

Physiological maturity of *Luffa cylindrica* (L.) Roem. Seeds¹

Maturação fisiológica de sementes de *Luffa cylindrica* (L.) Roem

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ABSTRACT - Maturation of seeds involves a systematic sequence of physical and physiological alterations observed from fecundation until they become independent of the mother plant. These alterations comprehend a set of steps which prepare them for a successful germination in the future, characterized by the synthesis and accumulation of reserves. Thus, the objective was to evaluate the development and physiological quality of seeds of *L. cylindrica* accessions during maturation. Fruits of *L. cylindrica* accessions were harvested from the 10th day after anthesis (DAA) until the 50th DAA, at 10-day intervals. In each harvest, fruit fresh weight was determined, and then the seeds were manually extracted and subjected to the following evaluations: 100-seed weight, seed water content, seed dry matter content, seedling emergence and emergence speed index. The experiment was carried out in a completely randomized design, with four replicates, in a 3 x 4 factorial scheme (three *L. cylindrica* accessions and four harvest periods). At 10 DAA, no seeds were formed in the fruits harvested from any the accessions. The best physiological performance of seeds of the *L. cylindrica* accessions, under the studied conditions, was found at 50 DAA.

Key words: Cucurbitaceae. Loofah. Plant genetic resources. Physiological quality.

RESUMO - A maturação das sementes envolve uma sequência ordenada de alterações físicas e fisiológicas verificadas a partir da fecundação até o ponto de se tornarem independentes da planta-mãe. Essas alterações compreendem um conjunto de etapas que preparam para o sucesso da futura germinação, caracterizada pela síntese e acúmulo de reservas. Dessa forma, objetivou-se avaliar o desenvolvimento e a qualidade fisiológica de sementes de acessos de *L. cylindrica* durante o processo de maturação. Para isto, os frutos de acessos de *L. cylindrica* foram colhidos a partir do décimo dia após a antese (DAA) até o quinquagésimo DAA, com intervalos de dez dias. A cada coleta, registrou-se a massa fresca dos frutos, em seguida as sementes foram extraídas manualmente e submetidas às seguintes avaliações: massa de 100 sementes, teor de água, conteúdo de massa seca das sementes, emergência de plântulas e índice de velocidade de emergência. O experimento foi conduzido em delineamento inteiramente casualizado, com quatro repetições, em esquema fatorial 3x4 (três acessos de *L. cylindrica* e quatro épocas de colheita). Aos dez DAA não foram observadas sementes formadas nos frutos colhidos em quaisquer dos acessos. O melhor desempenho fisiológico das sementes dos acessos de *L. cylindrica*, nas condições de estudadas, foram verificadas aos 50 DAA.

Palavras-chave: Cucurbitaceae. Bucha vegetal. Recursos genéticos vegetais. Qualidade fisiológica.

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INTRODUCTION

The genus *Luffa* comprises seven species, and *Luffa cylindrica* (L.) Roem. is the most cultivated (SCHAEFER; HEIBL; RENNER, 2009; SIQUEIRA; BRAS; DUFRENSE, 2010). In Brazil, despite being spread in all regions, it is still little exploited. It is a typical crop of family farming cultivated in the vast majority of properties with small areas, whose production is intended almost always to personal hygiene and manufacture of crafts (MAROUELLI; SILVA; LOPES, 2013). However, it can be used in many different ways; for example, the utilization of its fibers for their thermal characteristics (ANBUKARASI; KALAISELVAM, 2015), as rootstocks for other cucurbits (ITO *et al.*, 2014), production of biocatalysts (MARDER *et al.*, 2008), and as medicinal plant (PARTAP *et al.*, 2012; WADOOD *et al.*, 2013).

L. cylindrica reproduction is preferentially sexual, by either direct seeding in the field or production of seedlings for subsequent transplantation. Since the market does not yet have improved seeds, farmers produce their own seeds and exchange them with one another. This form of production is carried out without any specific management or control, so that the physiological quality of these seeds can be considered as low (AGUIAR *et al.*, 2014).

