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Physiological maturation in seeds of *Lophantera lactescens* Ducke[1]

Maturação fisiológica de sementes de *Lophantera lactescens* Ducke

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ABSTRACT - *Lophantera lactescens* Ducke (Malphigiaceae) is an arboreal plant that propagates by seed; once exclusive to the Amazon region, it is now widely distributed in South America. The aim of this work was to study the maturation process in seeds of *L. lactescens*, in order to determine the point of physiological maturity and the ideal time for harvesting. The field study was conducted at the Centre for Agrarian Sciences of the Federal University of Paraíba (CCA/UFPB), Campus II, in Areia, in the State of Paraíba (PB), where the fruit was harvested every two weeks over two consecutive years. The water content, external coloration, fruit size, fruit dry weight, germination and vigour (seed germination index, and seedling length and dry weight) were evaluated. The experimental design was completely randomised, with data submitted to analysis of variance and polynomial regression, as a function of the harvest time. For the two years of the study, the water content decreased during the maturation process, reaching 11% at 210 days after anthesis. Fruit dry matter increased during the process, with rises in germination and seed vigour, reaching a maximum value between 190 and 210 days after anthesis respectively. A light-brown coloration with wrinkling of the fruit pericarp is a good visual indicator for determining the point of physiological maturity of the seeds in this species. Under the environmental conditions found in Areia PB, the ideal time for harvest and the physiological maturity of the seeds of *L. lactescens* are reached from 165 to 186 days after anthesis.

Key words: Germination. Lanterneira. Maturity. Vigour.

RESUMO - *Lophantera lactescens* Ducke (Malphigiaceae) é uma planta arbórea que se propaga por sementes, exclusiva da região amazônica, mas amplamente distribuída na América do Sul. Diante disto, objetivou-se estudar o processo de maturação de sementes de *L. lactescens*, visando determinar o ponto de maturidade fisiológica e o momento adequado para a colheita. O estudo de campo foi conduzido no Centro de Ciências Agrárias da Universidade Federal da Paraíba (CCA/UFPB), Campus II, Areia, PB, realizando-se, quinzenalmente, 14 colheitas de frutos, por dois anos consecutivos. Avaliou-se o teor de água, coloração externa, tamanho dos frutos, massa seca dos frutos, germinação e vigor (índice de velocidade de germinação de sementes, comprimento e massa seca de plântulas). O delineamento experimental foi o inteiramente ao acaso, cujos dados foram submetidos à análise de variância e regressão polinomial, em função das épocas de colheita. O teor de água reduziu ao longo do processo de maturação, atingindo 11% aos 210 dias após a antese, nos dois anos estudados. A massa seca dos frutos aumentou no decorrer do processo, enquanto a germinação e o vigor das sementes aumentaram, atingindo valor máximo entre 190 e 210 dias após a antese, respectivamente. A coloração marrom clara com enrugamento do pericarpo dos frutos é um bom indicador visual para determinação do ponto de maturidade fisiológica das sementes desta espécie. Nas condições ambientais de Areia, PB, o ponto de colheita e maturidade fisiológica das sementes de *L. lactescens* é atingido entre 165 e 186 dias após a antese.

Palavras-chave: Germinação. Lanterneira. Maturidade. Vigor.

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INTRODUCTION

The species *Lophantera lactescens* Ducke, known locally as lanterneira, the Amazon *lofantera* and golden rain (PAOLI, 1997), belongs to family Malphigiaceae; it is endemic to the Amazon region, but is widely distributed in South America (JUDD *et al*., 1999), where it is found both in the interior of dense primary forest and in secondary formations (LORENZI, 2008). The plant is tree-like in size, and can reach between 10 and 20 metres in height, with fruit that are schizocarpic and dry, and seeds that are pyriform, anatropous, bitegmic and exarillate, with mucilaginous cotyledons (PAOLI, 1997). Flowering takes place between February and May, with the fruit ripening during September and October (LORENZI, 2008). The species is important in medicine, as it is able to lower malarial fever (ABREU *et al*., 1990). In recent years, the number of malaria cases in Brazil has again increased alarmingly. Despite a decline, the number of cases in 2008 was more than 300,000 nationwide. In the states of the Amazon region, transmission was 99.9%, with *Plasmodium vivax* the species responsible for almost 90% of cases (BRASIL, 2010). The plant is also is used in the permanent preservation of degraded areas, the urban landscaping of parks and gardens, and due to having fairly heavy and compact wood, in construction and for the manufacture of beams, ceilings and rafters used in joinery and carpentry (LORENZI, 2008).

