Original Research Article

Physiological Maturity and determination of the harvest time of *Vigna unguiculata* L. Walp.

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ABSTRACT

The maturation process of seeds is genetically controlled and involves an organized sequence of physiological changes from the fertilization until the complete independence from the plant. It is recommended that the harvest occurs in the ideal moment, this way the seeds can express their full potential, with maximum dry matter accumulation, reaching high potential of germination and vigor. The objective of this study was to determine the physiological maturity point of cowpea bean seeds (*Vigna unguiculata* L. Walp.), cv. Corujinha, aiming to indicate the best harvesting period, in order to guarantee greater germination potential and seed vigor. Cowpea pods were harvest from the third until the twenty-first day after anthesis, with intervals of three days between the harvests. After each harvest, the following evaluations were carried out: fruit and seed color, number of seeds per pod, pod and seed biometry, pod and seed moisture, water content, germination, germination speed index, length and dry matter of the shoots and roots. At 15 DAA, the seeds and fruits presented light green coloration, with maximum values of length, width, thickness, dry matter, germination percentage and germination speed index, at a vigor level. There was a gradual reduction of water content in the seeds and number of seeds up to 21 DAA. The highest values for shoot and root length were observed at 18 DAA, when seeds and fruits showed light brown color and for shoot and root dry matter at 21 DAA, with brown color. The physiological maturity of cowpea seeds was rapid and occurred between 15 and 21 DAA. The harvest is recommended at 15 days after anthesis, when the seeds present high germination and vigor.

Comment [AL1]: Please use one name. I suggest *pod* and change the *fruit* into *pod*. The description must be precise. In one place is under biometry, in another fruit biometry. Please check and correct the description for other alternative names.

Keywords: Cowpean bean, physiological quality, germination, vigor.

1. INTRODUCTION

The cowpea (*Vigna unguiculata* L. Walp.) is cultivated throughout the North and Northeast of Brazil, where it is considered the main component of the agricultural production of these regions, constituting an important source of income and subsistence for small farmers who practice agriculture. In addition, this crop is used as a staple food for the population, which consumes it in the form of green and dry grains, being very appreciated due to the fast cooking and nutritional aspects, such as the quantity of proteins [1].

The difficulty of obtaining seeds of good physiological quality is among the limiting factors in the production of cowpea, since the seeds are one of the main inputs of the agricultural production, where the quality is an important factor to obtain stands of uniform and vigorous plants, directly reflecting the yield [2].

To express its full potential, it is essential that the harvest occurs at the ideal moment, with maximum dry matter accumulation, reaching high germination and vigor potential [3]. Therefore, the study of the physiological maturation process of seeds is very important to determine the ideal harvest time and, consequently, obtain seeds of high physiological quality [4].

The seed maturation process is genetically controlled and involves an organized sequence of physical, biochemical, physiological and morphological changes, from the fertilization until its independence from the plant, these changes also include a set of preparatory steps for the process of germination, which are characterized by the synthesis and accumulation of nutrient reserves [5].

Several studies on the influence of physiological maturity on the seed quality and productivity of several crops have been carried out, such as the studies with pepper seeds (*Capsicum annuum* L.) [6] common bean (*Phaseolus vulgaris* L.) [7], ginger (*Sesamum indicum* L.) [8] and pumpkin (*Curcubita moschata* Duch) [9]. However, currently, for cowpea, there is little information on the maturation and the ideal harvest period of the seeds, justifying the need to perform this evaluation [2].

The objective of this study was to determine the physiological maturity point of cowpea seeds (*Vigna unguiculata* L. Walp.), cv. Corujinha, aiming to indicate the best harvest time, in order to guarantee greater germination potential and seed vigor.

Comment [AL2]: You have to write what indicators have been used to achieve this goal. This is what we know in the results.

