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

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9 **ABSTRACT**

10 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 harvested 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. The physiological maturity of cowpea seeds occurs rapidly, and the harvest is recommended at 15 days after anthesis, when the seeds show high germination and vigor.

11
12 **Keywords:** *Cowpean bean, physiological quality, germination, vigor.*

13 **1. INTRODUCTION**

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

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

24 To express its full potential, it is essential that the harvest occurs at the ideal moment, with
25 maximum dry matter accumulation, reaching high germination and vigor potential [3].
26 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 harvesting
42 time, in order to guarantee greater germination potential and seed vigor.

43 **2. MATERIAL AND METHODS**

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

49 According to Graussem's bioclimatic classification, the predominant bioclimate in the area is
50 the sub-dry Northeastern 3dfh with annual rainfall of approximately 1,400 mm. According to
51 Köppen's classification, the climate is characterized as warm and humid, with autumn-winter
52 rains. The average annual temperature ranges from 22 to 26 °C and relative humidity
53 between 75 and 87% [10]. During the conduction of the experiment the minimum
54 temperature was 20.3 °C and the maximum was 28.5 °C, with average relative humidity of
55 76.4%. According to Embrapa [11], the soil of the experimental area is classified as a typical
56 Psamitic Regolithic Neosols, of medium texture.

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

59 Soil preparation was performed by cleaning the area with garden hoes and opening of pits at
60 a depth of 4 cm, spaced 0.30 m between plants and 1.0 m between rows. The sowing
61 consisted of three seeds/pit, followed by thinning, leaving one plant/pit, the plants were
62 monitored periodically to follow the flowering, while the cultural treatments were
63 recommended for the crop.

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

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

73 After each harvesting, by using a sample of 40 pods the number of seeds per pod was
74 determined by manual counting, and the results were expressed as number of seeds per
75 pod⁻¹.

76 The water content of the pods and seeds were obtained by the stove method at 105 °C for
77 24 hours [12], using four replicates of 25 seeds and four replicates of 5 pods at each
78 harvesting period, the results were expressed in percentage.

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

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

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

96 For the germination speed index, daily counting were performed. **at the same time**, five to
97 eight days after the test, and the index was determined according to the equation proposed
98 by Maguire [13].

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

105 The experimental design used in the field was a randomized block, and completely
106 randomized at the laboratory, the results were submitted to analysis of variance and
107 polynomial regression to evaluate the characteristics described previously, the linear and
108 quadratic model were tested, where the significant model of higher order was selected to
109 express the results.

110

111 **3. RESULTS AND DISCUSSION**

112 Changes in the coloration of the pods and seeds were observed during the maturation
113 process (Table 1), varying from dark green to brown with small dark brown dots. According
114 to Lopez et al. [4], the color of the pods and seeds has been used as a good indicator of the

115 harvest point, however, environmental factors must be observed since the differences in
 116 coloring can also be caused by its influence.

117 **Table 1. Color of the pods and seeds of cowpea (*Vigna unguiculata* L. Walp.), cv.**
 118 **Corujinha, at different times of harvest.**
 119

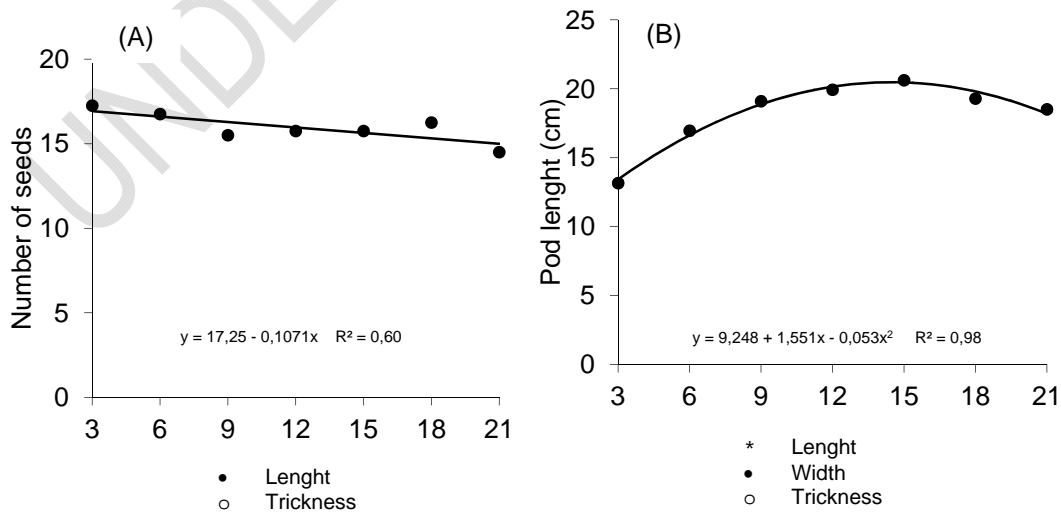
Harvest time	Days after anthesis	Color	
		Pods	Seeds
1 st	3	Dark green	Dark green
2 nd	6	Dark green	Dark green
3 rd	9	Light green	Dark green
4 th	12	Light green	Light green
5 th	15	Light green	Light green
6 th	18	Light brown	Light brown
7 th	21	Brown (dots)	Brown