Studies of family Malpighiaceae have been developed regarding the morpho-anatomy and ontogeny of the seeds (SOUTO; OLIVEIRA, 2008); for the species *L. lactescens*, studies have been carried out into the morpho-anatomy of the seeds and fruit (PAOLI, 1977), anatomy and lignification (AMARAL *et al*., 2012), and morphology of the pollen (DUTRA *et al*., 2014). As there are no reports on the physiological maturation of the seeds of this species, this study can therefore contribute to an understanding of the development, obtention and identification of seeds of better physiological quality, since they are used as propagating material for the species.

Maturation refers to various transformations that occur after the ovule is fertilised; it undergoes morphological, biochemical, physiological and functional changes that result in the formation of the mature seed (HEHENBERGER; KRADOLFER; KOHLER, 2012). Physical and physiological characteristics, including size, water content, germination, vigour and dry weight, are fundamental to the study of seed maturation (CARVALHO; NAKAGAWA, 2012).

When the seed reaches a maximum value for dry weight, germination and vigour, it can be said to be at the point of physiological maturity (PESKE; BARROS; ACHUCH, 2012). It is possible to determine this point by an analysis of the indicators that show alterations in the morphological characteristics, and the biochemical changes specific to a given species (MATA *et al*., 2013). When the seeds reach physiological maturity, they are detached physiologically from the mother plant (CARVALHO; NAKAGAWA, 2012).

With this in mind, the aim of this work was to study the maturation process in seeds of *L. lactescens*, in order to determine the point of physiological maturity and the ideal time for harvest.

MATERIAL AND METHODS

The field research was carried out at the Centre for Agrarian Sciences of the Federal University of Paraíba (CCA/UFPB), Campus II, in Areia, in the State of Paraíba (PB), and all determinations and tests were carried out in the Seed Analysis Laboratory (LAS) of the Department of Plant and Environmental Sciences (DFCA), located on Campus II, in the district of Areia, PB (6°58’12” S and 35°42’15” W), at an altitude of 575 m. According to the Köppen-Geiger classification, the climate in the district of Areia, is classified as tropical with a dry season (As) ending in August, and rainfall from December to January, (EXECUTIVE AGENCY FOR WATER MANAGEMENT OF THE STATE OF PARAÍBA - AESA, 2011).

To study physiological maturation in seeds of *L. lactescens*, four mother plants were selected in the region based on vigour, showing abundant fruiting and spaced 100 m apart. Cross- and self-pollination were used for seed production, while for fruit formation, natural and self-pollination systems were used, employing plants of good phytosanitary appearance and an average height of approximately 10-20 m. After verifying that 50% of the inflorescences were in anthesis, inflorescences throughout the canopy were marked using satin ribbon. Once marked, the plants were monitored, and every two weeks random measurements of four samples of 25 fruit were taken (ALVES *et al*., 2005).

Harvesting began 15 days after anthesis and continued up to 210 days, from 16 May to 14 November 2015 and from 13 May to 11 November 2016, at two-weekly intervals for a total of 14 harvests. The collections were carried out manually, being careful not to cause any mechanical damage. After harvesting, the fruit was packed in thermal boxes to avoid changes in water content and taken to the laboratory for the determinations and tests.

Biometric measurements were taken with a digital calliper, including length, width and thickness, from 15 up to 210 days after anthesis. One hundred fruit were randomly collected from different inflorescences, stored in thermal boxes and taken to the laboratory. These were then divided for later measurement, into four samples of 25 fruit for each harvest period and each year of the study, the results being expressed in millimetres.

Fruit coloration was classified visually as green, yellowish-green, light grey with light brown, and light brown, which occurred during the different stages of the maturation process. After each harvest, subsamples were packed into two different types of packaging (zip bags and glass) and stored in a freezer to preserve the coloration and photograph the 14 stages, based on variations in colour over the two years of study.

Water content was determined by the oven-drying method at 105 ± 3 °C for 24 hours (BRASIL, 2009), using four replications of 15 fruit for each harvest period over the two years. After drying, the samples were removed from the oven, placed in a desiccator and weighed on an analytical balance (0.001 g), with the results expressed as a percentage.

Dry weight was determined together with the water content for all harvest times. After remaining in the oven for 24 hours at 105 ± 3 °C (BRASIL, 2009), the four aluminium capsules containing the 15 fruit were weighed, and the results expressed in grams.

