

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

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: pod 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 pods 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 pods 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 physiological quality.

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Keywords: Cowpea 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

27 determine the ideal harvest time and, consequently, obtain seeds of high physiological
28 quality [4].

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

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

40 The objective of this study was to determine the physiological maturity point of cowpea
41 seeds (*Vigna unguiculata* L. Walp.), cv. Corujinha, aiming to indicate the best **harvest** time,
42 in order to guarantee **high physiological quality**.

43 **2. MATERIAL AND METHODS**

44 **2.1 Experimental Location**

45 The field experiment was performed with cowpea bean seeds, *Vigna unguiculata* cv.
46 Corujinha, between September 2015 and January 2016 at the Chã de Jardim Experimental
47 Farm of the Centro de Ciências Agrárias of the Universidade Federal da Paraíba (CCA-
48 UFPB), in Areia-Paraíba, located in the micro-region of the Paraíba, under the geographic
49 coordinates 6°58'12 "S and 35°42'15" W.

50 According to Graussem's bioclimatic classification, the predominant bioclimate in the area is
51 the sub-dry Northeastern 3dfh with annual rainfall of approximately **1.400 mm**. According to
52 Köppen's classification, the climate is characterized as warm and humid, with autumn-winter
53 rains. The average annual temperature ranges from 22 to 26 °C and relative humidity
54 between 75 and 87% [10]. During the conduction of the experiment the minimum
55 temperature was 20.3 °C and the maximum was 28.5 °C, with average relative humidity of
56 76.4%. According to Embrapa [11], the soil of the experimental area is classified as a typical
57 Psamitic Regolithic Neosols, of medium texture.

58 **2.2 Experimental Design**

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

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

68 **2.3 Evaluated Parameters**

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

71 After each harvest, four replicates of 15 pods and 25 seeds were submitted to direct
72 measurements with the aid of a digital caliper (0.01 mm accuracy), in which measurements
73 of length, width and thickness were performed, the results were expressed in millimeters,
74 only the length of the pod was expressed in centimeters.

75 After each harvest, by using a sample of 40 pods the number of seeds per pod was
76 determined by manual counting, and the results were expressed as number of seeds per
77 pod⁻¹. The color of the seed and pods were attributed according to the epiderm color in five
78 stages of maturity: Stage 1- Dark green; Stage 2- Light green; Stage 3- Light Brown; Stage
79 4- Brown; Stage 5- Brown with dark dots.

80 The water content of the pods and seeds were obtained by the stove method at 105 °C ± 3
81 °C for 24 hours [12], four replicates of 25 seeds and four replicates of 5 pods were used for
82 each harvest period, the results were expressed in percentage. The dry matter of the seeds
83 were obtained concurrently with the water content. After dried, the samples of seed and
84 pods were weighted in a analytical scale (0.001 precision), and the results expressed in
85 grams.

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

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

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

102 At the end of the germination test, the normal seedlings of each replicate were measured
103 with a ruler graduated in centimeters, the length of the seedlings were measured, and the
104 results were expressed in centimeters. The seedlings previously measured were packed in
105 Kraft paper bags type, taken to a stove regulated at 80 °C for 24 hours and, after that period,
106 weighed in an analytical scale with an accuracy of 0.001 g, the results were expressed in
107 grams.

108 **2.4 Data Analysis**

109 The experimental design used in the field was a randomized block, and completely
110 randomized at the laboratory, the results were submitted to analysis of variance and

111 polynomial regression to evaluate the characteristics described previously, the linear and
112 quadratic model were tested, where the significant model of higher order was selected to
113 express the results. The program Sisvar 5.0 was used to perform the statistics analysis at
114 the significance level of 5% probability ($P = .05$) [14].