2. MATERIAL AND METHODS

2.1 Experimental Location

The field experiment was performed with cowpea bean seeds, *Vigna unguiculata* cv. Corujinha, between September 2015 and January 2016 at the Chã de Jardim Experimental Farm of the Centro de Ciências Agrárias of the Universidade Federal da Paraíba (CCA-UFPB), in Areia-Paraíba, located in the micro-region of the Paraiba, under the geographic coordinates 6º58'12 "S and 35º42'15" W.

According to Graussem's bioclimatic classification, the predominant bioclimate in the area is the sub-dry Northeastern 3dfh with annual rainfall of approximately 1,400 mm. According to Köppen's classification, the climate is characterized as warm and humid, with autumn-winter rains. The average annual temperature ranges from 22 to 26 ºC and relative humidity between 75 and 87% [10]. During the conduction of the experiment the minimum temperature was 20.3 °C and the maximum was 28.5 °C, with average relative humidity of 76.4%. According to Embrapa [11], the soil of the experimental area is classified as a typical Psamitic Regolithic Neosols, of medium texture.

2.2 Experimental Design

For the soil preparation the area was cleaned with garden hoes and pits at a depth of 4 cm, spaced 0.30 m between plants and 1.0 m between rows were opened. Three seeds/pit were sown, after thinning, one plant/pit was left, the plants were monitored periodically to follow the flowering stage, while the cultural treatments were recommended for the crop.

Fifty-four days after sowing, when approximately 70% of the plants started the anthesis they were identified using wool yarns. The plants were monitored until fruiting and, every 3 days were harvested, with a total of seven harvests, manually performed, mechanical injuries in the pods and seeds were avoided. After harvested, the pods were packed in plastic bags, identified and sent to the laboratory.

2.3 Evaluated Parameters

The pod and seed biometry, and also their physiological quality, were evaluated in the Laboratório de Análise de Sementes, also located in the previously mentioned Center.

After each havest, four replicates of 15 pods and 25 seeds were submitted to direct measurements with the aid of a digital caliper, in which measurements of length, width and thickness were performed, the results were expressed in millimeters, only the length of the fruit was expressed in centimeters.

Comment [AL3]: Give accuracy; 0.01 g?

After each harvest, by using a sample of 40 pods the number of seeds per pod was determined by manual counting, and the results were expressed as number of seeds per pod^{-1}.

The water content of the pods and seeds were obtained by the stove method at 105 ºC for 24 hours [12], using four replicates of 25 seeds and four replicates of 5 pods at each harvest period, the results were expressed in percentage.

Comment [AL4]: Did you weigh the samples. On what type of weight and with what accuracy. What mass had the sample. These percentages are not clear.

The samples were placed in a stove at 105 ± 3 °C for 24 hours [12], after that, the dry matter of the pods and seeds were determined together with the water content, at all harvest time. The results were expressed in grams.

Comment [AL5]: Why this procedure is described separately. After all, when determining the moisture content, you have the same data. Why do you enter the dry matter content in grams. It does not make sense. The dry mass is given in percent. It should be a relative value due to the initial mass.

The germination test was performed following the requirements of the Rules for Seed Analysis - RSA [12], using 200 seeds per treatment, distributed in four replicates of 50 seeds, placed in a paper towel substrate (germitest®) moistened with sterilized distilled water in a quantity equivalent to 2.5 times the dry paper weight, distributed on two sheets of paper, covered by a third and organized in the form of rolls, which were packed in transparent plastic bags to avoid the loss of water by evaporation. The rolls were placed in germination chamber of the Biological Oxygen Demand type (B.O.D.) regulated at a constant temperature of 25 °C. The counting was performed five to eight days after the test, considering the normal seedlings were considered, characterized by having a long, thin primary root coated with absorbent hairs along the entire surface, well defined lateral roots and well developed shoot, presenting the potential to continue its development and give rise to normal plants, the results were expressed in percentage.

The first germination counting was carried out concurrently with the germination test, the germinated seeds were counted on the 5th day after sowing [12].

For the germination speed index, daily countings were performed, five to eight days after the test, and the index was determined according to the equation proposed by Maguire [13].