The germination test was carried out on 100 seeds divided into four replications of 25 seeds each and treated with Captan® fungicide in the proportion of 240 g 100 kg^{-1} seeds. The seeds were sown in acrylic boxes (11.0 x 11.0 x 3.5 cm) in blotting paper moistened to 2.5 times its dry weight as substrate. The boxes were placed under daylight fluorescent lamps (4 x 20 W) in a Biological Oxygen Demand (BOD) germinator, regulated to an alternating temperature of 20-30 °C and a photoperiod of 16 hours light and 8 hours darkness respectively (BRASIL, 2009). Daily counts were taken of the germinated seeds until the 82nd day after the start of the test, using the criterion of normal seedlings as described in the Rules for Seed Analysis (BRASIL, 2009), with the results expressed as a percentage.

The germination speed index was carried out together with the germination test by a daily count of the germinated seeds always taken at the same time; the number of normal seedlings was then determined. The index was calculated as per the formula proposed by Maguire (1962).

Seedling length and dry weight were determined at the end of the germination test. The length of the shoot and primary root of normal seedlings was measured using a graduated rule (mm), and the results expressed in centimetres. After measuring, the roots and shoots were separated with scissors, packed in Kraft paper bags and placed in an air circulation oven at 65 °C to constant weight (48 hours). They were then removed from the oven, placed in desiccators and weighed on an analytical balance (0.001 g), with the results expressed in grams.

A completely randomised design was used, with the data submitted to analysis of variance (ANOVA) for the two years of the study, and to polynomial regression analysis for the harvest times, using the largest order regression model (R^2), so that all possible occurrence estimates were expressed. To carry out the statistical analysis, the SISVAR software (FERREIRA, 2007) was used.

RESULTS AND DISCUSSION

From the data on fruit length in *L. lactescens*, a quadratic fit was seen, with an increase in this variable being noted during the maturation process. The maximum value during 2015 was 4.92 mm, obtained 136 days after anthesis, followed by a decrease after that period. According to the data, it was found that fruit length during 2016 did not fit any of the models under test (linear or quadratic), with a single mean value of 4.19 mm (Figure 1A).

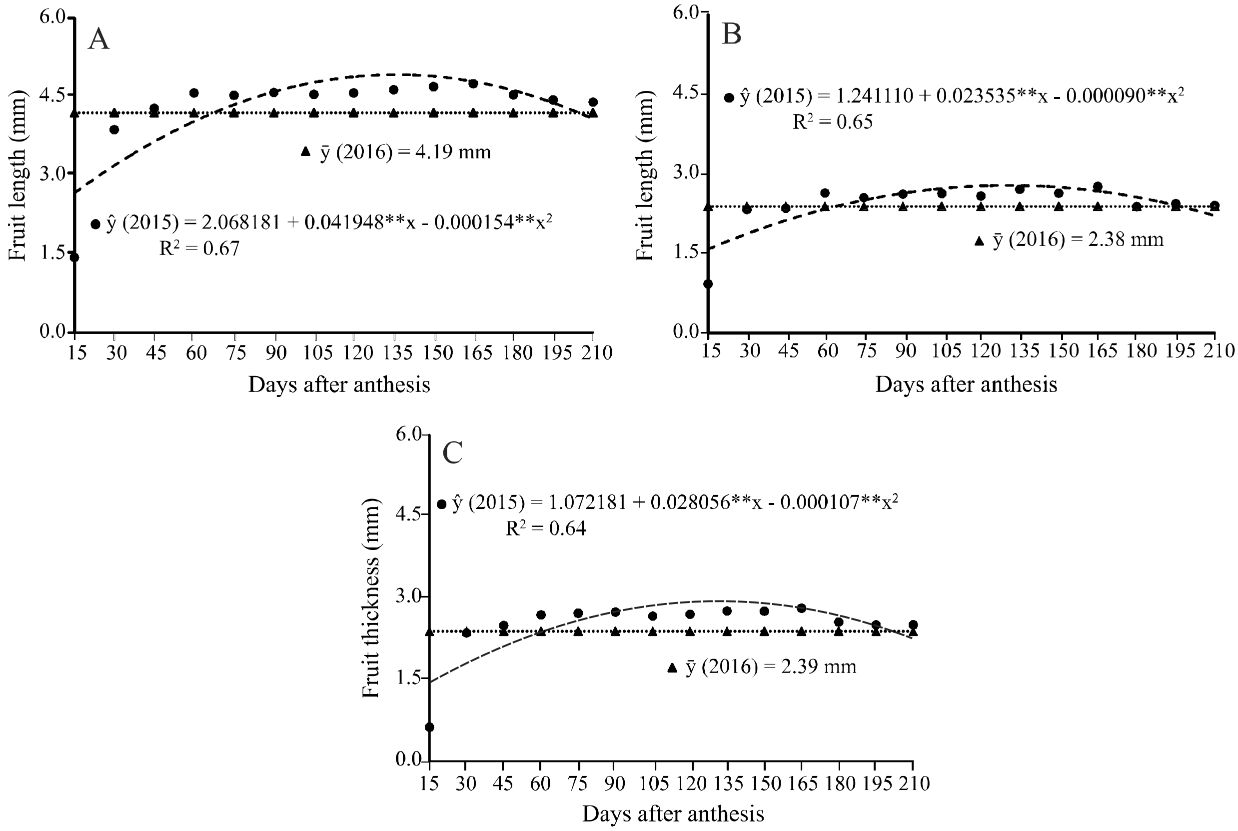
The seeds grow rapidly in size, reaching the maximum in a short time compared to the total duration of the maturation period; this rapid growth is the result of the multiplication and development of the cells that make up the embryonic axis and reserve tissue. In many species, there is a marked reduction in the size of the seeds at the end of the maturation process, probably due to their marked and rapid dehydration (NOGUEIRA *et al*., 2013; SILVA *et al*., 2015).

