

## MATURATION STAGES OF FRUITS AND PHYSIOLOGICAL SEED QUALITY OF *Physalis ixocarpa* BROT. EX HORMEN<sup>1</sup>

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**ABSTRACT-** *Physalis* is represented by species that produce fruits with relevant potential for national and international marketing. The species are normally grown from seeds, so maturation studies are important to assist the point of harvest, as well as seeds with a high physiological potential, since the fruits remain enclosed within a cup throughout their development. Despite the nutraceutical importance of fruits and medicinal potential of many species of *Physalis* information on the physiological seeds quality are scarce. This study aimed to monitor the physical and physiological changes of maturation and the viability of seeds during fruit development stages. The fruits of *P. ixocarpa* were collected in 15, 25, 35, 45, 55 days after anthesis (DAA). Length, diameter, fresh weight, skin and cup coloring characterization, TSS content, number and weight of seeds per fruit were obtained at each development stage. We also evaluated and correlated the coloring and pigment content of the cup (chlorophyll *a* and *b*). Water content, dry matter weight and germination rate were measured from fresh and dry seeds (room conditions, 72 hours). The main physical and physiological changes during maturation occurred within 35 DAA. The maximum physiological seeds quality was achieved within 45 DAA when the cup is completely or partially broken with coloring between yellowish-green and light-yellowish-brown and the ripe fruit in dark-green.

**Keywords:** sowing, point of harvest, seeds weight, GSI.

## MATURAÇÃO DE FRUTOS E QUALIDADE FISIOLÓGICA DE SEMENTES DE *Physalis ixocarpa* BROT. EX HORMEN

**RESUMO:** *Physalis* é representado por espécies que produzem frutos com relevante potencial de comercialização nacional e internacional. As espécies são normalmente cultivadas a partir de sementes, e estudos de maturação são importantes para auxiliar na obtenção do ponto de colheita e de sementes com elevado potencial fisiológico, visto que os frutos permanecem encerrados dentro de um cálice durante todo o seu desenvolvimento. Apesar da importância nutracêutica dos frutos e do potencial medicinal de muitas espécies de *Physalis*, informações sobre a qualidade fisiológica de sementes são escassas. Este trabalho teve como objetivo acompanhar as mudanças físicas e fisiológicas da maturação e a viabilidade das sementes durante os estádios de desenvolvimento do fruto. Os frutos de *P. ixocarpa* foram coletados aos 15, 25, 35, 45, 55 dias após a antese (DAA). Em cada estágio de desenvolvimento foi obtido o comprimento, diâmetro, peso fresco, caracterização da coloração da casca e do cálice, teor de SST, número e peso de sementes por fruto. Foram também avaliados e correlacionados a coloração e o teor de pigmentos (clorofilas *a* e *b*) do cálice. As sementes frescas e dessecadas (condições ambiente, 72 h) foram avaliadas quanto ao conteúdo de água, peso da matéria seca e taxa de germinação. Observou-se que as principais mudanças físicas e fisiológicas na maturação ocorreram até 35 DAA. A máxima qualidade fisiológica das sementes foi alcançada aos 45 DAA, indicando que a melhor época de coleta é quando o cálice encontra-se completamente ou parcialmente rompido, de coloração verde-amarelado a marrom-amarelado-claro e os frutos maduros de coloração verde-escuro.

**Termos de indexação:** semeadura, ponto de colheita, peso de sementes, IVG.

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## INTRODUCTION

*Physalis* is highlighted as a genus with diverse potential and biotechnological uses (DKHIL et al., 2014; FISCHER et al., 2014), especially fruits of *Physalis ixocarpa*, which in the Brazilian horticulture scenario may be present as exotic and with an aggregate and significant market value.

In Mexico commonly known tomatillo, 'tomate de cascara' and 'tomate verde', the *P. ixocarpa* fruits are widely used in the popular cuisine like salads, soups, stews and green sauces, being so far the most extensively cultivated species in this country, which represents a valuable food, medicinal and economic resource (SANTIAGUILLO-HERNANDEZ; YANEZ, 2009). Published data have shown that besides being sources of essential nutrients to human health, such as vitamin C and B3, carbohydrates, proteins and minerals (BOCK et al., 1995). The ethanol extract of the fruit was active against *Staphylococcus aureus* (KHAN et al., 2016) and showed chemo-preventive activity (MALDONADO et al., 2011). This information showed the need for more accurate studies related to the farming, especially the fruit maturation peculiarities and seeds viability of that species used as a starting point for further investigations, as well as new discoveries of their potential.