At the end of the germination test, the normal seedlings of each replicate were measured with a ruler graduated in centimeters, the length of the seedlings were measured, and the results were expressed in centimeters per seedlings. The seedlings previously measured were packed in Kraft paper bags type, taken to a stove regulated at 80 °C for 24 hours and, after that period, weighed in an analytical scale with an accuracy of 0.001 g, the results were expressed in g.plantula^{-1}.

Comment [AL6]: And what do they express?

2.4 Data Analysis

The experimental design used in the field was a randomized block, and completely randomized at the laboratory, the results were submitted to analysis of variance and polynomial regression to evaluate the characteristics described previously, the linear and quadratic model were tested, where the significant model of higher order was selected to express the results. The program Sisvar 5.0 was used to perform the statistics analysis at the significance level of 5% probability (*P* = .05). [14].

Comment [AL7]: I suggest you do a correlation analysis between the indicators.

3. RESULTS AND DISCUSSION

Changes in the coloration of the pods and seeds were observed during the maturation process (Table 1) and varied from dark green to brown with small dark brown dots. According to Lopez et al. [4], the color of the pods and seeds has been used as a good indicator of the harvest point, however, environmental factors must be observed since the differences in coloring can also be caused by its influence.

Comment [AL8]: Is this an objective or subjective evaluation? The method should be described in Material and methods if the result is described in this chapter.

Table 1. Color of the pods and seeds of cowpea (*Vigna unguiculata* L. Walp.), cv. Corujinha, at different times of harvest.

Harvest time	Days after anthesis	Color	
		Pods	Seeds
1st	3	Dark green	Dark green
2nd	6	Dark green	Dark green
3rd	9	Light green	Dark green
4th	12	Light green	Light green
5th	15	Light green	Light green
6th	18	Light brown	Light brown
7th	21	Brown (dots)	Brown

For the number of seeds per fruit, a decreasing linear behavior is observed as a function of the harvesting time (Figure 1A) and, in relation to the size of the pods and the seeds, the data were adjusted to the quadratic model, with maximum length of (20.6 cm), width (8.8 mm) and thickness (11.8 mm) of the pods obtained at 15 days after anthesis (Figures 1A and B). For the seeds, the maximum length (12 mm), width (8.6 mm) and thickness (7.0 mm) were also verified at 15 days after anthesis (Figure 1D).

Similar results were found by Botelho et al. [7] when studying the ideal harvest time for beans (*Phaseolus vulgaris* L.) where was verified a direct relation between seed size and physiological quality, in which seeds of lower size negatively influenced the seed quality of the lot.

Padua et al. [15] also verified that larger seeds originated higher soybean plants than plants originated from smaller seeds. According to Carvalho and Nakagawa [16], larger seeds were better nourished during their development, have well-formed embryos and a greater amount of reserves, with greater potential for germination and more vigorous plants when compared to smaller seeds.