115 3. RESULTS AND DISCUSSION

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

121 **Table 1. Color of the pods and seeds of cowpea (*Vigna unguiculata* L. Walp.), cv.**
122 **Corujinha, at different times of harvest.**
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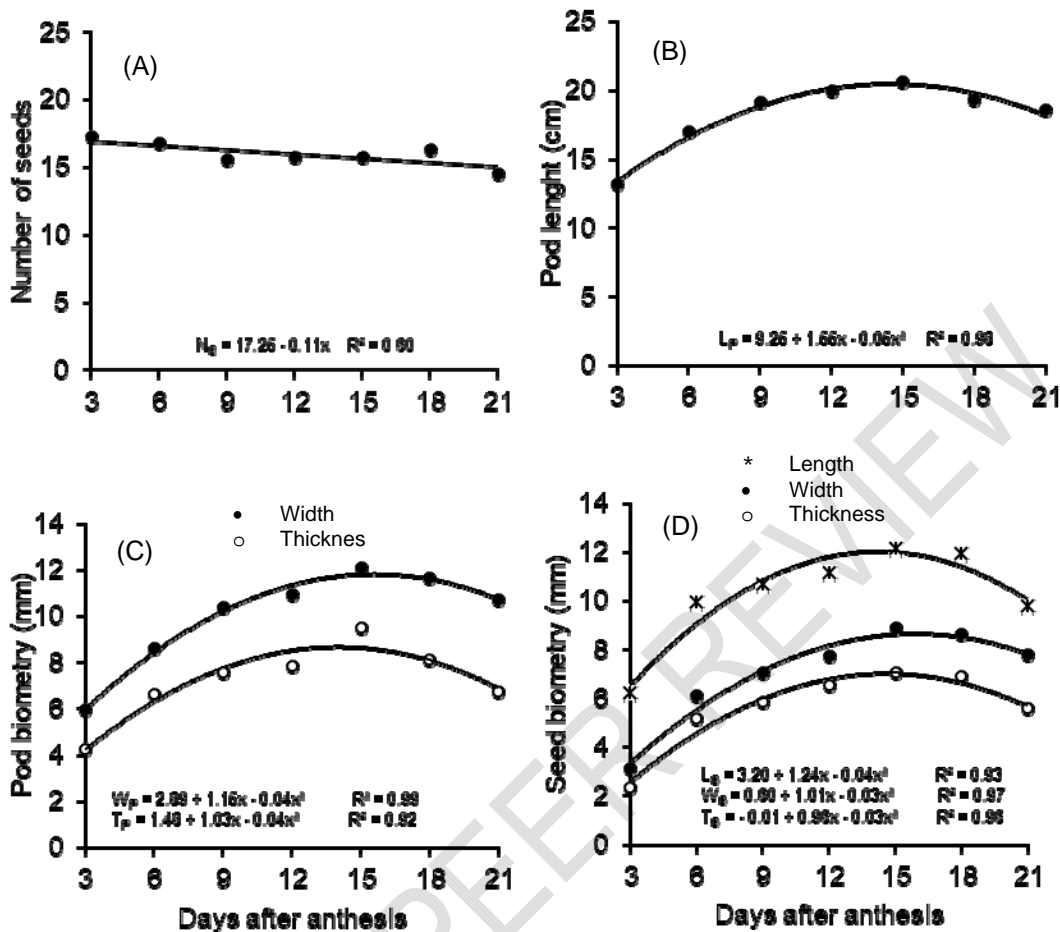
Harvest time	Days after anthesis	Color	
		Pods	Seeds
1 st	3	Dark green	Dark green
2 nd	6	Dark green	Dark green
3 rd	9	Light green	Dark green
4 th	12	Light green	Light green
5 th	15	Light green	Light green
6 th	18	Light brown	Light brown
7 th	21	Brown (dots)	Brown

124

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

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

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



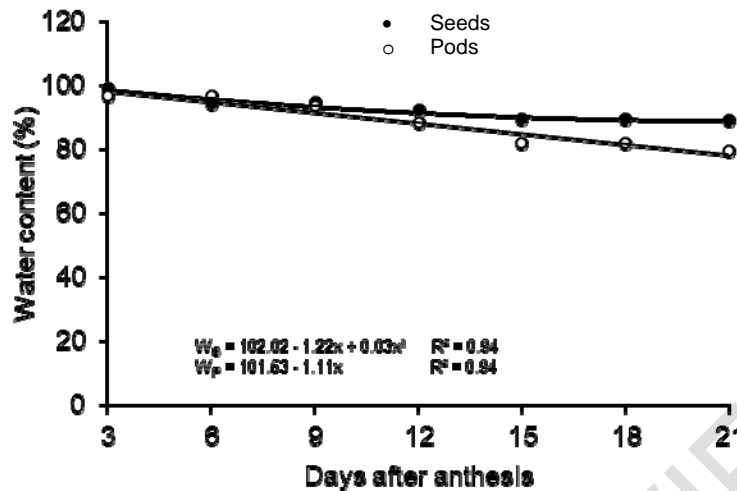
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142 Figure 1. Number of seeds (A), pod length (B), pod biometry (C) and seed biometry (D)
 143 of Cowpea bean (*Vigna unguiculata* L. Walp.), cv Corujinha, at different times of
 144 harvest.