Studies have been performed aiming to understand the answers to the stress, storage, conservation and identification of the best moment of harvest to obtain vigorous seeds of *P. ixocarpa* due to the fact that its spread occurs mainly by seeds (CALZADA-LOPEZ et al., 2014; PICHARDO-GONZÁLEZ et al., 2014).

However, species that shows flowering and fruiting occurring discontinuously as *P. ixocarpa* make difficult and sometimes impossible to anticipate the ideal point of the fruit harvest and the physiological seeds maturation, since the same plant shows fruits in different stages of maturation (JUSTINO et al., 2015).

Information on obtaining and production of *P. ixocarpa* seeds are scarce even its being an important aspect for the success of a culture. Thus, studies are necessary to identify and compare the main changes during maturation of fruits in order to determine a harvest index for seeds production in high level.

Thus, the main purpose of this study was to monitor and determine the physiological seeds maturation of *P. ixocarpa* during development and maturation of the fruit trying to predict the best harvest moment.

## MATERIALS AND METHODS

### *P. ixocarpa* farming

Experiments were carried out from April to August 2014 at the Horto Florestal Experimental Unit of the State University of Feira de Santana (UEFS), BA, Brazil, located at 12°16'00" south latitude and 38°58'00" west longitude, showing altitude of 234 meters. The regional climate is dry sub-humid, mega-thermal (C2rA'a) with average temperature at 24 °C and average rainfall around 848 mm annual, according to the classification of Thornthwaite and Matther (1955) (Climatological station of UEFS, 2015).

Seeds from the previous year grown in the same location were used for seedlings production. Sowing occurred in plastic containers of 300 ml completed with a commercial substrate (Plantmax<sup>®</sup>) placed in a greenhouse and under irrigation by microsprinkler. Thinning was performed after emergence and expansion of cotyledons by keeping a seedling per container until the transplanting with an average of 15 to 20 days after sowing, when seedlings show the first couple of eophylls. Before transplanting, the pits (15 cm deep) received 50g of organic fertilizer. Sixty seedlings of *P. ixocarpa* were arranged in five lines, spaced 0.8 m between plants and 2.0 m between lines.

The staking of air branches were performed in X format during farming according to the recommendations of Muniz et al. (2011). Dripper spaced every 0.8 m were used as irrigation system. Emulsifiable oil solution (1%) of a commercial neem was used for pests controlling, which was diluted in water in a portion of 5 mL L<sup>-1</sup>, applied with hand sprayer in the beginning of plant development, when a higher incidence of pest attack was observed.

During flowering stage, from the anthesis (flower opening) an average of eight flowers per plant was identified, then, the fruit development was monitoring. The identification period did not exceed 15 days cultivation at that stage.

### *Physical-chemical analysis and fruit maturation*

The physical-chemical analysis was performed by a set of fruit + cup collected with five (5) stages of development, corresponding to 15, 25, 35, 45 and 55 days after anthesis (DAA). At each stage 50 fruits were randomly harvested in different positions from the plant. A color chart RHS (THE ROYAL HORTICULTURAL SOCIETY, 2001) was utilized to determine the predominant colors of the fruits skin and cup. The weight average of the fresh

weight (g), the transverse diameter (measured in the middle portion of the fruit), and longitudinal (measured from apex to base) of the fruit (mm) were used for sampling through an analytical balance known as Shimadzu AY220 (0.001g) and digital caliper of Cosa brand (0.01mm), respectively. The total soluble solids (TSS) was indirectly measured by placing a fruit juice drop in the manual refractometer prism of Atago brand in a temperature correction of 20 °C and expressed in °Brix. After that analysis, a randomized sample was performed consisting of five repetitions and each one was represented by 10 fruits. An average number and seeds weight per fruit were defined after processing and drying.

The cup coloring was related with the content of chlorophylls *a* and *b* and carotenoids. The pigments were extracted by using dimethyl sulfoxide (DMSO P.A.) and analyzed in triplicate. Six foliar discs of known field were removed from the cup, immersed in screw-tubes containing 5 mL of the organic extractor. The tubes were in dark and in ambient temperature conditions for approximately 48 hours according to methodology adapted by Cruz et al. (2007). Absorbances were read in Bel Spectro S05 in lengths of 480, 649 and 665 mm. Another six discs, with the same surface were used to determination of weight dry matter (DM). The content of chlorophyll *a* and *b*, and carotenoids (mg g<sup>-1</sup>) were determined according to the equations proposed by Wellburn (1994).