The initial step for a successful planting of any crop propagated sexually is the use of seeds of known origin and superior quality, and it depends directly on the harvest period of the fruits and physiological maturity of the seeds.

The study on maturity is carried out to determine the ideal point of harvest, aiming at production and quality of seeds. Seeds reach physiological maturity when maximum germination and vigor coincide. The time at which this point occurs varies among species and within each species, according to the cultivar and environmental conditions (MAIA *et al.*, 2011).

Studies on physiological maturity of seeds of other cucurbits such as pumpkin (FIGUEIREDO NETO *et al.*, 2014), gherkin (MEDEIROS *et al.*, 2010), zucchini (MARROCOS *et al.*, 2011) and cucumber (NAKADA *et al.*, 2011) can be found in the literature. However, there are no reports on the physiological maturity of *L. cylindrica* seeds, and research is essential to obtain seeds with high physiological quality, an important requirement for a successful plantation.

Given the above, the objective of the present study was to determine the physiological maturity of seeds of *L. cylindrica* accessions, collected in the Rio Grande do Norte state, Brazil.

MATERIAL AND METHODS

The experiments were carried out in the Experimental Vegetable Garden, at the Laboratory of Plant Genetic Resources and at the Laboratory of Seed Analysis of the Department of Plant Sciences of the Federal Rural University of the Semi-Arid Region (UFERSA), Mossoró, RN, Brazil (5° 11'S, 37° 20'W and altitude of 18 m). According to Köppen's classification, the climate of the region is BSW^h (very hot, with rainy season in the summer extending to autumn) (CARMO FILHO; ESPÍNOLA SOBRINHO; MAIA NETO, 1991).

Seeds of three accessions were subjected to a treatment to overcome dormancy, which consisted in a cut with pruning shears at the opposite end to the hilum (OLIVEIRA *et al.*, 2012), and planted on polystyrene trays with 128 cells. The seeds were washed in running water and immersed in sodium hypochlorite solution (1 mL L⁻¹), neutral detergent (1 mL L⁻¹) and water for disinfection. Tropstrato HT[®] was used as substrate, indicated for the production of cucurbit seedlings. After sowing, the trays were kept in greenhouse and irrigated twice a day. Transplanting was carried out when the seedlings had two true leaves, approximately 25 days after sowing.

In the field, no experimental design was adopted and the plants of each accession were grown in continuous rows, at spacing of 5 m between plants and 3 m between rows. A trellis system was used to grow the plants, which were trained using stakes until reaching the wires, to prevent branches from interlacing and facilitate correct identification of flowers in the plots.

A drip irrigation system was adopted and the cultivation practices (weeding and monitoring of pests and diseases) were carried out according to crop needs.

Along the cycle, the female flowers of each accession were tagged on the day of their anthesis, in the morning. The flowers were not protected, so natural pollination probably occurred. After the fruits began to develop, harvests were performed at 10, 20, 30, 40 and 50 days after anthesis (DAA).

After harvest, the fruits were characterized at the Laboratory of Plant Genetic Resources of UFERSA. Fruit fresh weight (FFW) was determined by weighing the whole fruits on an electronic scale with precision of two decimal places, and the results were expressed by the mean values of the samples in grams (g).

As the seeds were extracted from the fruits, the test was set up at the Laboratory of Seed Analysis of UFERSA, to evaluate the following parameters: 100-seed weight (100SW) - eight subsamples of 100 seeds, recently

extracted from the fruits, were weighed on electronic scale with precision of two decimal places and the results were adjusted to the moisture content and expressed by the mean values of the samples in grams (g); seed water content – two subsamples of 30 seeds recently extracted from the fruits were separated, weighed and dried in an oven at 105 ± 3 °C for 24 h (BRASIL, 2009), and the results were expressed by the mean values of the samples in percentage (wet basis); seed dry matter - obtained by weighing, after drying in an oven at 105 ± 3 °C for 24 h, in two replicates of 30 seeds each, and the results were expressed by the mean values of the samples in grams/30 seeds; seedling emergence - four subsamples of 50 seeds from each treatment were treated to overcome dormancy (OLIVEIRA *et al.*, 2012), planted on polystyrene trays of 128 cells, kept in greenhouse at room temperature and, at 15 days after sowing, the number of normal seedlings emerged was recorded and the values were expressed as percentage of normal seedlings emerged; emergence speed index - determined along with the seedling emergence test, by counting every day, at the same time, the number of seedlings emerged until stabilization. Calculations were carried out using the formula employed by Maguire (1962).

