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# Physiology of Growth and Development of Fruits and Seeds of Common Beans

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**Abstract**

Common beans are one of the most economically important legumes in the world. The determination of the ideal harvesting period may coincide with the maximum seed quality and vigor. Thus, the objective was to evaluate the physiology of growth and development of fruits, seeds and seedlings of two cultivars of common beans. To this end, two cultivars of common beans were used: the “Macarrão Trepador” and “Rasteiro Fartura” harvested every five days after anthesis (DAA). The pods were harvested and sent to the laboratory for seed analysis and extraction. In the laboratory, biometric characteristics of fruits and seeds, physiological quality (germination and vigor) and chemical composition of seeds were evaluated. The physiological maturity of “Macarrão Trepador” and “Rasteiro Fartura” cultivars occurred at 35 DAA, during which the seeds had the maximum dry matter and the minimum water content. The chemical composition of the seeds of both cultivars was similar, except for lignin, whose content was higher in “Rasteiro Fartura” cultivar at 25 DAA. The color and dry mass of fruits and seeds, germination percentage, first germination count, germination speed index and average germination time are the indicators that help in determining the physiological maturity point.

**Keywords:** physiological maturity, seed quality, vigor

## 1. Introduction

Common beans (*Phaseolus vulgaris* L.) are one of the most important crops for human consumption, being one of the main sources of protein consumed in the world (Carbas et al., 2020). Pods can reach maximum size when seed development is still insignificant, after this stage the seed increases in dry matter and length. As the seeds mature, they harden, become fiber rich and less palatable, and it is necessary to evaluate the physical and chemical changes that occur during pod maturation (Carrão -Panizzi et al., 2019).

Physiological maturity is described as the stage of development when a plant or part of the plant will remain ontogenic even if separated; and commercial maturity is defined as the stage of development when a plant or part of the plant has the prerequisites for use by consumers for a specific purpose, some aspects can be used in the form of parameters such as the color and size of a particular item and therefore its grade. Some physical characteristics of quality, texture, content, taste and nutrient or chemical attributes are also used to determine maturity (Shewfelt, 2014).

Seeds accumulate carbohydrate, lipid and protein reserves, and embryonic pattern formation, reserve accumulation, acquisition of desiccation tolerance, and dormancy are the main seed development programs and are important agronomic traits that define their quality (Devic & Roscoe, 2016). These nutrients are hidden features that affect consumer perception. Its composition varies greatly in raw materials due to genetics, pre-harvest factors, maturity in crop and post-harvest management conditions (Shewfelt, 2014).

The identification of the ideal harvesting point is essential, because the longer the plant time in the field after maturation, the greater the loss of seeds during harvesting, which could compromise 50% of the planting if not done at the right time; therefore, the seeds should be harvested close to the physiological maturity time (Ferreira et al., 2017). Paradoxically when early harvesting results in poor longevity and low vigor, this is because not all vigor characteristics of the seed have been developed. Therefore, it is necessary to harvest the seeds before they are fully mature, as it will ensure the harvest before dispersion (Leprince et al., 2017).

The objective of this work was to evaluate the physical, physiological and chemical changes, as well as to determine the ideal harvesting stage during the common beans fruit and seed ripening process.

## 2. Method

The field for seed production was installed in the Olericulture module in the experimental farm Olho d´agua of the Center of Agrarian Sciences of the Federal University of Paraíba, Areia, Paraíba, Brazil (06º57’30” S, 35º45’33,8” W, altitude of 503 m). The company Hortivale donated the pod bean seeds of the “Rasteiro Fartura” and “Macarrão Trepador” cultivars.

The spacing between beds was 1 m and between plants was 0.40 cm between plants. Four seeds were sown at a depth of 3 cm spaced 0.40 cm. The beds were spaced 1 m apart. After emergence, thinning was performed, leaving two plants/pit. Weed control was manual and drip irrigation was done for both cultivars. Tutors were placed for the “Macarrão Trepador” cultivar 20 days after sowing (DAS).