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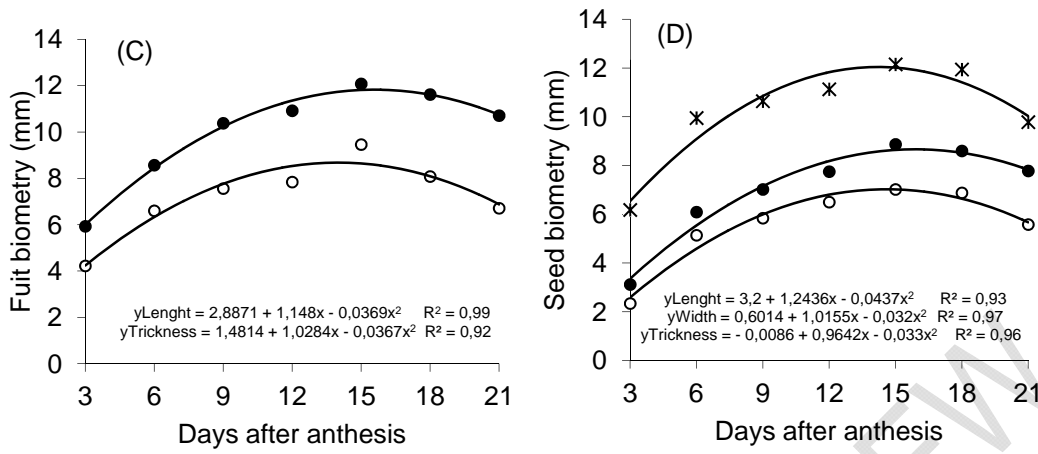
121 For the number of seeds per fruit, a decreasing linear behavior is observed as a function of
 122 the harvesting time (Figure 1A) and, in relation to the size of the pods and the seeds, the
 123 data were adjusted to the quadratic model, with maximum length of (20.6 cm), width (8.8
 124 mm) and thickness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

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

131 Padua et al. [14] also verified that larger seeds originated higher soybean plants than plants
 132 originated from smaller seeds. According to Carvalho and Nakagawa [15], larger seeds were
 133 better nourished during their development, have well-formed embryos and a greater amount
 134 of reserves, with greater potential for germination and more vigorous plants when compared
 135 to smaller seeds.



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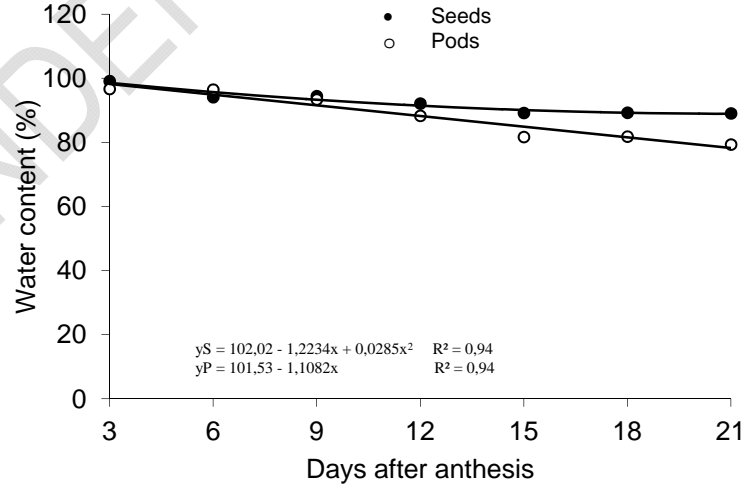


