Physiological maturity of pumpkin seeds

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Received 19 January, 2015; Accepted 18 June, 2015

The objective of this study was to evaluate the physiological changes of pumpkin seeds during the maturation process. Thereby, the study was conducted in the municipality of Juazeiro, at the experimental area of the State University of Bahia. Seeds of the cultivar ‘Maranhão’ were harvested from fruits at regular intervals, every 10 days, from 15 to 60 days after anthesis (DAA). The experimental design was completely randomized with six treatments (15, 25, 30, 40, 50 and 60 DAA) and four repetitions. For each period, the fruits were assessed visually and average weights were determined, and the seeds extracted from fruits were evaluated for the following tests: moisture content, dry matter of the seeds, germination, electrical conductivity, field emergence, emergence speed index. The seeds reached physiological maturity at 50 days after anthesis, although the best time to begin harvesting fruit of this cultivar is between 50 to 60 DAA. At this time the seeds moisture content is low enough to allow the harvest.

Key words: Germination, harvest, seed quality.

INTRODUCTION

In vegetable seeds production must ensure maximum quality and it should be as or more intense than other crop seeds, specially, because of the unit cost of the some of these species is high and there is a provided economic return.

In the latter case, it is important to remember that, in recent years, the cost and productive advantages, qualitative and quantitative of the hybrids have played an important role in development of the agricultural sector. Therefore, hybrid seeds are usually more expensive than other types of seeds because hybrid seeds production involves more advanced technology and as well as more laborious work and thus, it requires the best physical, physiological and sanitary quality as well as maximizing its use. Thereby, it is of great importance to determine the best time to harvest seeds, but also the best storage conditions which will make them last longer.

Considering that, when harvested, the seeds are detached from the mother plant, which until that time was their natural environment. After harvesting, men are responsible for the tasks of conserving the seeds in the best conditions throughout time. However, seed storage starts before the harvesting operation when the seeds have reached their point of physiological maturity (Costa et al., 2006). Harvesting fruit for the purpose of seed extraction in cucurbits is normally done when the fruits...
are ripe, however, it can be performed even before complete maturation, and followed by post-harvest storage (Bisognin et al., 1999). Therefore, it is of fundamental importance to determine the best time to harvest the fruits combined with the post-harvest storage for obtaining seeds of high physiological quality. The post-harvest fruit storage is related to the fact that the seeds continue to ripe if they have not completed their maturity in the field, reaching maximum levels of germination and vigor (Morocco et al., 2011).

Research findings with other cucurbits indicated different behaviors regarding optimal harvest time and post-harvest storage. The most positive on germination and vigor were obtained when the fruits harvests were done early for pumpkin cv. ‘Menina Brasileira’ (Morocco et al., 2011), and for Italian squash (Alvarenga et al., 1991).

For cucurbits, which have fleshy fruits, the difficulty is to know the time when their seeds reach the point of maximum physiological quality. For this family the process of seed maturation continues after harvest, reaching maximum levels of germination and vigor after undergoing a rest period, which varies among different species (Vidigal et al., 2006; Dias et al., 2006). Therefore, the objective of this work was to determine the physiological maturity of seeds of pumpkin.

**MATERIALS AND METHODS**

The experiment was conducted in the experimental field of vegetables at the Department of Technology and Science of the University of the State of Bahia/ UNEB, situated in Juazeiro-BA during the period December 2012 to March 2013. Seeds of pumpkin cv. Maranhão were used. The soil of the experimental area soil classified as Vertisol and soil samples were collected and sent for chemical analysis to the Laboratory of Soils Analysis of the UNEB and the results obtained were as follows: pH in water = 6.5; P = 69.64 mg.dm$^{-3}$; K = 0.34 cmolc.dm$^{-3}$; Ca = 6.4 cmolc.dm$^{-3}$; Na = 0.06 cmolc.dm$^{-3}$ and Mg = 1.1 cmolc.dm$^{-3}$. The municipality of Juazeiro is located at 09°24’ latitude and 40°30’ longitude WGR, and an altitude of 368 m. The climate is semi arid according to Koppen classification. The meteorological data of the area, collected during conduction of the experiments are shown in Table 1.