145 The water content of the pods presented a linear behavior and the seed water content
 146 presented a quadratic behavior according to the harvest times, in which, in the first **harvest**,
 147 at three days after the anthesis, the water content was high in the pods (96.7%) and seeds
 148 (99.0%). Then, there was a gradual decrease until the last **harvest**, 21 days after anthesis
 149 (18% for pods and 10% for seeds) (Figure 2). Botelho et al. [7] found similar results, and
 150 verified a decrease in water content of common bean seeds during the physiological
 151 maturation process.

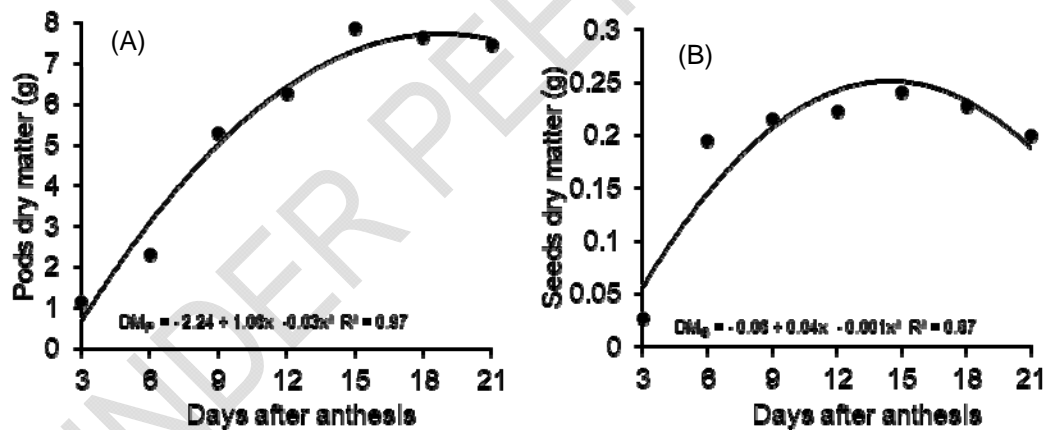
152 However, the water content at the time of **harvest** was high and this permanence for a long
 153 period can negatively affect the storage and commercialization of the seeds, which can
 154 result in the reduction of the physiological quality, cause deformations and favor conditions
 155 for the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



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

160 For the pods and seeds dry matter, data were adjusted to quadratic models, with maximum
 161 values of 7.85 and 0.240 g, respectively, reached at 15 days after anthesis (Figure 3A and
 162 3B). In the same harvesting time (15 days after anthesis) the maximum dry matter was
 163 observed in the pods and seeds, the water content of the seeds was high, above 80%, and
 164 the germination percentage reached the maximum values.



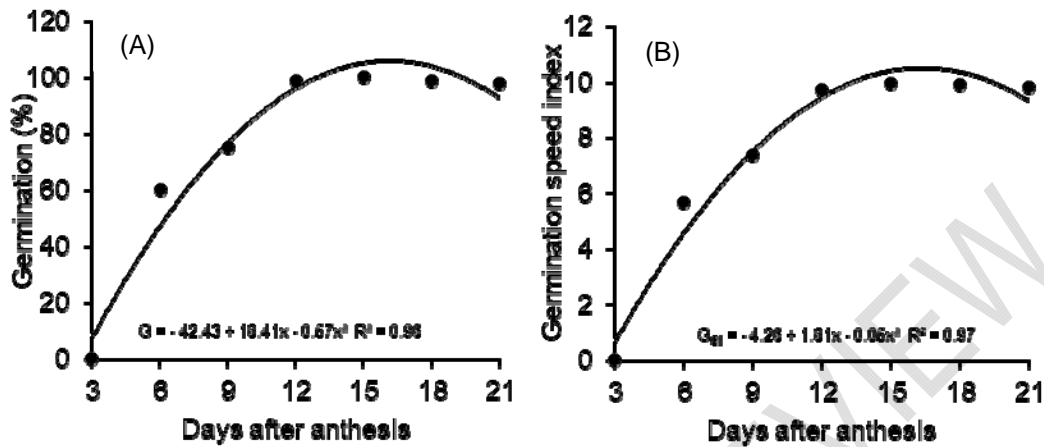
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166 **Figure 3. Dry matter of the pods (A) and seeds (B) of Cowpea bean (*Vigna unguiculata***
 167 **L. Walp.), cv Corujinha, at different times of harvest.**

168 A similar behavior was described by Eskandari [17] in seeds of *Vigna sinensis*, Botelho et al.
 169 [7] and Bolina et al. [18] in common bean seeds and Nogueira et al. [2] in cowpea seeds.