Forty days after sowing, when 50% of the plants had their flowers open, the flowers were marked. Marking occurred daily until the last day of flowering. Fruit harvesting was performed at intervals of five days after anthesis (5, 10, 15, 20, 25, 30, 35 DAA), totaling seven harvests for both cultivars. After harvesting, the fruits were packed in plastic bags and taken to the Seed Analysis Laboratory (LAS) of the Center for Agricultural Sciences of the Federal University of Paraíba. The determination of moisture and the evaluation of seed physiological quality was determined from the third (15 DAA) harvest, considering that at 5 and 10 DAA the seeds were not fully formed.

The variables length, width and thickness of 100 seeds and 100 fruits were determined at all harvesting times (5, 10, 15, 20, 25, 30, 35 DAA) with a 0.01 mm precision digital caliper. Fruit and seed color were evaluated by color chart made according to the maturation season, for both pods and seeds. While the variables water content and dry mass of fruits and seeds, by the stove method at 105 ± 3 °C for 24 hours (Brazil, 2009), using four fruit sub-samples (5 fruits/sample) and five grams of seeds.

The germination test was performed at the end of each harvest, with four repetitions of 50 seeds in previously sterilized germitest® paper and placed in rolls in B.O.D. (*Biological Oxygen Demand*) at a constant temperature of 25 °C and a photoperiod of 12 hours. The substrate was moistened with distilled water in an amount equivalent to 2.5 times its dry weight. Concomitant to the germination test, the first germination count was evaluated, the germination speed index was established according to Maguire (1962) and the average germination time determined following Labouriau (1983).

At the end of the experiment, the length of the roots, shoots and seedlings was established with the aid of a graduated ruler, the values being expressed in centimeters (cm). Subsequently, the separated parts were placed in Kraft paper bags and put to dry in a forced air circulation oven at 65 ºC until reaching constant weight, with the results expressed in g seedlings$^{-1}$.

The fruits were oven dried at 55ºC for 72 hours until constant weight. Crude protein (PB) (AOAC, 1998), neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (Van Soest et al., 1991) were determined at the Laboratory of Food Analysis and Animal Nutrition (LAANA), UFPB, Areia – PB.

The obtained results were submitted to analysis of variance and polynomial regression, testing the linear and quadratic model, being selected to explain the results, the significant model of higher order. The variables studied were analyzed using the statistical analysis program Sisvar 5.6 (Ferreira, 2014).

## 3. Results

As for the color of the pods, they vary in color as a function of maturity advance, where the fruits of “Macarrão Trepador” cultivar showed dark green color at 25 days after anthesis, turning to yellow and orange at 30 and 35 DAA (Fig. 1). For the “Rasteiro Fartura” cultivar, the dark green and medium tones were found from 5 to 30 days, turning yellowish green at 35 DAA (Figure 1).

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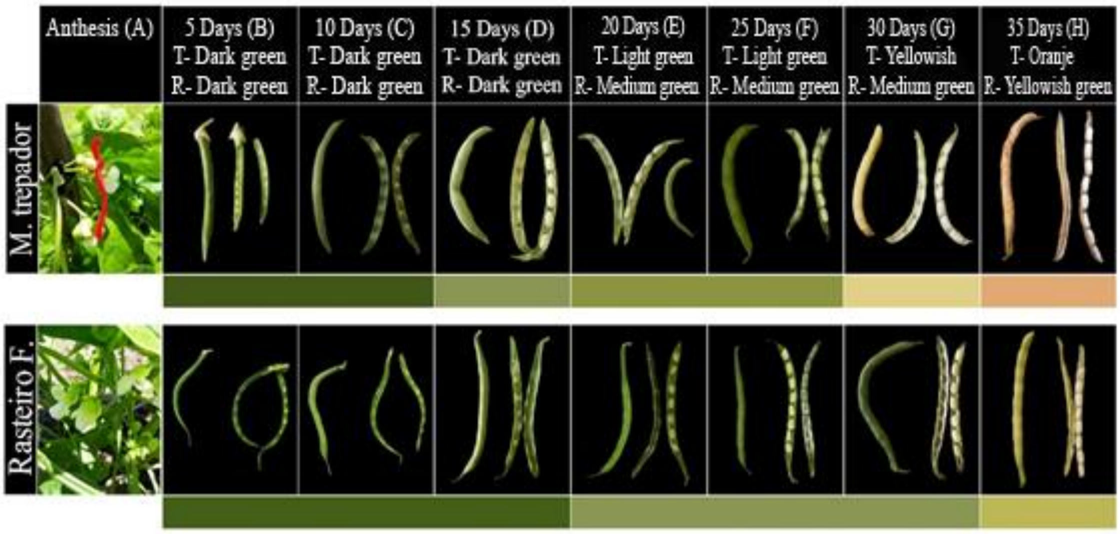