#### **Physiological seeds quality**

The water content of seeds was defined in two moments after harvest: a) when recently taken from the fruits (fresh seeds) and b) after drying for 72 hours at ambient condition. Dry seeds remained separated in Eppendorf, packed in glass pots at a temperature of 5°C ± 1 to obtain seeds in all evaluated stages. The measured water content was based by ISTA (2007) to each maturation stage (DAA) 200 seeds (50 seeds by repetition) incubated at 103 ± 2 °C for 17 ± 1 hours at air circulation. The difference between initial and final weight was used to calculate water content (%).

The seeds viability in different stages of development and two conditions after seeds harvest (fresh and dry) was determined by germination test performed in controlled conditions of temperature and photoperiod determined for other species of *Physalis* (SOUZA et al., 2011; 2014). The tests were carried out in chambers with photoperiod of 12 hours adjusted in alternating temperature of 20-30°C for 21 days. Daily evaluations were performed and seeds that produced radicle with at least 2 mm long were considered germinated. At the end of the test,

the germination curves were compared at different stages of development, the average time (AT) and the germination speed index (GSI) of fresh and dry seeds.

The germination test was randomized with 4 replicates of 24 seeds. Shapiro-Wilk and Levene tests were used for testing data for normality and homogeneity, being transformed with  $\arcsin\sqrt{(x/100)}$  when applied. Data were submitted to variance analysis and Tukey test at 5% of probability was used for comparison between the averages. The data was statistically analyzed using the SISVAR software (FERREIRA, 2011).

## **RESULTS AND DISCUSSION**

The fruits of *P. ixocarpa* showed significant growth in length and diameter within 35 days after anthesis (DAA), achieving an average of 34.5 and 42 mm, respectively. From this period any investment in the fruit growth does not differ significantly from later stages (Table 1). The results are similar to the higher values obtained by varieties of *P. ixocarpa* evaluated by Rodríguez-Burgos et al. (2011). Analyzing the length and diameter data we observed that the increase of these characteristics occurs simultaneously with the fruit maturation, which was also observed by Cantwell et al. (1992) who accompanied the growth of *P. ixocarpa* fruits. Sbrussi et al. (2014) also observed the similar result during maturation of *P. peruviana* fruits.

The weight of fresh fruits showed the same growth trend observed in the analysis of length and diameter (Table 1) not showing significant differences within 35 DAA and in the following stages. Since the anthesis, the increase in the weight of fresh fruits occurred continuously from 7.4 g to 34.2 g, an increase of almost five times the initial weight in just three weeks of growth. Similar results were observed by Cantwell et al. (1992) and Rodríguez-Burgos et al. (2011) working with same species.

According to Aizat et al. (2014), the starch stored in the fruits was hydrolyzed during maturation process, which increases the soluble solids content. This characteristic is very important for the majority species of *Physalis*, especially those that are consumed often fresh. The content of soluble solids in fruits of *P. ixocarpa* after 35 DAA showed values approximately 5 °Brix (Table 1) and were similar to those reported for the species, ranging between 5 to 6.5 °Brix according to Cruz-Álvarez et al. (2012) and Jiménez-Santana et al. (2012). The values found were lower than those obtained for other species of *Physalis*, e.g. *P. peruviana* whose mature fruit

reached 13-14 °Brix (RODRIGUES et al., 2014) and *P. angulata* with 12 °Brix (OLIVEIRA et al., 2011). Differently from species of *Physalis* described before, *P. ixocarpa* fruits are used as vegetables with similar purposes of the tomato (*Solanum lycopersicum*). In general, TSS values in tomato plant vary around 5 °Brix (ARAUJO et al., 2014) showing that the value found in the study is according to the expected for mature fruits used as vegetables.

According to data in Table 1, fruits of *P. ixocarpa* intended for consumption and processing can be harvested within 35 days of development, which showed the highest weights and physical measurements of growth, besides the maximum value of °Brix.