The data on fruit width in *L. lactescens* also fitted the quadratic model, with a maximum value in 2015 of 2.78 mm, reached 131 days after anthesis. In 2016, it was found that the data for this same variable did not adjust to the linear or quadratic models, showing one mean value of 2.38 mm only, probably due to environmental conditions, since maturation is influenced by several factors, especially those of climate (Figure 1B).

For fruit thickness during 2015, as for length and width, it was found that the data fitted the quadratic model, where the maximum value (2.91 mm) was seen 131 days after anthesis. In relation to 2016, the data on fruit thickness did not fit any of the models under evaluation (linear or quadratic), with a single mean value of 2.39 mm (Figure 1C).

The reduction in both fruit length and width over the two years of the study may be related to dehydration; as the end of maturation approaches, there is a marked decrease in water content as a function of higher temperatures during the process, as can be seen

Figure 1 - Length (A), width (B) and thickness (C) in fruit of *Lophantera lactescens* harvested at different times during 2015 and 2016, Areia, PB



in Figure 2. Physiological changes during the process of fruit maturation may promote variations in biometric values (ABUD *et al*., 2013), while oscillations in size can be caused by climate factors such as a change in the timing of the fruiting process between individuals during the study (MATA *et al*., 2013).

The data on water content for the fruit of *L. lactescens* were seen to fit the linear model, for which, in the harvest started 15 days after anthesis (the first harvest) an increase of 85 and 84% was seen in 2015 and 2016 respectively. Continuing the maturation process, there was a progressive reduction, which in the final harvest (210 days after the anthesis) assumed values of 11 and 3% in 2015 and 2016. This water content favours dispersal of the species, which is classified as a sinzoochore (SALOMÃO; ROSA; MORAIS, 2007).

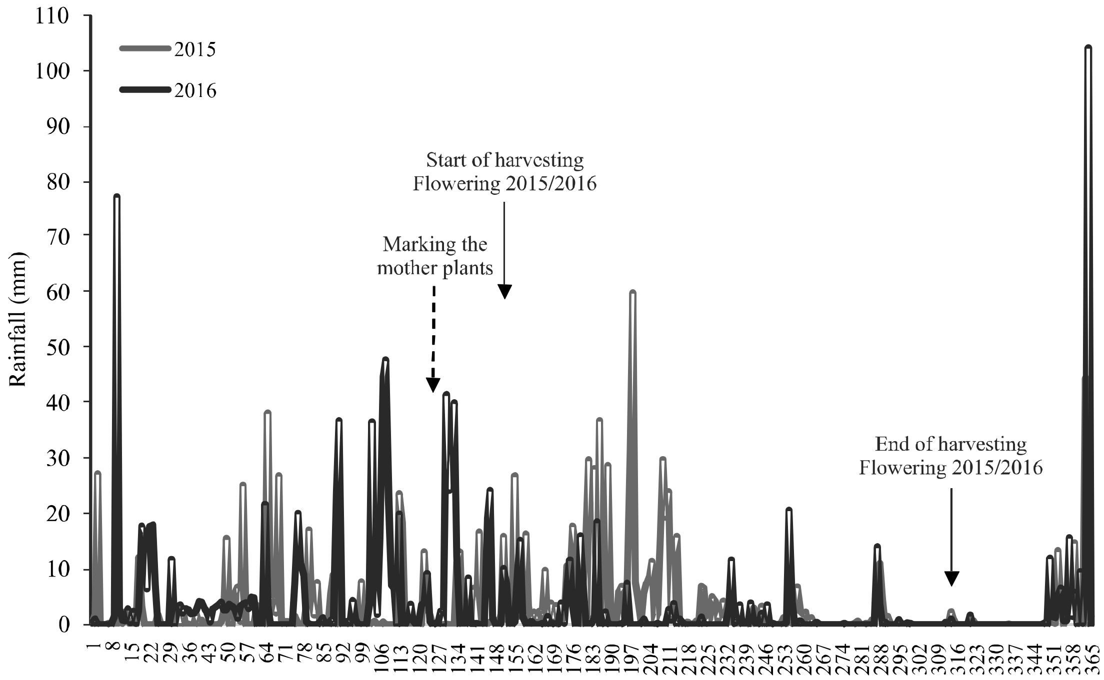
The water content of the fruit, with an R^2 of 0.96 over the two years of study, can be seen in Figure 3A, thereby having a large influence on maturation, and differing from the R^2 for fruit length (0.67), width (0.65) and thickness (0.64) in 2015. Furthermore, the data did not fit the regression models in 2016, which can be attributed to a lack of size uniformity.