Figure 1. Fruit coloring of *Phaseolus vulgaris* L., "Rasteiro Fartura" and "Macarrão Trepador" cultivars at 0 (A), 5 (B), 10 (C), 15 (D), 20 (E), 25 (F), 30 (G) and 35 (H) days after anthesis

The variation in color was also observed in the seeds of cultivar Macarrão Trepador, with increasing physiological maturity, showing a dark green color until 15 days after anthesis, passing from there, the whitish coloration until completely white at 35 DAA (Figure 2). The "Rasteiro Fartura" cultivar showed dark to light green variation from 5 to 30 DAA, becoming at the end of the seed maturation period to have whitish color.

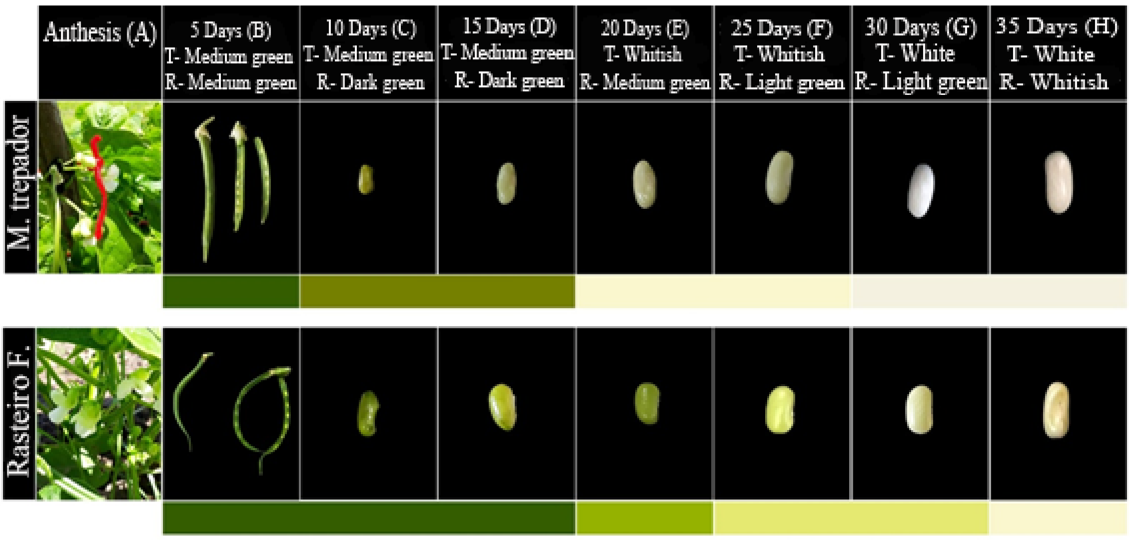
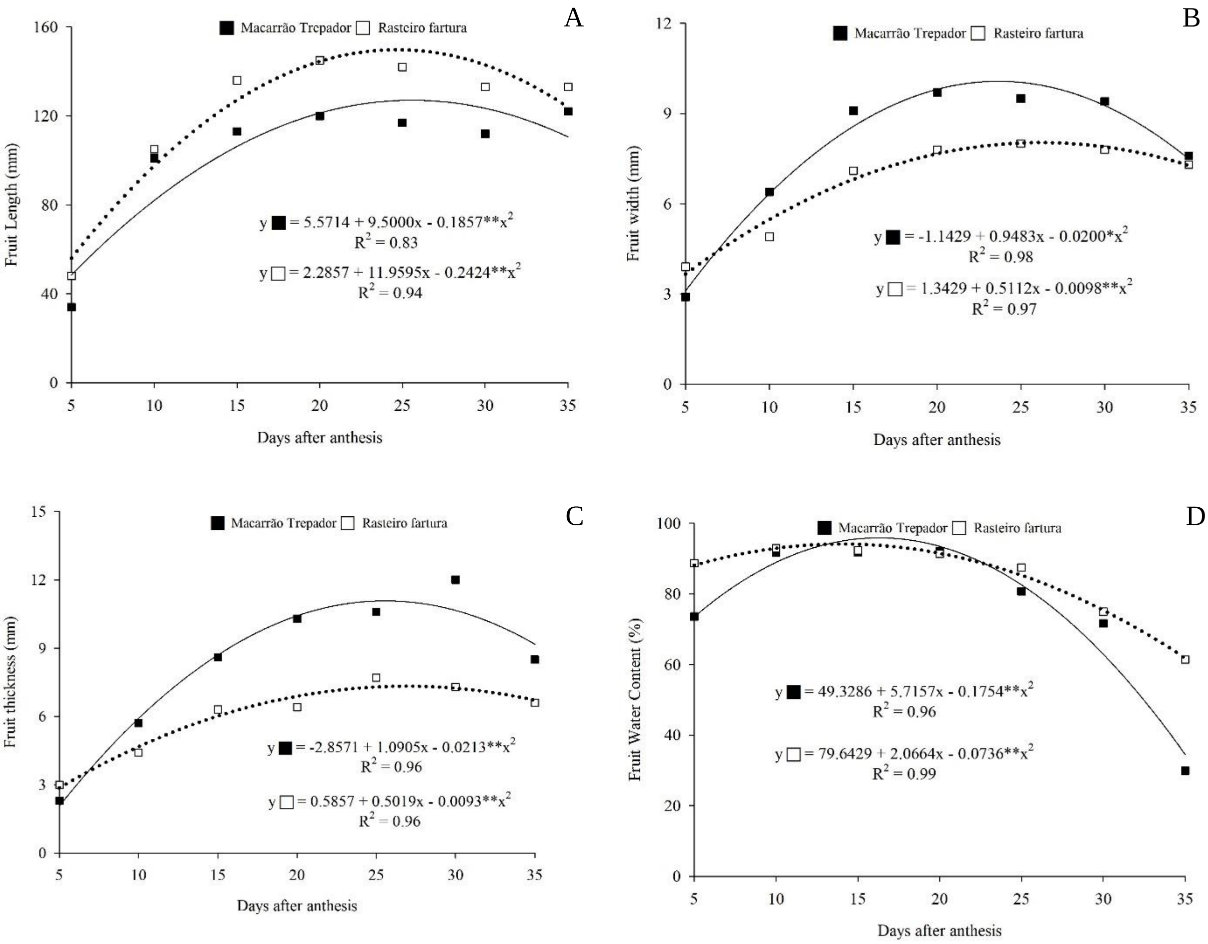



Figure 2. Coloring of fruits and seeds of *Phaseolus vulgaris* L., "Rasteiro Fartura" and "Macarrão Trepador" cultivars at 0 (A), 5 (B), 10 (C), 15 (D), 20 (E), 25 (F), 30 (G) and 35 (H) days after anthesis

Figure 3 presents the data corresponding to the physical characterization of pod bean fruits. For the number of seeds per fruit, it was found that the data did not fit the statistical models tested, containing on average 6 seeds per fruit. The "Macarrão Trepador" cultivar presented the highest values for length (127 mm), width (10 mm) and thickness (34.5 mm) at 25, 24 and 35 DAA. For the "Rasteiro Fartura" cultivar, the values of 149.7 and 8.0 mm were observed at 26 DAA for length and width and 7.35 mm for pod thickness at 27 DAA (Figure 3A, 3B and 3C).

Fruit water content was higher at 16 DAA (96%) for "Macarrão Trepador" cultivar and at 14 DAA (94%) for "Rasteiro Fartura" cultivar, decreasing to 35 DAA, respectively (Figure 3D). This reduction in water content coincides with the dry mass gain of the pods, where in both cultivars there were increments due to the increase of the development stage, reaching maximum values 48,4 and 45,6 g at 30 DAA for the "Macarrão Trepador" and "Rasteiro Fartura" cultivars, respectively (Figure 3E).