No changes were observed during maturation of fruit skin coloring of *P. ixocarpa*. Different that other species of *Physalis*, the fruits of *P. ixocarpa* remains green during all the development period, differing only in green intensity color. A color changing was observed in the beginning of the development, from dark green color (144A-RHS) to yellowish-green (145A-RHS) as it achieved the last stage of maturation (55 DAA) (Figure 1).

According to Cantwell et al. (1992) the content of chlorophyll and carotenoid in the pulp decreases with the development and these pigments are related to changes in the green intensity along during the maturation of *P. ixocarpa* fruits. The same trend pigment decrease was observed when comparing the cup color with chlorophylls contents; cup color changed from yellowish-green (145A-RHS) with 35 DAA, to partially light-yellowish-brown (158A-RHS) with 45 DAA, ending the maturation with the cup predominantly light-yellowish-brown and crumbly appearance (55 DAA) (Figure 2). This breaking characteristic of the cup can be attributed to higher measures of length and diameter of the fruits obtained at this stage (Table 1). The pigment quantity in the cup is inversely proportional to the increase of weight in fresh fruits (Tables 1 and 2), showing higher contents in the early stages and decreasing too fast in the last three stages of maturation, indicating the cup participation in the production, and probably in the partitioning of assimilated for mass increases during the fruit development.

One of the species characteristics belonging to *Physalis* genus is the presence of the cup acting as a protective structure until its dehydration color changing and small disruptions indicating for the majority of the species at harvest of ripe fruit (BALAGUERA-LOPEZ et al., 2014). *P. ixocarpa* shows changes in the cup much more pronounced than the color of the fruits, different from *P. angulata*

and *P. peruviana*, which have a completely brownish and orange color at the time of harvest (CARVALHO et al., 2014; SBRUSSI et al., 2014).

No significant differences were observed between development stages for the number of seeds produced, showing vary between 221 and 270 seeds per fruit (Table 1). Although the number of seed is a characteristic genetically determined by species, the weight reached during the development stages is a result of nutritional and physiological conditions of the plant. During embryogenesis and seed development there is a great demand of assimilated, which in the final phase of maturation corresponds to the maximum weight of the seeds, due to the complete formation of the embryonic axis and endosperm tissue. As shown in Table 1, *P. ixocarpa* seeds reached the highest weight during the last three maturation stages (35 to 55 DAA), with approximately 503, 419, and 474 mg, respectively, without differences between them, but with falling trend in the advanced stage of maturity. Rodriguez-Burgos et al. (2011) found similar results in ripe fruit of Quetaro and Mahone variety of *P. ixocarpa*, with 0.555g and 0.515g, respectively.

As shown in Figure 2, the dry weight of the seeds has increased rapidly in the early stages of development. This result was observed in both fresh and dry seeds. With 35 DAA, the dry matter accumulation occurred slowly in the seeds, reaching the maximum of reserves deposition within 45 DAA, coinciding with reductions of water content, which characterizes the desiccation phase, and preparation for trophic links interruption with the mother plant. Physiologically, this would be the seed harvest point in their maximum physiological quality. However, the seeds of *Physalis* are inserted in fleshy fruits, and water content reductions shall only occur after seeds withdrawal and processing (CARVALHO et al., 2014; PÉREZ-CAMACHO et al., 2008). Seeds of *P. ixocarpa* reached the maximum dry weight with high water content, between 30-50%, in such circumstances, the metabolism remains active, and a seed deterioration process can start if not promptly harvested (KONG et al., 2014). Thus, the delay in fruits harvesting may lead to the decrease of physiological seeds potential, causing damage in vigor and longevity.

Changes in the water content occurred inversely to dry matter accumulation. The moisture content of the seeds decreases as the fruits and seeds have matured, both in fresh seeds as in dry one (Figure 2). As expected, fresh seeds showed a high water content at the beginning of development, reaching 90% and with reduction of 40% until the

last stage of maturation. Generally seeds harvested with high water content deserve close attention when we want to maintain longevity and vigor. The final drying shall be gradually to avoid membrane damage before storage. Pérez-Camacho et al. (2008) observed a fast reduction in humidity between 35 and 42 days after flowering for *P. ixocarpa*, and the maintenance of humidity around 45% up to the final evaluated stage. According to these results, although there are significant reductions in water content, it is still high in fresh seed of *P. ixocarpa*, even in the last evaluated stages (Figure 2A).