In tillage practices were performed plowing and disking followed by harrowing and plant fertilization was performed according to the recommendations of the results of soil analysis (Cavalcanti, 1998). Irrigation water was applied once daily, using drip emitters with 1.8 Lh$^{-1}$. Pumpkin seeds sowing was carried out with the cultivar ‘Maranhão’ using polystyrene trays containing 128 cells filled with Plantmax® commercial substrate. At 12 days after sowing, the transplanting of seedlings was performed (when the plants have two true leaves). The seedlings were planted at a spacing of 2.0 m x 1.0 m. Other agronomical practices were performed based on the crop needs for optimal production (Filgueira, 2008). The crop cycle was checked daily for monitoring plant growth. The flowers were tagged with colored ribbons on the day of anthesis and their fruits were harvested in accordance with pre-established ages. Immediately after harvest, the fruits were sent to the Laboratory Storage of Agricultural Products, and kept for 10, 20 and 30 days at room temperature around 28°C. After this period, the seeds were removed and then placed to determine the moisture content and dry mass. The seeds were washed and subjected to disinfection with sodium hypochlorite solution of 1% for three minutes to eliminate contaminant, and placed to dry at room temperature (27 to 30°C) for 12 h in the laboratory (Kikut, 2005). Seed quality was evaluated by the following tests:

**Moisture content:** Was performed with four subsamples (and four replicates) of 0.5 g of seeds per treatment at 105 ± 3°C oven method for 24 h, according to the Rules for Seed Analysis - RAS (Brasil, 2009) and the results expressed in percentage.

**Dry weight of seeds:** Was determined in two subsamples of 30 seeds (and four replicates) based on the final outcome of the seeds after drying at 105 ± 3°C for 24 h (Brasil, 2009) and results were expressed in g 30$^{-1}$ seeds.

**Germination:** Was performed using four subsamples of 50 seeds (and four replicates) that were sown in germitest paper towel rolls, moistened with an amount of water equivalent to 2.5 times the weight of dry paper and packed in transparent plastic bags and kept in a germination chamber at a temperature of 25°C. The evaluations were performed daily until the eighth day after sowing, determining the percentage of normal seedlings (Brasil, 2009).

**Electrical conductivity:** Was performed using four subsamples of 50 seeds (and four replicates). The seeds were weighed and placed in plastic cups to soak containing 75 ml of distilled water and kept in a BOD incubator for 4 h at 30°C (Terres et al., 1998). Readings were taken and the results expressed as μS.cm$^{-1}.g^{-1}$ seed.

**Field emergence of seedlings (EC):** Four subsamples of 50 seeds (four replicates) were sown 0.5 cm deep in plastic trays containing washed and sterilized sand, moistened to 60% capacity retention. Trays were kept in a green house by a 70% of lighting and at an average temperature of 28°C. The number of emerged seedlings was counted daily until the eighth day (Maguire, 1962). The experimental design was completely randomized with six treatments (15, 25, 30, 40, 50 and 60 DAA) and four replications. Statistical analysis was performed using the Sisvar software (2000). All variables were subjected to regression analysis and curve fitting based on the age of the fruits.

| Year | Month  | RH average (%) | Temperature (°C) | Precipitation (mm) |
|------|--------|----------------|------------------|-------------------|
| 2012 | December | 62.4 | 26.5 | 187.2 |
| 2013 | January  | 58.1 | 28.3 | 55.4 |
| 2013 | February | 56.4 | 29.1 | 51.3 |
| 2013 | March    | 71.5 | 27.8 | 153.0 |

Table 1. Meteorological data of the area during the experiment.
RESULTS AND DISCUSSION

The average fruit weight ranged from 1163.81 g at 15 days after anthesis (DAA) to 2319.65 g at 60 DAA, that is, increased gradually during the period of fruit development (Figure 1A). In early stages of fruit growth, the dry mass was small, having intensified from 30 DAA, during which an average increase of 18%. It was opposed to the final period, when the fruits showed a higher accumulation of dry weight, with an average increase of 57%.

According to Medeiros et al. (2010), the accelerated growth phase corresponds to the stage of predominant cell expansion and the stage of ripening. Similar behavior was observed in other cucurbits such as squash (Medeiros, 2006; Vidigal et al., 2007) watermelon (Grangeiro et al., 2005), and cantaloupe (Villanueva et al., 2000; Giehl et al., 2008) (Figure 1).

There was significant increase in dry weight of the pumpkin seeds, cv. Maranhão, from 15 to 60 days, which corresponded to the last harvesting of fruits (Figure 1B). The dry mass of the seeds is considered by many authors, one of the surest measures of seed maturity. The seed reaches physiological maturity when it reaches its maximum dry weight (Costa et al., 2006).

The early seed development is characterized by relatively slow dry mass accumulation (Figure 1B) as it is at this stage that dominated the division and cell expansion, responsible for setting up the appropriate structure for receiving the substances transferred from the mother plant. The next phase is characterized by the accumulation of dry mass that intensifies until it reaches its maximum, which occurs when the seeds still have relatively high water contents (Marcos Filho, 2005).

Accordingly, Costa et al. (2006) found in hybrid squash fruits that the seeds gain mass until 50 DAA, occurring...
stabilization from this point.

The water content of seeds extracted from freshly harvested fruits decreased as age of the fruit increased. At 15 DAA the seeds showed average water content of 93.44%, and at 60 DAA this number decreased to 30.54%. There was increased dehydration from 30 DAA, although the seeds have reached the end of the observation period even with high water content. This may have occurred because they are fleshy fruits with high water content, which was also observed by other authors in Italian squash fruit (Alvarenga et al., 1991), pepper (Vidigal et al., 2009a,b) and tomato (Vidigal et al., 2006).