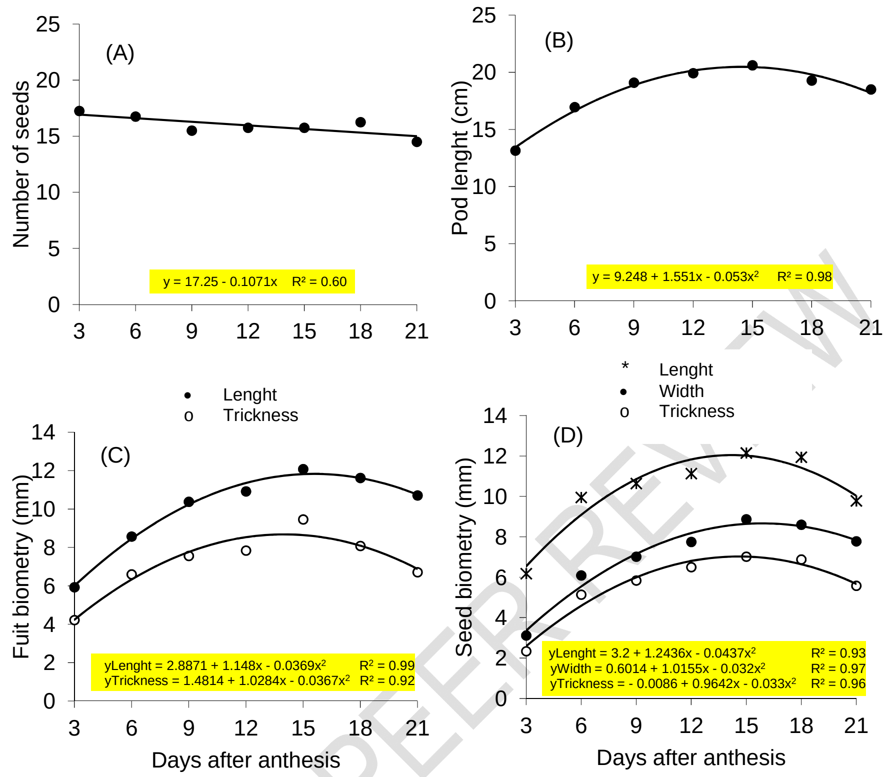

Figure 1. Number of seeds (A), pod length (B), Fruit biometry (C) and biometry of the seeds (D) of Cowpea bean (*Vigna unguiculata* L. Walp.), cv Corujinha, at different times of harvest.

The water content of the pods presented a linear behavior and the seed water content presented a quadratic behavior according to the harvest times, in which, in the first harvesting, at three days after the anthesis, the water content was high in the pods (96.7%) and seeds (99.0%). Then, there was a gradual decrease until the last harvest, 21 days after anthesis (18% for pods and 10% for seeds) (Figure 2). Botelho et al. [7] found similar results, and verified a decrease in water content of common bean seeds during the physiological maturation process.

However, the water content at the time of harvesting was high and this permanence for a long period can negatively affect the storage and commercialization of the seeds, which can result in the reduction of the physiological quality, cause deformations and favor conditions for the development of fungi, which are factors responsible that accelerate the deterioration process [3].

Comment [AL9]: Are all regression coefficients in the quadratic model statistically significant? I suggest that the functions for indicators should have specific designations, eg. instead of everywhere y write a designation for length *l*; length seed - l_s, pod length – l_p (correct the fruit for the pod), etc. for other physical quantities. For time (day) t. Constants in equations should have such accuracy, as is the accuracy of measurement, eg. 2.8871 is too accurate, just 2.89.
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Correct errors in length and thickness.

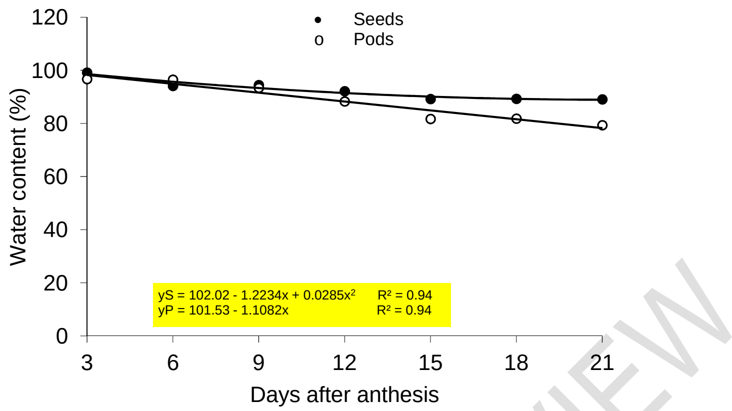

Figure 2. Water content of the pods and seeds of Cowpea bean (*Vigna unguiculata* L. Walp.), cv Corujinha, at different times of harvest.