Values below 9% for water content of dry seed at stage from 25 to 55 DAA (Figure 2B) are close to the used as reference for the maintenance of physiological and germinative capacity (WALTERS, 2015). These data are important for making decisions of seeds drying methods for conservation purposes. According to the development stage of *Physalis* seeds the low water content had an increase of germination capacity, keeping the physiological potential and decreases the loss of viability (CARVALHO et al., 2014; PEREZ-CAMACHO et al., 2008; SOUZA et al., 2014).

*P. ixocarpa* seeds showed some germination potential 25 days after anthesis and higher values were observed with 45 DAA, at the same period that seeds reached maximum weight and physiological maturity. The lowest seeds germinated rate in the early stages occurred because of the embryonic axis immaturity which seeds are still investing in tissue formation and probably does not acquired desiccation tolerance capacity, because it would be accumulating reserves to be used during the germination process. According to Perez-Camacho et al. (2008) the increase in desiccation tolerance capacity occurs in

the final stages of *P. ixocarpa* seed development, likely when sugar content is high and intracellular membranes stabilization, improving the embryo's performance during germination. Figure 3 shows that 45 DAA point of physiological maturity, the relative frequency of germination was found to the left showing unimodal peak at greater speed and uniformity of germination of *P. ixocarpa*. These results are related to vigor and germination rate and are important parameters of comparison when your goal is to conserve seeds and to use them at other times.

The pre-drying of seeds had no influence in the germination rate when related to an advanced stage of development (Figure 2). However, it provided a quite germination, reaching 70% of total (Figure 3). Perez-Camacho et al. (2008) observed similar results, in which drying increased the seed germination capacity of *P. ixocarpa* from 28 days to 63 days after flowering.

Seeds remained fresh have decreased in average time (AT) and increased in germination speed index (GSI) with the seeds development, where AT of 2.4 days and GSI of 11.5 days were the best results obtained within 55 days after anthesis (Table 3). As for dry seed, the AT of 6.2 days decreased to 2.4 days, when stages of 35 and 55 DAA were compared. The higher GSI was observed in the last two stages of development, achieving approximately 11 and 10.9 days, respectively. The results confirmed the physiological seed quality of *P. ixocarpa* with 45 DAA, reflecting the higher seed vigor and the similarity to those observed by Perez-Camacho et al. (2008) in which the highest speed indexes were obtained in harvested seeds with 42 days of flowering.

**TABLE 1-** Length (L), diameter (D), fresh weight (FW), content of total soluble solids (TSS), seed weight (SW) and number of seeds (NS) in different stages of development of *P. ixocarpa* fruits.

| DAA | L<br>(mm) | D<br>(mm) | FW<br>(g) | TSS<br>(°Brix) | SW<br>(mg) | NS    |
|-----|-----------|-----------|-----------|----------------|------------|-------|
| 15  | 21.9 c    | 25.8 c    | 7.50 c    | 2.4 b          | 87.7 c     | 241 a |
| 25  | 29.0 b    | 36.4 b    | 20.8 b    | 3.2 b          | 282.3 b    | 243 a |
| 35  | 34.5 a    | 42.0 a    | 34.2 a    | 4.6 a          | 502.6 a    | 271 a |
| 45  | 33.8 a    | 44.2 a    | 37.4 a    | 5.1 a          | 419.2 a    | 221 a |
| 55  | 35.9 a    | 44.0 a    | 38.3 a    | 5.0 a          | 474.1 a    | 250 a |

DAA= days after anthesis. Averages followed by the same letter in the column, for each variable analyzed, do not differ by Tukey test, 5% of probability.

**TABLE 2** - Content of chlorophyll *a*, *b*, total and carotenoids of cups of *P. ixocarpa* fruits in different stages of development.