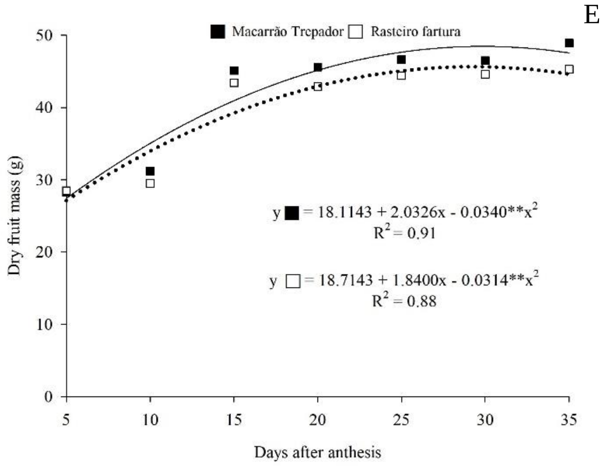



Figure 3. Fruit physical characterization: length (A), width (B), thickness (C) water content (D) and pod dry matter (E) of *Phaseolus vulgaris* L., at different harvest times

The physical characterization of pod bean seeds demonstrates that seed development follows the behavior observed during fruit ripening. For seed length, it was observed that the "Manteiga Trepador" cultivar stood out with the highest values (14.2 mm) at 27 DAA, while the "Rasteiro Fartura" cultivar with the maximum length (11.5 mm) at 32 DAA (Figure 4A). While the seed width and thickness of the "Manteiga Trepador" cultivar the values of 6.3 and 6.1 mm were recorded at 26 DAA. The "Rasteiro Fartura" cultivar, the largest increments (4.84 mm at 30 DAA and 5.63 mm at 29 DAA) were obtained for width and thickness, respectively (Figure 4B and 4C).

The water content of the seeds in both cultivars was reduced over the days after anthesis, with variations ranging from 86.4 to 54.2% with the "Macarrão Trepador" and "Rasteiro Fartura" cultivars, respectively (Figure 4D). Seed dry mass increased linearly as a function of DAA, following the seed dehydration process, obtaining the maximum increments at 35 DAA, with 3.46 and 1.08 g for "Macarrão Trepador" and "Rasteiro Fartura" cultivars, respectively (Figure 4E).