The experimental design was completely randomized with four replicates, in a 3 x 4 factorial scheme, in which the first factor corresponded to three accessions and the second to four harvest periods (20, 30, 40 and 50 DAA). All variables analyzed were subjected to analysis of variance using the program SISVAR V5.3, regression analysis and curve fitting as a function of fruit age using the program TableCurve 2D V5.01. Tukey test at 0.05 probability level was used to compare the means when significant effect was observed for the factor accession.

RESULTS AND DISCUSSION

In fruits harvested at 10 DAA, no seeds were formed. Seeds could be extracted to set up the tests only at 20 DAA.

The interaction between fruit harvest periods and *L. cylindrica* accessions had significant effect (0.05 probability level) on fruit fresh weight. Effect of the interaction between the fruit harvest periods and *L. cylindrica* accessions was also observed on seed dry matter content at 0.01 probability level (Table 1).

The analysis of variance (Table 1) revealed significant effects of accessions at 0.01 probability level on fruit fresh weight and seed dry matter, and at 0.05 probability level on 100-seed weight.

High coefficients of variation were found for seedling emergence (100.35%) and emergence speed index (103.92%). These high coefficients were probably influenced by the heterogeneity of the genetic material of the studied accessions, which are still segregating.

According to the interaction of the effect of *L. cylindrica* accessions as a function of fruit harvest periods on fruit fresh weight, the accession BUCHA12, at 20 DAA, had highest fruit fresh weight, showing more accentuated initial growth compared with the others (Table 2). From 40 DAA, the accessions did not differ from one another regarding fruit fresh weight (Table 2).

Along the maturation process, the fresh weight of the fruits of the *L. cylindrica* accessions decreased as fruit age increased (Figure 1A). Such reduction in fresh weight of the fruits is due to their dehydration during maturation.

Water content in the seeds of the *L. cylindrica* accessions decreased with fruit ripening. At 20 DAA,

Table 1 - Summary of analysis of variance for the variables: fruit fresh weight (FFW), 100-seed weight (100SW), seed dry matter (SDM), seed water content (SWC), seedling emergence (EM) and emergence speed index (ESI) of three accessions of *Luffa cylindrica* (L.) Roem. used to determine the physiological maturity of the seeds

Source of Variation	DF	Means Square					
		FFW	100SW	SDM	SWC	EM	ESI
Harvest Periods (E)	3	250.771,08**	165,86**	3,09**	1.733,39**	3.555,56**	14,89**
Accessions (A)	2	119.736,99**	35,40*	1,46**	57,17 ^{ns}	361,00 ^{ns}	2,11 ^{ns}
Interaction E x A	6	17.977,38*	5,75 ^{ns}	0,47**	47,22 ^{ns}	141,56 ^{ns}	0,68 ^{ns}
Residual	36	7.014,04	7,58	0,09	24,17	211,72	0,94
Overall Mean		372,37	18,99	2,18	59,42	14,5	0,94
CV (%)		22,49	14,49	14,21	8,27	100,35	103,92