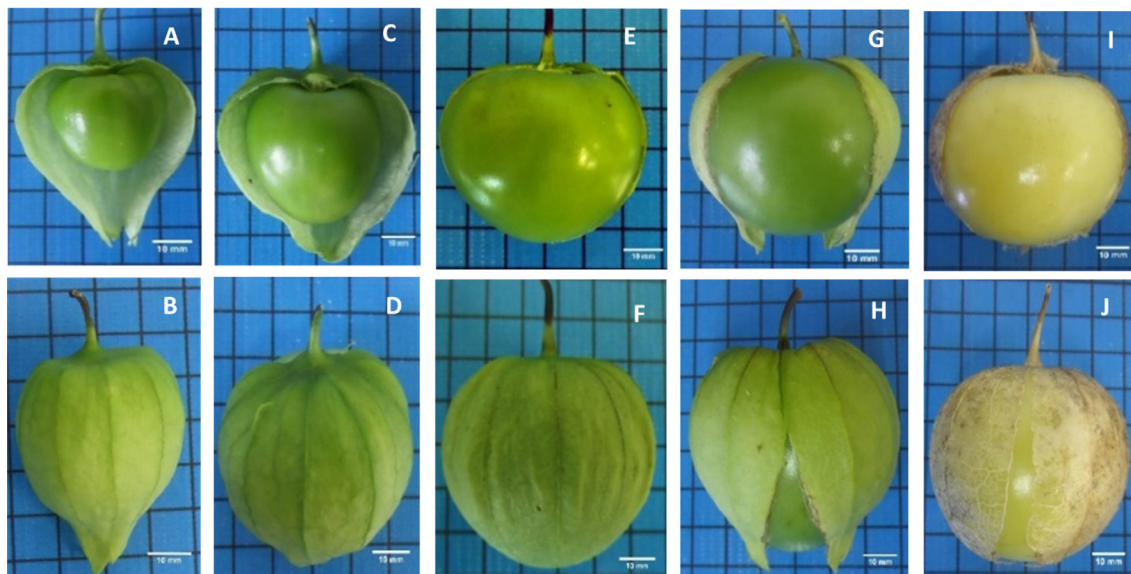
| DAA | Chl <i>a</i> (mg g <sup>-1</sup> ) | Chl <i>b</i> (mg g <sup>-1</sup> ) | Carot (mg g <sup>-1</sup> ) | Chl total |
|-----|------------------------------------|------------------------------------|-----------------------------|-----------|
| 15  | 1.60 c                             | 0.97 b                             | 0.35 b                      | 2.58 d    |
| 25  | 1.82 c                             | 0.99 b                             | 0.38 b                      | 2.82 cd   |
| 35  | 1.06 bc                            | 0.50 a                             | 0.17 a                      | 1.56 bc   |
| 45  | 0.36 ab                            | 0.20 a                             | 0.19 a                      | 0.55 ab   |
| 55  | 0.09 a                             | 0.26 a                             | 0.07 a                      | 0.35 a    |

DAA= days after anthesis. Averages followed by the same letter in the column, for each variable analyzed, do not differ by Tukey test, 5% of probability.

**TABLE 3** - Average time (AT) and germination speed index (GSI) of fresh and dry seeds in different stages of development of *P. ixocarpa* fruits.

| DAA | Fresh seeds |         | Dry seeds |         |
|-----|-------------|---------|-----------|---------|
|     | AT          | GSI     | AT        | GSI     |
| 15  | 0.00        | 0.00    | 0.00      | 0.00    |
| 25  | 10.37 d     | 0.43 d  | 0.00      | 0.00    |
| 35  | 7.84 c      | 3.27 c  | 6.20 b    | 4.36 b  |
| 45  | 5.62 b      | 5.22 b  | 2.26 a    | 10.96 a |
| 55  | 2.36 a      | 11.54 a | 2.36 a    | 10.85 a |

DAA= days after anthesis. Averages followed by the same letter in the column, for each variable analyzed, do not differ by Tukey test, 5% of probability.

**FIGURE 1**- Stages of development and maturation of fruits and cup coloring of *P. ixocarpa* with 15 (A;B), 25 (C;D), 35 (E;F), 45 (G;H) and 55 (J;I) days after anthesis.

Source: Author's photos.