The high initial water content and subsequent reduction are related to the importance of water in the processes of transporting assimilates that accumulate during the seed maturation process. At the beginning, the water content of the seeds is high, around 80%; during the course of the process, this is reduced until a hygroscopic balance is reached (MATHEUS; LOPES; CORRÊA, 2011). From then on, the mother plant is no longer seen to control the water content of the seed (CARVALHO; NAKAGAWA, 2012).

The data on fruit dry weight in *L. lactescens* fitted the quadratic regression model. In 2015, the maximum dry weight accumulation (0.171 g) was seen 165 days after anthesis; in 2016, the maximum value for dry weight was 0.202 g 186 days after anthesis, with a later reduction in this value. At that moment the fruits may have detached from the mother plant, so that no more nutrients were received from the plant once the plant-fruit connection ended. The fruit was then only connected to the mother plant physically. There may also have been a degradation of the reserves, since a decrease was seen in dry weight.

L. lactescens is a species with dry fruit, so dry matter accumulation in the fruit is higher, as can be seen

Figure 2 - Daily rainfall data obtained from the weather station at the Centre for Agrarian Sciences of the Federal University of Paraíba during 2015 and 2016, Areia, PB



in Figure 3B, where the respective values for R^2 were 0.98 and 0.99 (in 2015 and 2016 respectively), thereby having a great influence on maturation.

At the moment the seed reaches the point of physiological maturity it displays the maximum dry matter weight, and is therefore at maximum potential and consequently minimal deterioration. The dry matter index is always considered in studies that aim to determine the point of physiological maturity in seeds (SILVA *et al*., 2015).