The seeds from fleshy fruits reach physiological maturity with high water content, tending to the stability, close to physiological maturity (Marcos Filho, 2005). In this kind of fruit, the seeds do not normally pass through the phase of fast dehydration, or suffer large fluctuations in its water content as a function of relative humidity (Dias, 2001). It occurs because of the constitution of fleshy fruit with thick flesh, maintaining high water content inside the fruit, as well as reducing the interference of increased relative humidity. According to Welbaum and Bradford (1988), although the water content of the seeds is used as an adequate indicator of physiological maturity, it is not a proper indicator of physiological maturity because of genetic and environmental influences. However, similar results were verified by Alvarenga et al. (1991), working with Italian pumpkin. Similarly, in squash cultivar ‘menina brasileira’, the water content observed at the end of the study period (at 60 DAA) was 50% and it was considered high (Morocco et al., 2011), confirming the results obtained to the fruits of pumpkin cv. Maranhão (Figure 2C).

According to Nakada et al. (2011) seeds were harvested at 30 days after anthesis had a water content around 70% and dry matter accumulation was still quite low (30%). Data corroborate those found for pumpkin cv. Maranhão. The obtained values for electrical conductivity (Figure 1D) indicated that there was initially a large amount of leachate, but it decreases as increases days to harvest the fruit.

Electrical conductivity ranged from 144.72 μS.cm⁻¹.g⁻¹ at 15 DAA seeds to 38.12 μS.cm⁻¹.g⁻¹ at 60 DAA and remained decreasing, although with less intensity from 30 DAA, indicating that there is an organization and greater

Figure 2. Germination (A and C) and Emergence Speed Index (ESI) (B and D) of pumpkin seeds, cv. ‘Maranhão’, depending on the storage of fruits after harvest at 50 (●) and 60 (▲) DAA, evaluated in BOD and greenhouse.
integrity of cell membranes during the fruit harvest. In tomato, the values of electrical conductivity (EC) observed for the seeds extracted from fruits harvested 60 DAA indicated that the seeds were already fully formed (Vidigal et al., 2006).

In cucumber, Nakada et al. (2008) obtained similar results in seeds harvested at 30 DAA, observing values of 71 μS.cm⁻¹.g⁻¹ at 30 and 16 DAA μS.cm⁻¹.g⁻¹ at 55 DAA. According to Medeiros et al. (2010), the values of electrical conductivity for gherkin fruits were high. The values ranged from 2321.57 μS.cm⁻¹.g⁻¹ at 15 DAA seeds to 1556.19 μS.cm⁻¹.g⁻¹ at 40 DAA. These results indicate that the seeds initially had lower physiological quality, releasing great amounts of leachate as a result of the low structure and selectivity of the membranes. Later, there was a reduction in the leaching of solutes due to the proper structuring of cell membranes with the approach of physiological maturation. These values can vary in larger or smaller intervals depending on the length of the vegetative cycle and cucumber species studied.

Based on these results, it is recommended to harvest the pumpkin fruits cv. Maranhão, from 60 DAA, if they are not stored. However, when these data with those obtained in the germination rate and speed of seedling emergence in BOD and a greenhouse tests are compared, it appears that a period of 30 days is required for the fruits harvested from 60 DAA to have seeds with considerable germination.

In the present study it is possible that, after reaching the maximum dry weight, pumpkin seeds still needed an additional period of thirty days, to structuring and differentiation of their tissues and then to express their maximum germination potential, unlike what established by Popinigis (1985) stating that maximum germination is reached just before the seeds reach maximum dry matter.

In Figure 2 it can be seen that the storage of the fruits harvested at 60 DAA have similar trends with respect to results obtained in the BOD and greenhouse. Seed germination was increasing as the storage time was increased, but without damages in the fruits. This result is similar to that found by Costa et al. (2006) when stored fruits of squash hybrid. It is observed that the seeds stored in the fruits during 30 days and at 60 days after anthesis will get an increase of 25% germination in BOD conditions, in relation to 50 days.

Another point to be highlighted is the minimum difference of germination curves of Figure 2, increasing the difference between the result of the ESI in the BOD and greenhouse. Therefore, in BOD seed germinates faster and its percentage of germination is higher compared to the seeds placed in greenhouse.

Conclusions

Pumpkin fruits can be harvested at 60 DAA for obtaining seeds with maximum physiological quality. Pumpkin seeds reach physiological maturity in the period between 50 and 60 DAA, when they have the lowest water content and electrical conductivity practically stable. After harvest, the storage of fruits is essential to ensure seed quality and a period of 30 days is recommended.

Conflict of Interest

The authors have not declared any conflict of interest.

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