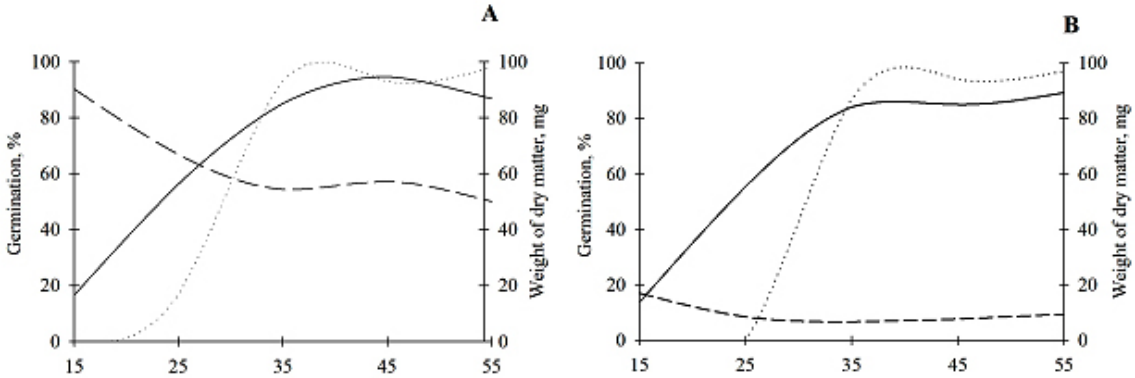


FIGURE 2- Germination (%) (.....), water content (%) (- - -) and weight of dry matter (mg) (—) of fresh (A) and dry (B) seeds in different stages of development of *P. ixocarpa* fruits.

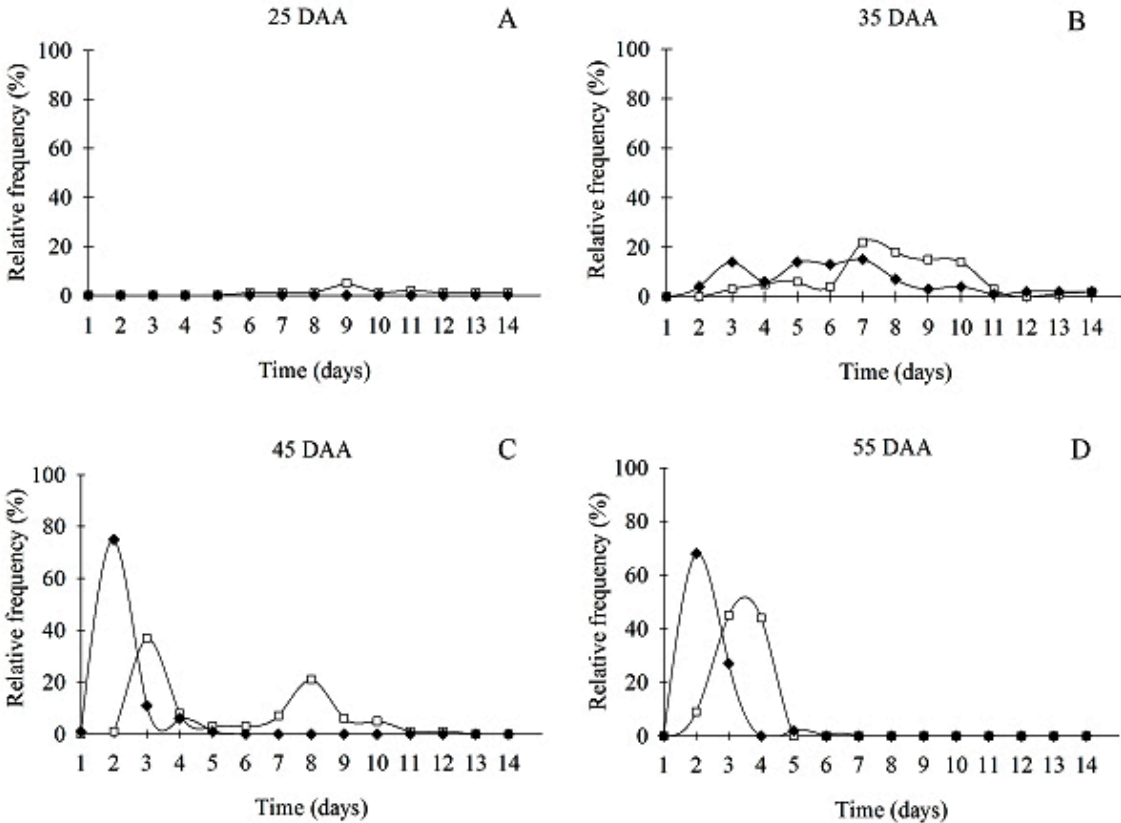


FIGURE 3- Relative frequency (%) of germination of fresh (□) and dry (◆) seeds in different stages of development of *P. ixocarpa* fruits.

## CONCLUSION

*P. ixocarpa* seeds of higher physiological quality were achieved with 45 days after anthesis and can be harvested when the cup becomes disruption and predominantly yellowish-green and dark-green fruits. On the other hand, the fruits intended for use as vegetables due to its physic-chemical characteristics shall be harvested 35 days after anthesis.

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