For the pods and seeds dry matter, data were adjusted to quadratic models, with maximum values of 7.85 and 0.240 g, respectively, reached at 15 days after anthesis (Figure 3A and 3B). In the same harvesting time (15 days after anthesis) the maximum dry matter was observed in the pods and seeds, the water content of the seeds was high, above 80%, and the germination percentage reached the maximum values.

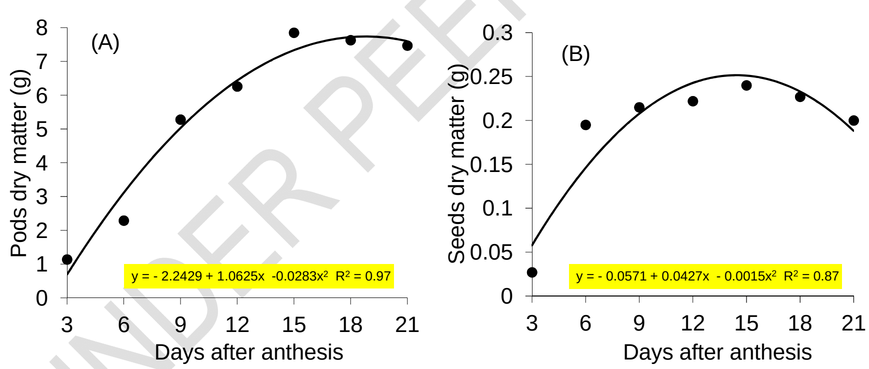

Figure 3. Dry matter of the pods (A) and seeds (B) of Cowpea bean (*Vigna unguiculata* L. Walp.), cv Corujinha, at different times of harvest.

Comment [AL10]: If the dry mass will be in percent, then please prepare one Figure with two graphs. In Fig. (B), the ordinate has commas. Please, change to dots. This is English and a separator for decimal numbers in the form of dots is required. Please change in the system settings to draw such coordinates.

A similar behavior was described by Eskandari [17] in seeds of *Vigna sinensis*, Botelho et al. [7] and Bolina et al. [18] in common bean seeds and Nogueira et al. [2] in cowpea seeds.

The germination percentage and germination speed index were adjusted to the quadratic model, with the highest values observed at 15 days after anthesis (100% and 9.9, respectively), remaining high until the last day of evaluation (21 days after anthesis) (Figure 4A and B). Nogueira et al. [2] evaluated the development and physiological quality of cowpea seeds, cv. BRS Guariba, during the maturation process, observed that at 14 days after anthesis, the seeds reached their highest percentage of germination and germination speed index, remaining stable until the last harvest, corroborating with the results obtained in this work.

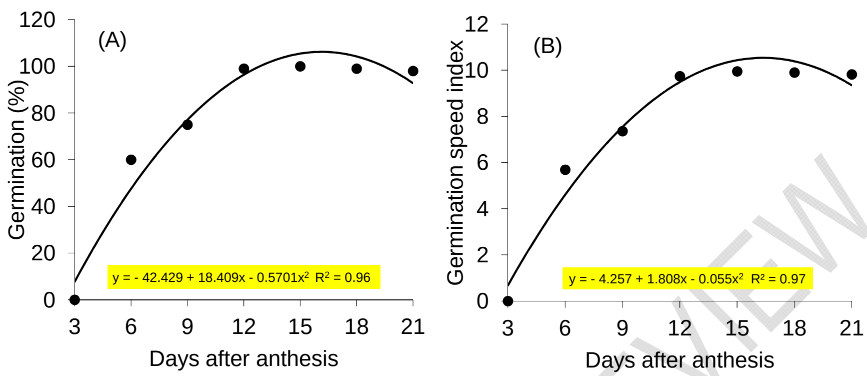

Figure 4. Germination (A) and germination speed index (B) of seeds of Cowpea bean (*Vigna unguiculata* L. Walp.), cv Corujinha, at different times of harvest.

Comment [AL11]: How is the difference between these indicators. What the new explains the second. Only one indicator is cleared.