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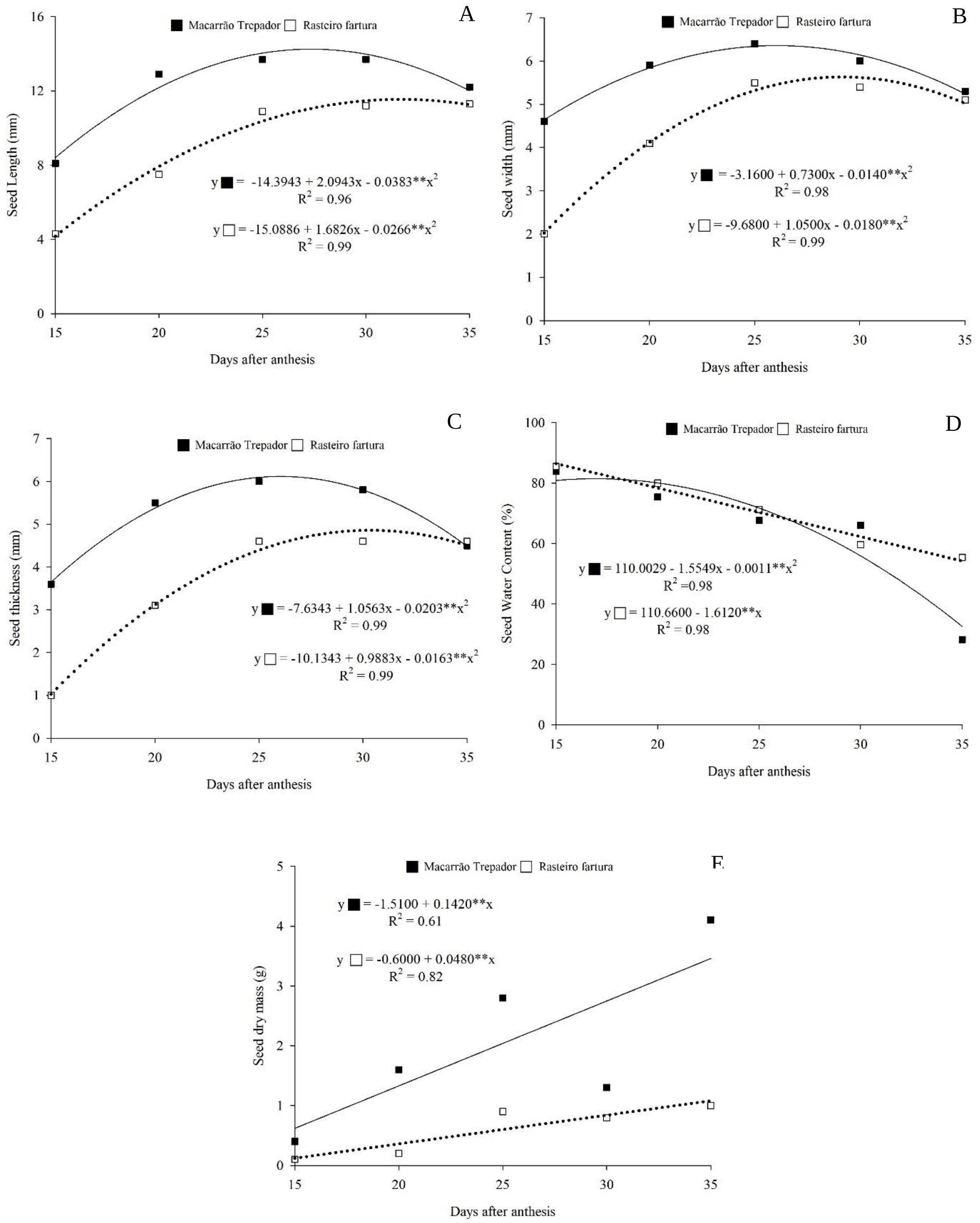


Figure 4. Physical characterization of seeds: length (A), width (B), thickness (C) water content (D) and pod dry matter (E) of *Phaseolus vulgaris* L., at different harvest times

Germination and germination speed index were influenced by seed maturation period, with no difference between cultivars. For both variables, advancing the days after anthesis promotes highest percentage of germinated seeds (94%) and the highest rate of germination process speed (4.67), reaching the maximum values at 35 DAA (Figure 5A and 5B).

For the First Germination Count, germination increased as the seed maturation stage increased, reaching the maximum values at 35 DAA, with 94 and 96.9% for “Macarrão Trepador” and “Rasteiro Fartura” cultivars, respectively (Figure 5C). Despite having high germination at the first count, both “Macarão Trepador” and “Rasteiro Fartura” cultivars had a longer time (5.23 and 5.43) for seeds to germinate at 35 DAA (Figure 5D).