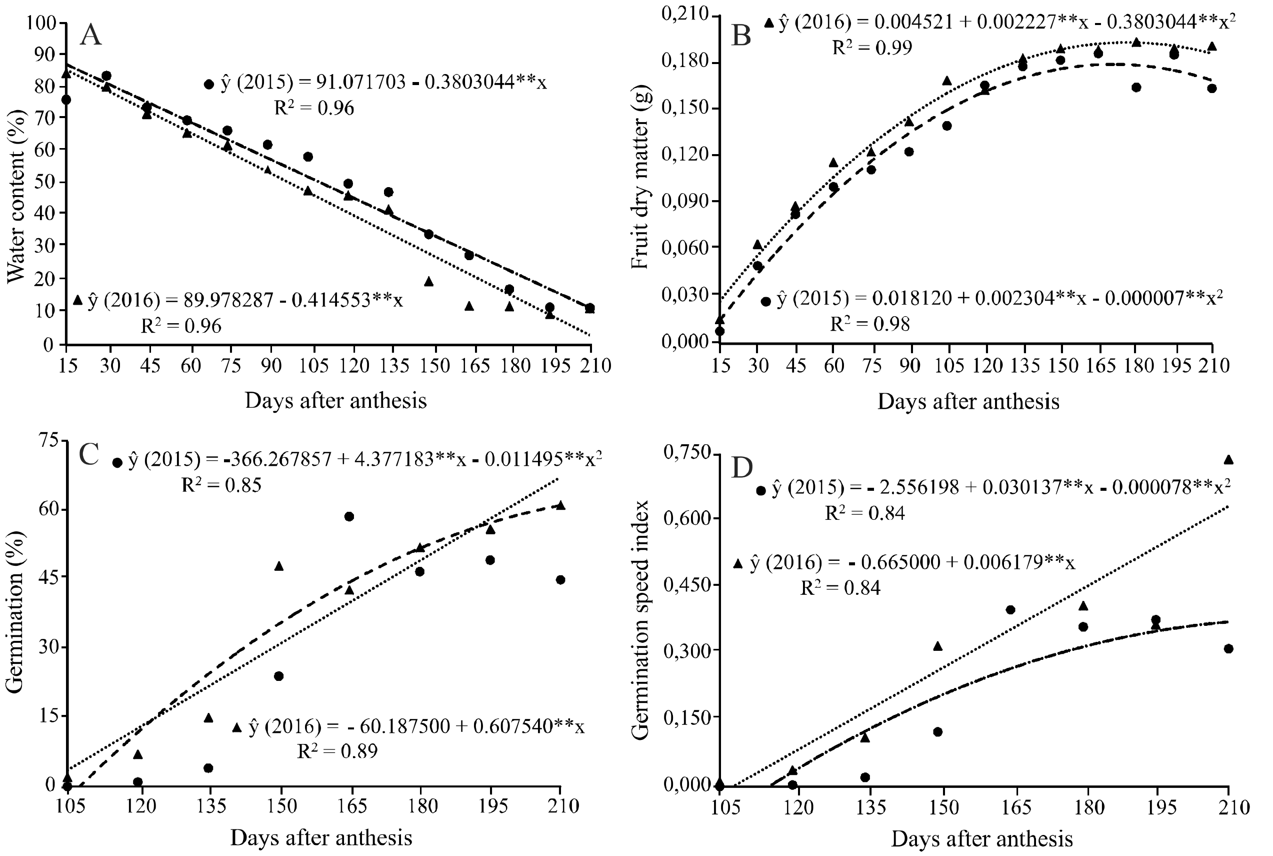
In relation to percentage germination in the seeds of *L. lactescens*, the data fitted the quadratic model in 2015, where the maximum value (50%) was seen 190 days after anthesis. For the seeds harvested in 2016, the data fitted the linear model, with an increase in germination during the days after anthesis, reaching a value of 67% after 210 days, the remaining seeds were not evaluated for dormancy. Germination in family Malpighiaceae is generally characterised as late; in the *Byrsonima* genus, this process may take up to a year to begin (DUKE, 1965) (Figure 3C).

During the maturation process, seed germination increases, reaching maximum percentage close to the immobilisation of the dry matter flow from the plant to the seed, as seen in species that present dormancy, caused by an imbalance in the action of substances which promote or inhibit germination at the time of dry matter accumulation (MARCOS-FILHO, 2015).

To define maximum dry matter accumulation, Ellis and Pieta Filho (1992) suggested the term "mass maturity", which is not related to the maximum physiological quality that may occur a little before or after mass maturity. In seeds of *Poincianella pyramidalis* (Tul.) L.P. Queiroz (LIMA *et al*., 2012), *Inga striata* Benth. (MATA *et al*., 2013) and *Poincianella pluviosa* (DC.) L.P.Queiroz (SILVA *et al*., 2015), maximum physiological quality does not generally coincide with maximum dry matter accumulation. Maximum germination percentage in the seeds of *L. lactescens* did therefore not coincide with maximum dry matter accumulation, as changes in the physiological quality of the seeds may still occur after maximum dry matter accumulation.

In 2015, the data on the rate of seed germination in *L. lactescens* fitted the quadratic model, with the maximum value (0.355) seen 193 days after anthesis, after which there were gradual reductions in vigour, probably due to the seeds being physiologically detached from the mother plant and therefore subject to environmental conditions that may promote physiological changes but may also promote losses due to deterioration in the field. In 2016, the data fitted the linear model, where there was a continuous increase in vigour until 210 days after anthesis

Figure 3 - Water content (A) and fruit dry weight (B), percentage germination (C) and rate of germination (D) in seeds of *Lophantera lactescens* harvested at different times during 2015 and 2016, Areia, PB



(0.738), at which time the fruit displayed a light-brown coloration with wrinkling of the pericarp (Figure 3D).

The data on primary root length in seedlings of *L. lactescens* fitted the linear model in 2015, with increases in the root as embryo development progressed, reaching a maximum value (1.31 cm) 210 days after anthesis. In 2016, the data fitted the quadratic model, achieving the greatest length (1.01 cm) 206 days after anthesis (Figure 4A).

For shoot length in seedlings of *L. lactescens*, the data fitted the quadratic and linear models in 2015 and 2016 respectively, similar to primary root length in the seedlings. In 2015, the greatest length (2.53 cm) was seen 198 days after anthesis, while in 2016, the length gradually increased with development of the embryo; the maximum value of 3.54 cm was seen 210 days after anthesis (Figure 4B).