170 The germination percentage and germination speed index were adjusted to the quadratic
 171 model, with the highest values observed at 15 days after anthesis (100% and 9.9,
 172 respectively), remaining high until the last day of evaluation (21 days after anthesis) (Figure
 173 4A and B). Nogueira et al. [2] evaluated the development and physiological quality of
 174 cowpea seeds, cv. BRS Guariba, during the maturation process, observed that at 14 days

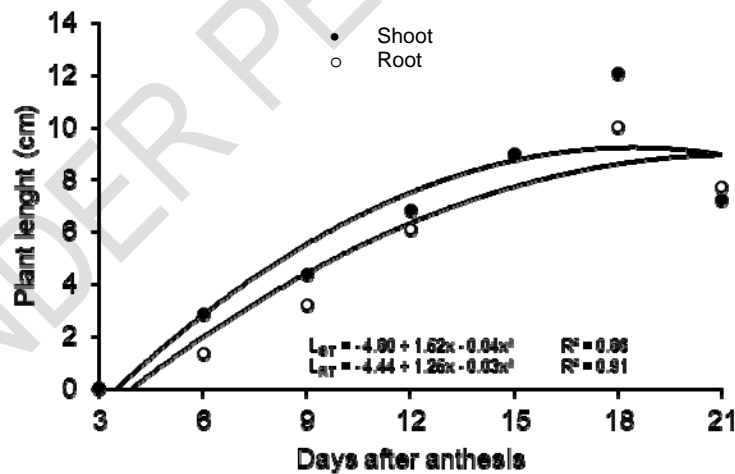
175 after anthesis, the seeds reached their highest percentage of germination and germination
 176 speed index, remaining stable until the last harvest, corroborating with the results obtained in
 177 this work.



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

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

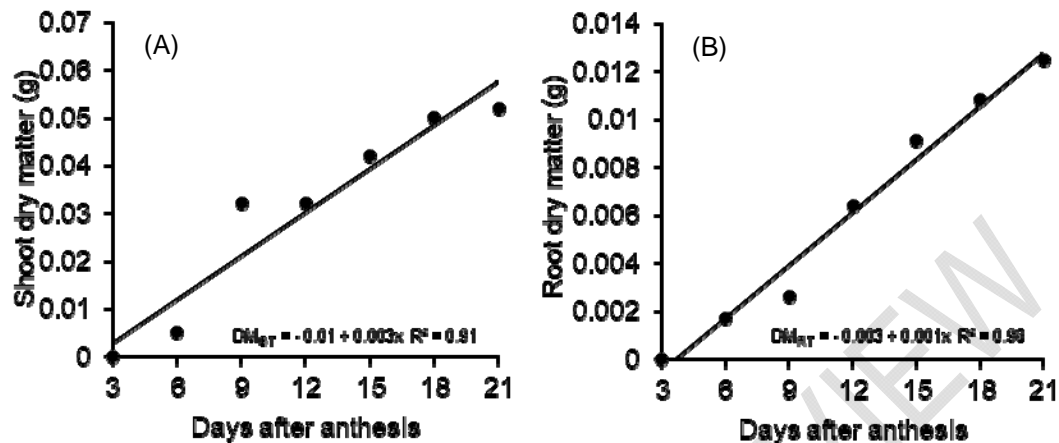


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186 **Figure 5. Shoot and root length of seedlings of Cowpea (*Vigna unguiculata* L. Walp.),**
 187 **cv Corujinha, at different times of harvest.**

188 For the shoot (Figure 6A) and root (Figure 6B) dry matter, a linear and increasing behavior
 189 was observed as a function of the harvest time, reaching its maximum value (0.052 g for
 190 shoot and 0.0125 g for root), at the last harvest, at 21 days after anthesis, which is due to

191 the metabolic and catabolic events of accumulation in the reserves tissue throughout the
192 development of the seed.



193

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

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

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

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

209 **4. CONCLUSION**

210 **The physiological maturity of cowpea bean seeds, cv. Corujinha is fast and occurred**
211 **between 15 and 21 days after the anthesis, the period that correspond to 15 days after the**
212 **anthesis is the best period for the harvest of this cultivar, which coincides with a high quality**
213 **physiological.**

214 **COMPETING INTERESTS**

215 Authors have declared that no competing interests exist.

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