137

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

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

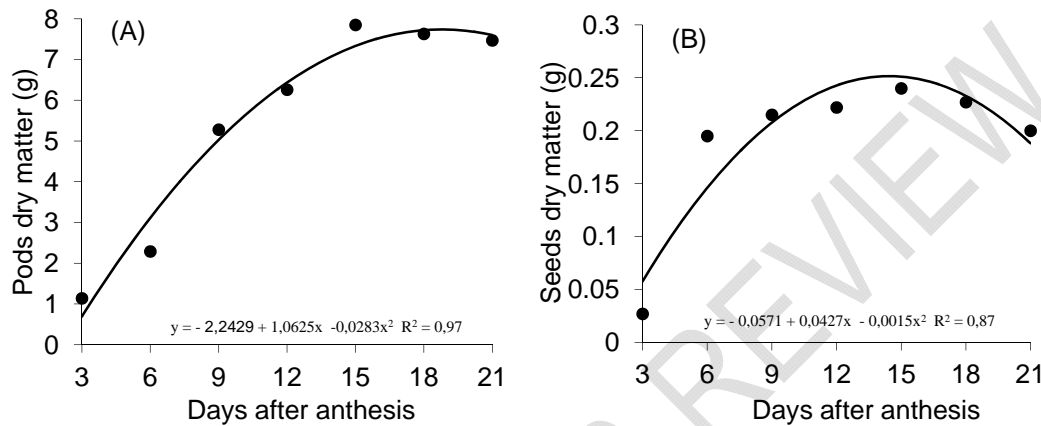
148 However, the water content at the time of harvesting was high and this permanence for a
 149 long period can negatively affect the storage and commercialization of the seeds, which can
 150 result in the reduction of the physiological quality, cause deformations and favor conditions
 151 for the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



153

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

156 For the pods and seeds dry matter, data were adjusted to quadratic models, with maximum
157 values of 7.85 and 0.240 g, respectively, reached at 15 days after anthesis (Figure 3A and
158 3B). In the same harvesting time (15 days after anthesis) the maximum dry matter was
159 observed in the pods and seeds, the water content of the seeds was high, above 80%, and
160 the germination percentage reached the maximum values.

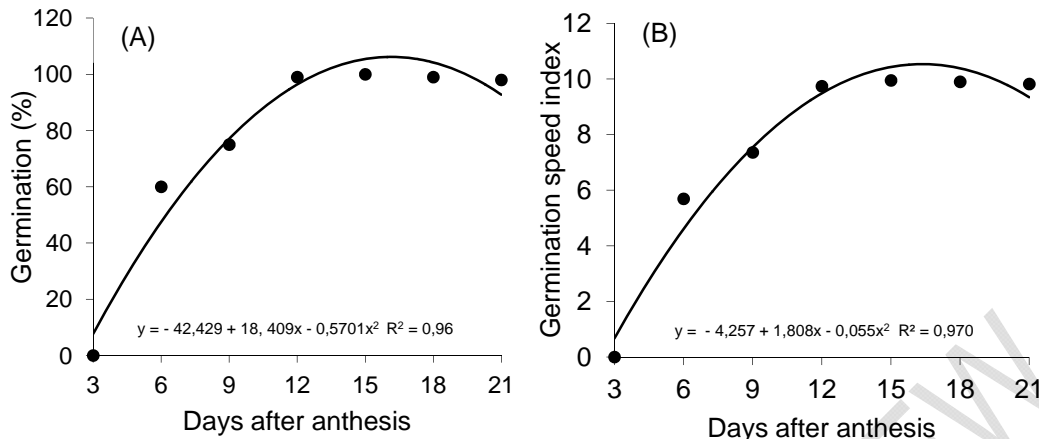


161

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

164 A similar behavior was described by Eskandari [16] in seeds of *Vigna sinensis*, Botelho et al.
165 [7] and Bolina et al. [17] in common bean seeds and Nogueira et al. [2] in cowpea seeds.