The shoot and root length data (Figure 5) were also adjusted to the quadratic model, where a gradual increase was observed during the maturation process, with an estimated maximum value for shoot (12.06 cm) and root (10.02 cm) at 18 days after the anthesis and, with a subsequent small decrease.

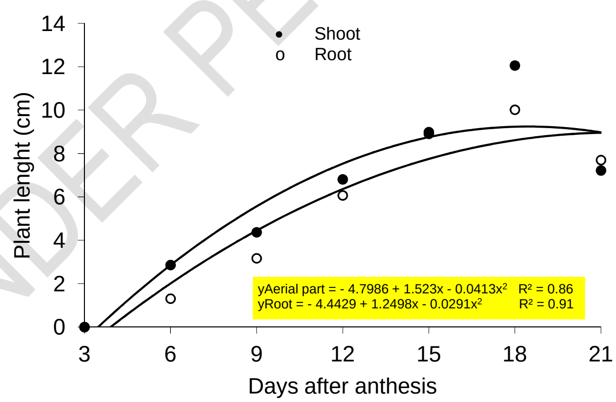

Figure 5. Shoot and root length of seedlings of Cowpea (*Vigna unguiculata* L. Walp.), cv Corujinha, at different times of harvest.

For the shoot (Figure 6A) and root (Figure 6B) dry matter, a linear and increasing behavior was observed as a function of the harvest time, reaching its maximum value (0.052 g for shoot and 0.0125 g for root), at the last harvest, at 21 days after anthesis, which is due to the metabolic and catabolic events of accumulation in the reserves tissue throughout the development of the seed.

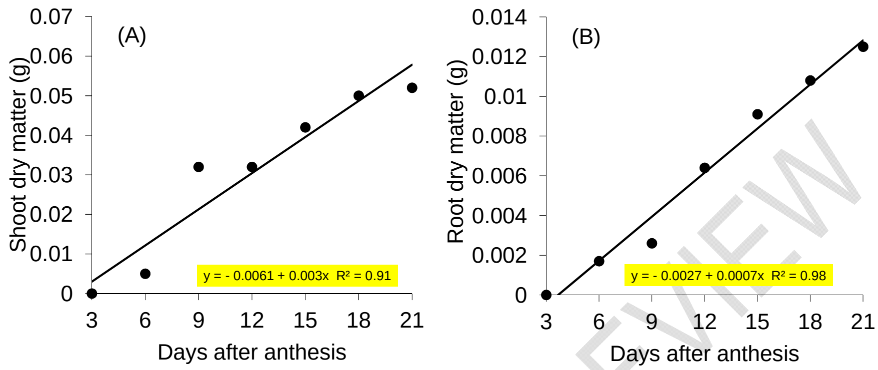

Figure 6. Shoot (A) and root (B) dry matter of seedlings of Cowpea bean (*Vigna unguiculata* L. Walp.), cv Corujinha, at different times of harvest.

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A direct relation between the seed size (Figure 1D) and physiological quality results could be verified, where the seeds of higher size were also those with higher percentage of germination (Figure 4A and 4B) and vigor (Figure 5 6A and 6B). According to Carvalho and Nakagawa [16], the size of the seeds may influence the germination and vigor, since larger seeds were well nourished during their development, usually have well-formed embryos, have larger amounts of reserves and are potentially more vigorous.

Similar results were also observed by Padua et al. [15], where they evaluated the influence of soybean seed size on the initial growth of plants and their effect on yield, they observed that larger seeds presented higher percentages of germination, vigor and produce plants with higher height at the harvest time, with higher yield, when compared to smaller seeds.

Therefore, it is important to harvest the seeds when they reach their maximum size, considering that it will result in higher seed quality, uniformity, more vigorous and productive plant stands.

4. CONCLUSION

The physiological maturity of cowpea bean seeds, cv. Corujinha is fast and occurred between 15 and 21 days after the anthesis, the period that correspond to 15 days after the anthesis is the best period for the harvest of this cultivar, which coincides with a greater germination and vigor.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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