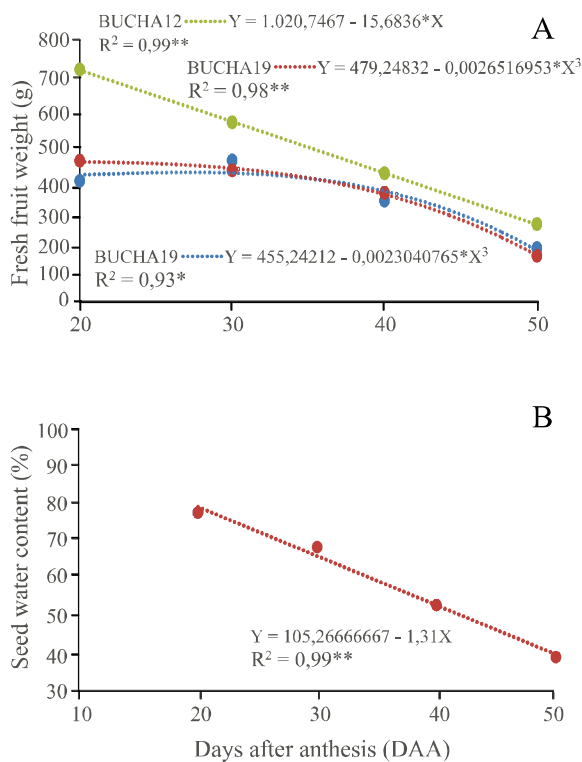
^{ns} Not significant, ** and * significant at 0.01 and 0.05 probability levels by F test, respectively

Table 2 - Effect on fruit fresh weight (g) of *Luffa cylindrica* (L.) Roem. accessions as a function of fruit harvest period

Harvest Periods (DAA)	Fruit fresh weight (g)		
	BUCHA06	BUCHA19	BUCHA12
20	369,38 b	431,15 b	710,32 a
30	431,54 ab	401,90 b	548,51 a
40	309,65 a	332,90 a	392,47 a
50	163,44 a	139,87 a	237,32 a

*Means followed by the same letter in the row do not differ by Tukey test at 0.05 probability; DAA = Days after anthesis

Figure 1 - Fruit fresh weight (A) and seed water content (B) of *Luffa cylindrica* (L.) Roem. accessions as a function of fruit age



the mean water content in the seeds was 77.5%, which decreased to 39% at 50 DAA (Figure 1B).

The high initial water content of *L. cylindrica* seeds in the first harvest and its subsequent decline can be related to the importance of water in the transport of photosynthates. According to Marcos Filho (2015), water plays an important role in seed formation and maturation, and its content decreases along the entire process, but remains sufficiently high to transport dry matter from the plant to the seeds. While the seeds are accumulating reserves, their dehydration is slow, but it accelerates when they reach the maximum point of dry matter accumulation.

In cucumber, ‘Ômega’ hybrid, Nakada *et al.* (2011) observed that seed water content was close to 70% at 30 DAA, but decreased to 33% at 45 DAA. In studies on the physiological maturity of gherkin seeds, Medeiros *et al.* (2010) observed that seed water content decreased as fruit age increased. For pumpkin, cv. ‘Jacarezinho’, Figueiredo Neto *et al.* (2014) found that seed water content also decreased during maturation. In zucchini, cv. ‘Menina Brasileira’, Marrocos *et al.* (2011) observed an initially high water content, with reductions during fruit ripening.

According to the interaction of the effect of *L. cylindrica* accessions as a function of harvest periods on dry matter accumulation in the seeds (Table 3), the

Table 3 - Means of the follow-up test for the interaction between *Luffa cylindrica* (L.) Roem. accessions and fruit harvest periods on seed dry matter content

Harvest Periods	Seed dry matter content (g/30 seeds)		
	BUCHA06	BUCHA19	BUCHA12
20 DAA	1,77 a	1,30 a	1,47 a
30 DAA	2,81 a	1,55 b	1,78 b
40 DAA	2,51 ab	2,34 b	2,89 a
50 DAA	2,95 a	2,51 ab	2,25 b

*Means followed by the same letter in the row do not differ statistically by Tukey test at 0.05 probability level; DAA = Days after anthesis.

accessions did not differ initially (20 DAA) with respect to seed dry matter.