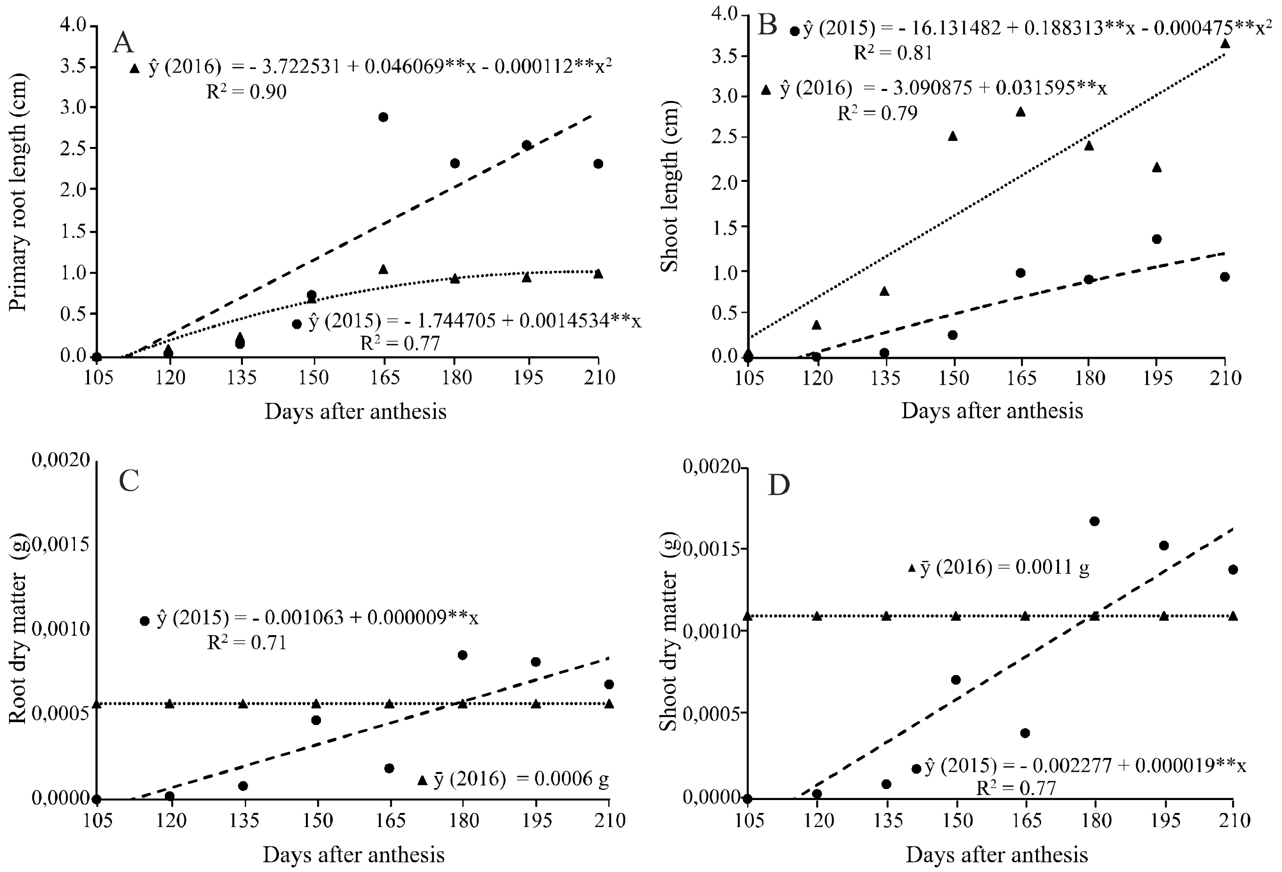
The data for root dry matter in the seedlings fitted the linear model in 2015, showing a continuous increase of this index during embryo development, the maximum value (0.000827 g) was obtained 210 days after anthesis, similar to the data for primary root length, which also fitted the same model. In 2016, the data on root dry mass in the seedlings did not fit any of the models under test (linear or quadratic), (Figure 4C), showing only one mean value of 0.0006 g.

The shoot dry matter data for seedlings of *L. lactescens* fitted the linear model in 2015, showing an increase in dry matter content as embryo development progressed; the greatest value (0.001713 g) was seen 210 days after anthesis.

The seedlings probably reached maximum dry matter during the period the seed finished accumulation of the reserves obtained in the course of the maturation process by the mother plant, possibly finally reaching the point of physiological maturity. However, in 2016 the data did not fit the linear or quadratic models, (Figure 4D), with a mean value of 0.0011 g.