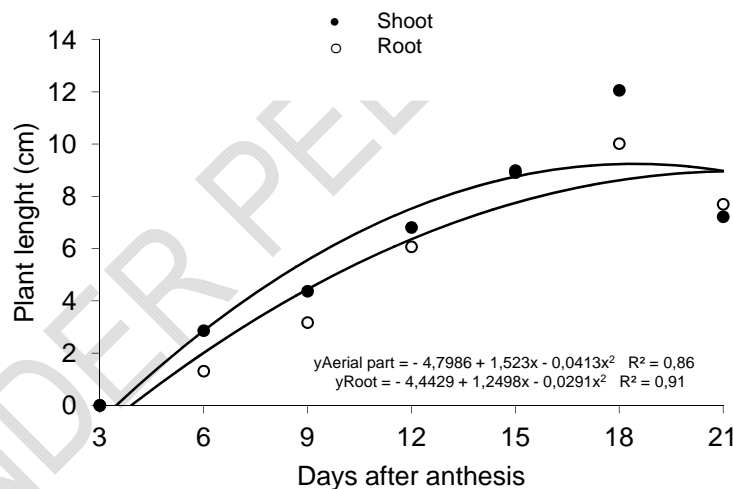
166 The germination percentage and germination speed index were adjusted to the quadratic
167 model, with the highest values observed at 15 days after anthesis (100% and 9.9,
168 respectively), remaining high until the last day of evaluation (21 days after anthesis) (Figure
169 4A and B). Nogueira et al. [2] evaluating the development and physiological quality of
170 cowpea seeds, cv. BRS Guariba, during the maturation process, observed that at 14 days
171 after anthesis, the seeds reached their highest percentage of germination and germination
172 speed index, remaining stable until the last harvest, corroborating with the results obtained in
173 this work.



174

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

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

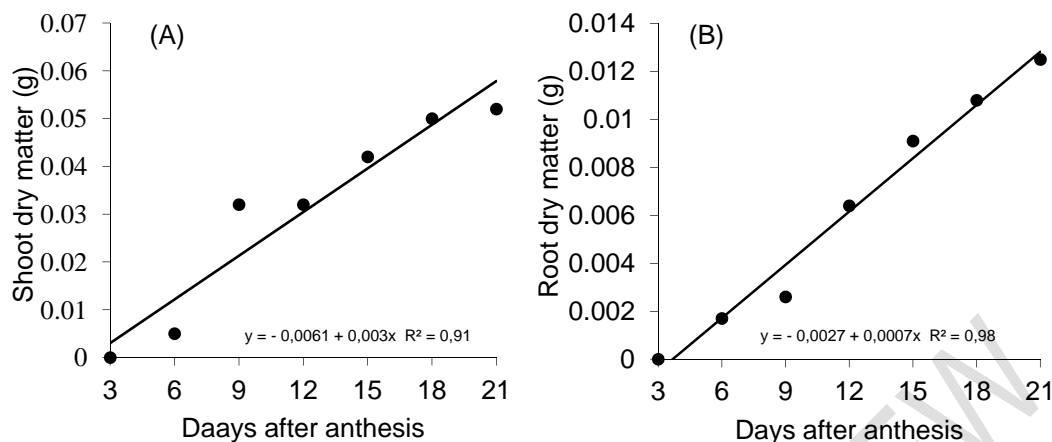


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182 **Figure 5. Shoot and root length of seedlings of Cowpea (*Vigna unguiculata* L. Walp.),**
 183 **cv Corujinha, at different times of harvest.**

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

189



190

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

193 A direct relation between the seed size (Figure 1D) and physiological quality results could be
 194 verified, where the seeds of higher size were also those with higher percentage of
 195 germination (Figure 4A and 4B) and vigor (Figure 5 6A and 6B). According to Carvalho and
 196 Nakagawa [15], the size of the seeds may influence the germination and vigor, since larger
 197 seeds were well nourished during their development, usually have well-formed embryos,
 198 have larger amounts of reserves and are potentially more vigorous.

199 Similar results were also observed by Padua et al. [14], where they evaluated the influence
 200 of soybean seed size on the initial growth of plants and their effect on yield, they observed
 201 that larger seeds presented higher percentages of germination, vigor and produce plants
 202 with higher height at the harvesting time, with higher yield, when compared to smaller seeds.

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

206 **4. CONCLUSION**

207 The seeds of Cowpea bean, cv. Corujinha, reached the physiological maturity at 15? after
 208 anthesis which coincides with a higher germination and vigor, being recommended as the
 209 best period for harvest of this cultivar.

210 **COMPETING INTERESTS**

211 Authors have declared that no competing interests exist.

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