As maturation progressed, the accession BUCHA12 reached maximum seed dry matter already at 40 DAA, whereas the BUCHA06 and BUCHA19 reached their maximum points at 50 DAA (Table 3). This behavior can be explained by the fact that dry matter accumulation in a seed is initially slow, for a short period, immediately followed by a phase of fast and constant accumulation, until a maximum point is reached. The point of physiological maturity of the seeds is close to the point of maximum dry matter accumulation and varies even within the same species (CARVALHO; NAKAGAWA, 2012).

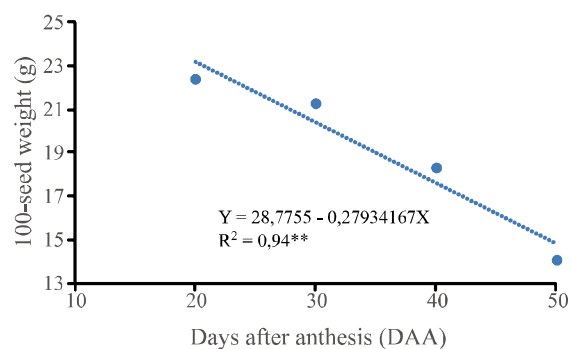
Similar results of dry matter accumulation were observed in seeds of other cucurbits. In studies on the physiological maturity of gherkin seeds, Medeiros *et al.* (2010) observed that seed dry matter increased until 40 DAA and its greatest increment occurred between 25 and 35 DAA. This moment coincided with the period of greatest reduction in seed water content, being considered as the probable point of physiological maturity in gherkin seeds. In a study on cucumber, 'Ômega' hybrid, Nakada *et al.* (2011) observed that at 30 DAA seed dry matter was close to 30%, a value considered as low by the authors; approximately at 45 DAA, this variable reached 67% and decreased from this point on. For pumpkin, cv. 'Jacarezinho', Figueiredo Neto *et al.* (2014) observed that the dry matter accumulation in the seeds increased significantly from 15 DAA to 60 DAA. Costa, Carmona and Nascimento (2006) found that, in seeds of hybrid pumpkin, dry matter tended to accumulate until 50 DAA and remained stable from this point on. Marrocos *et al.* (2011), studying zucchini, cv. 'Menina Brasileira', observed that the dry matter accumulation in the seeds increased during the entire period studied, reaching maximum at 60 DAA, and the period of greatest increment occurred between 20 and 40 DAA.

The 100-seed weight of the *L. cylindrica* accessions decreased as fruit age increased, from 22.38 g at 20 DAA to 14.06 g on average among the accessions, at 50 DAA (Figure 2).

From 40 DAA, the decrease in 100-seed weight was more accentuated, following the pattern of seed water content. In this same period, dry matter accumulation in the seeds of the accessions reached the highest mean values observed, with maximum at 50 DAA (Figure 2).

Unlike other cucurbits, in which the weight tends to increase with fruit age, as observed in hybrid pumpkin (COSTA; CARMONA; NASCIMENTO, 2006) and cucumber (NAKADA *et al.*, 2011), the weight of seeds of the *L. cylindrica* accessions decreased as a function of

Figure 2 - 100-seed weight of *Luffa cylindrica* (L.) Roem. accessions as a function of fruit age

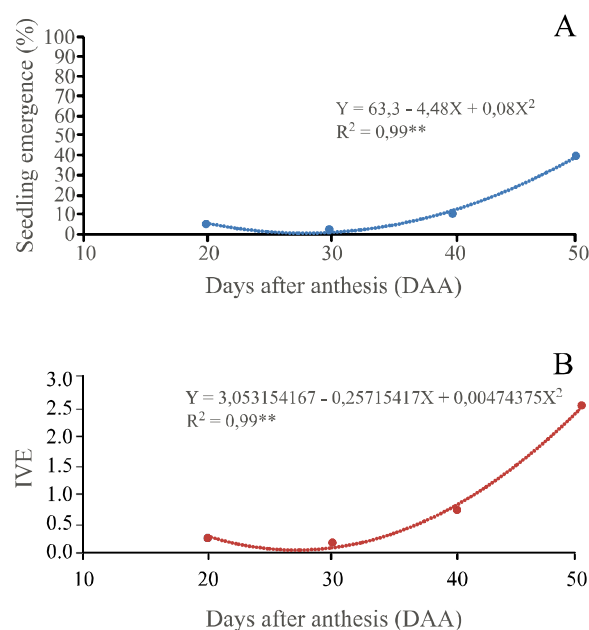


fruit age. This probably occurs because it is a fruit that dehydrates rapidly, whereas the other examples cited are fleshy fruits.