Considering that vigour is associated with the seed´s capacity for accumulating reserves, one of the main tests for evaluating seed vigour is seedling dry matter, followed by its distribution for seedling development and growth. It is worth noting that seed vigour may have a direct influence on the ability of the plant to accumulate

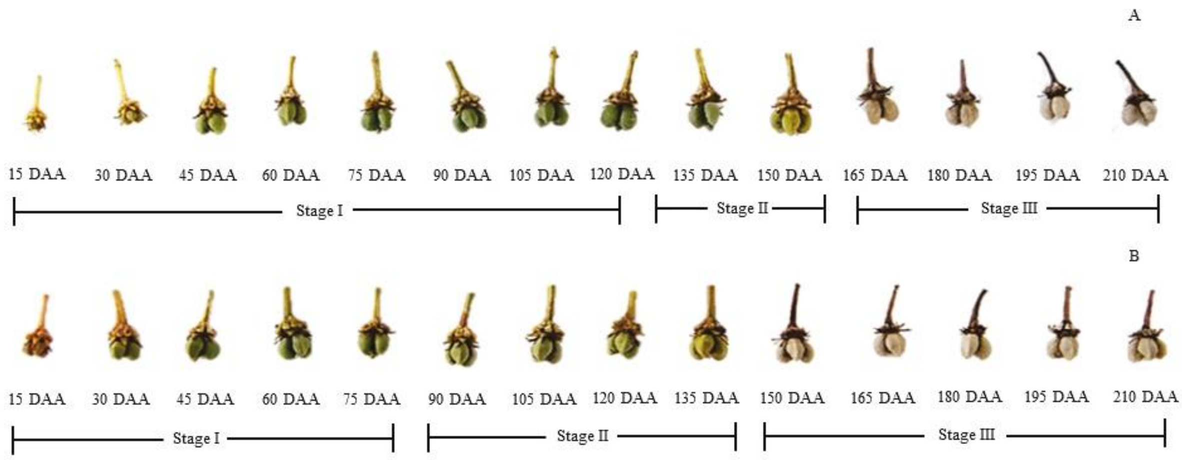
Figure 4 - Length of the primary root (A) and shoots (B), and root (C) and shoot (D) dry matter in seedlings of *Lophantera lactescens* from seeds harvested at different times during 2015 and 2016, Areia, PB



dry matter, with the seed reserves, together with their adequate mobilisation, only promoting initial growth in the seedling for a relatively short time after emergence (HENNING *et al*., 2010).

For the maturation index based on fruit coloration (Figure 5A), in 2015 it was found that from 15 to 120 days after anthesis, coloration varied for tones of green only, i.e. from very light green, to light green, green and dark

Figure 5 - Coloration in fruit of *Lophantera lactescens* from 15 to 210 days after anthesis (DAA) during 2015 (A) and 2016 (B), Areia, PB



green (Stage I). From 135 to 150 days after anthesis, the coloration ranged from yellowish-green to light yellowish-green (Stage II), while from 165 to 210 days after anthesis, the coloration went from light grey and light brown to light brown with wrinkling of the pericarp, when the fruit was preparing for dispersal (Stage III).

In 2016, fruit coloration also varied for the green tones, i.e. from very light green, to light green, green and dark green; however, from 15 to 75 days after anthesis only (Stage I). Between 90 and 135 days after anthesis, the coloration varied from yellowish-green to light yellowish-green (Stage II), while from 150 to 210 days after anthesis, the coloration went from light grey and light brown to light brown with wrinkling of the pericarp (Stage III) (Figure 5B). It could be seen that the process of physiological maturation in seeds of *L. lactescens* was accompanied by visible changes in the external appearance and coloration of the fruit and seeds, thus corroborating the results of Castro, Godoy and Cardoso (2008).

Fruit coloration was an important variable for indicating physiological maturity in seeds of *Jatropha curcas* Linn (RUBIO *et al*., 2013). In *Moringa oleifera* Lamarck, physiological maturity in the seeds was reached during the fourth stage of maturation, with dehiscent fruit of a dark-brown coloration, showing that fruit coloration can be used to evaluate the physiological maturity of the seeds (AGUSTINI *et al*., 2015).

CONCLUSIONS

1. Under the environmental conditions of Areia, PB, the ideal harvest time and the point of physiological maturity of seeds of *L. lactescens* is reached between 165 and 186 days after anthesis;
2. A light-brown fruit coloration with wrinkling of the pericarp is a good visual indicator for determining the point of physiological maturity in seeds of this species;
3. The water content and fruit dry matter are the most efficient indicators for determining the ideal harvest time and the physiological maturity of seeds of *L. lactescens*.

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