	46.0[a]	111.0[a]	5.7[a]	15.2[ab]
Paraguaçu	22.8[a]	87.5[a]	2.8[a]	57.0[a]	46.0[a]	108.0[a]	6.7[a]	17.0[a]
CV (%)	12.11	4.66	5.54	4.60	8.81	11 .78	22.82	9.70

* Averages followed by the same lower case letter in the column do not differ from each other by the Tukey test at 5% of probability (P<0.05%).

the evaluated parameters of root length and seedling total length, and along with the Oracília genotype, the shoot length means were higher than the others. The Paraguaçu genotype was statistically similar to Oracília and Gonçalo in the seedling dry mass evaluation; nevertheless, the Bento and Filomena genotypes were statistically similar to Gonçalo and Oracília.

According to Nakagawa (1999), in order to have an accurate batch quality evaluation, it is important that jointly with the results from seedling growth test, the germination is also considered. This statement is also valid to situations where the batches present a high germination percentage and a low value of seedling average length, as well as a batch with low germination percentage but with high seedling average length value.

In emergence speed index tests, leaf number, trunk diameter, seedling height, root length and root dry mass there were no significant differences in vigor among the evaluated genotypes. The shoot dry mass parameter presented significant differences between the evaluated materials showing a difference in vigor. The Paraguaçu, Filomena, Bento and Oracília genotypes were statistically similar and had higher means. In the seed emergence results, there were statistical differences among the evaluated genotypes, in which Paraguaçu genotype presented a higher mean in relation to the Gonçalo one (Table 6).

The seedling emergence percentage in field was similar to germination test, only differing for Filomena genotype that presented 32.5% in the germination test (Table 3) and 85.5% in the emergence test (Table 6). Silva et al. (2008), when evaluating physic nut seeds, found 79% of emergence and 1.86% of ESI, while Braga (2010) found 45 to 85% of emergence and a variation of 2.60 to 5.10 of ESI. Valdés-Rodríguez et al. (2013b), evaluating varied soil texture effect on non-toxic *Jatrophas curcas* L. seed germination, observed that the highest seedling survival occured in sand substrate (99%), followed by sandy-loam (99%) and clayey loam (87%). According to Marcos Filho (1999), the emergence of seedlings in field depends directly on the batch history and environment conditions. As these conditions usually are not controllable, the evaluation of the physiological quality of the seeds must be effectively performed to enable the precise identification of the batches with higher potential to be established in field.

Conclusions

The seed size did not influence the physiological quality of the evaluated genotypes. The tests that better differentiated the genotypes were the accelerated ageing, seedling length and dry mass, shoot emergence and dry mass. In the face of the conducted evaluations, in general, the Paraguaçu genotype presented desirable characteristics, being highlighted from the other genotypes.

Conflict of Interest

The authors have not declared any conflict of interests.

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