Such reduction in 100-seed weight along the studied period can also be attributed to the continuous dehydration of the seeds and consequently to the reduction in their weight. In the first periods of fruit harvest, at 20 and 30 DAA, seedling emergence was low, 5 and 2%, respectively, on average among the tested accessions of *L. cylindrica* (Figure 3A).

Expressive increases in emergence were only observed in fruits harvested at 50 DAA, when the mean emergence among the studied accessions reached 40% (Figure 3A).

Figure 3 - Seedling emergence (A) and emergence speed index (ESI) (B) in seeds of *Luffa cylindrica* (L.) Roem. accessions as a function of fruit age



Seeds that are not yet mature can germinate but do not result in vigorous seedlings like those that would be obtained from seeds harvested at the point of physiological maturity (CARVALHO; NAKAGAWA, 2012). When the seeds of the *L. cylindrica* accessions were not yet at physiological maturity, seedling emergence occurred at 20, 30 and 40 DAA, but at low percentage. Seed germination is also influenced by the phenomenon of dormancy, making it difficult to evaluate this characteristic according to the authors mentioned above. Seed vigor, expressed by seedling emergence, increased substantially in the period between 40 and 50 DAA, from 10 to 40% (Figure 3A).

Similarly, in the pumpkin cv. 'Jacarezinho', Figueiredo Neto *et al.* (2014) observed that the seeds of the fruits harvested and not stored at ages of 15, 25 and 30 days did not germinate, which occurred only from 50 and 60 days, reaching 37 and 47%, respectively. Marrocos *et al.* (2011), studying zucchini, cv. 'Menina Brasileira', observed that seed germination did not occur at 20 DAA and, at 30 DAA, 54% of the seeds were germinated. Thus, germination percentage also increased, with certain stability between 40 and 50 DAA, reaching maximum of 86% at 60 DAA. In cucumber, cv. 'Pérola', Barbedo *et al.* (1997) observed that seedling emergence in the field varied from 0% at 20 DAA to 91% at 40 DAA. In cucumber, 'Ômega' hybrid, Nakada *et al.* (2011) observed that the germination of seeds that did not undergo artificial drying varied from 23% at 30 DAA to 95% at 50 DAA. In gherkin, Medeiros *et al.* (2010) observed that germination percentage increased gradually, reaching highest point (90%) at 30 DAA and decreasing from this point on.

The emergence speed index increased during the entire period and the best results were obtained when *L. cylindrica* fruits were harvested at 50 DAA (Figure 3B). These results coincided with those obtained in the emergence test, so that in the period between 40 and 50 DAA the expression of seed vigor, by the emergence speed index, became more evident. Similar results occurred in gherkin (MEDEIROS *et al.*, 2010), cucumber, 'Ômega' hybrid (NAKADA *et al.*, 2011), zucchini, cv. 'Menina Brasileira' (MARROCOS *et al.*, 2011) and pumpkin, cv. 'Jacarezinho' (FIGUEIREDO NETO *et al.*, 2014).

Under the studied conditions, the seeds of the *L. cylindrica* accessions reached maximum dry matter accumulation and minimum water content at 50 DAA. Maximum seedling emergence and highest emergence speed indices were also found at this same date. Therefore, considering these characteristics as indication of physiological maturity, the seeds of the *L. cylindrica* accessions, under the studied conditions, reached physiological maturity at 50 DAA.

CONCLUSIONS

Maximum physiological quality of seeds of the *L. cylindrica* accessions occurs at 50 DAA.

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