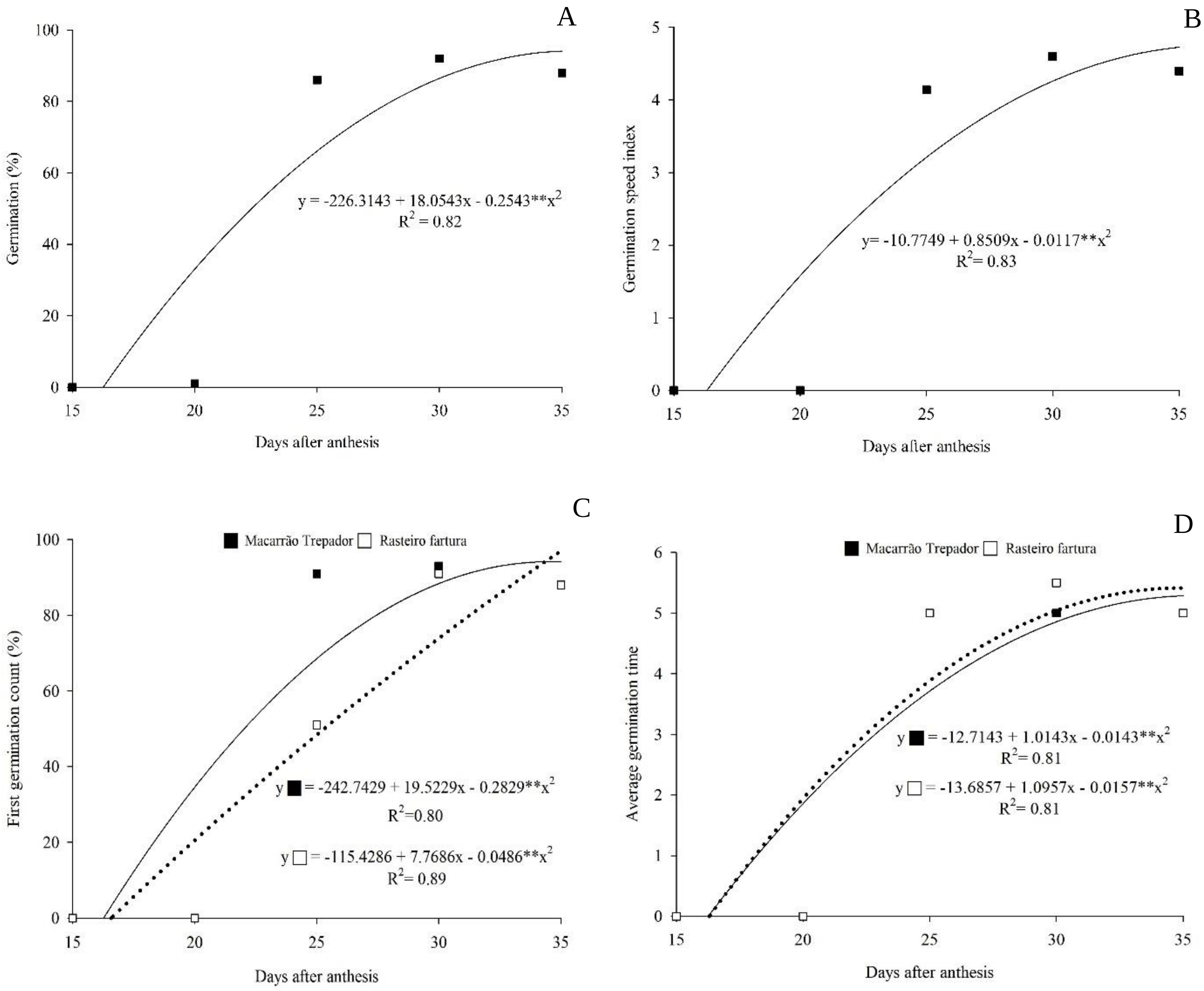


Figure 5. Germination (A), Germination Speed Index (B), First Germination Count (C) and Average Germination Time (D) of *Phaseolus vulgaris* L., at different harvest times

Regarding the root length of the seedlings, it was found that the “Macarrão Trepador” cultivar was more vigorous, reaching the maximum growth of the primary root (15.7 cm) at 35 DAA. While the “Rasteiro Fartura” cultivar showed linear growth, reaching the maximum increment of 8.36 cm (Figure 6A). Similarly, shoot and seedling growth occurred, where both cultivars exhibited linear growth as a function of days after anthesis, with maximum values of 10.8 and 9.1 cm and 26.5 and 17.4 cm for “Macarão Trepador” and “Rasteiro Fartura” cultivars, respectively (Figure 6B and 6C).

Root, shoot and seedling dry matter contents in both cultivars were higher than 35 DAA, where the “Macarrão Trepador” cultivar presented values of 3.0; 0.47 and 16.0 g, respectively. For the “Rasteiro Fartura” cultivar the maximum increments of 0.10 were obtained; 1.74 and 1.85 g for root, shoot and seedling dry mass (Figure 6D, 6E and 6F).

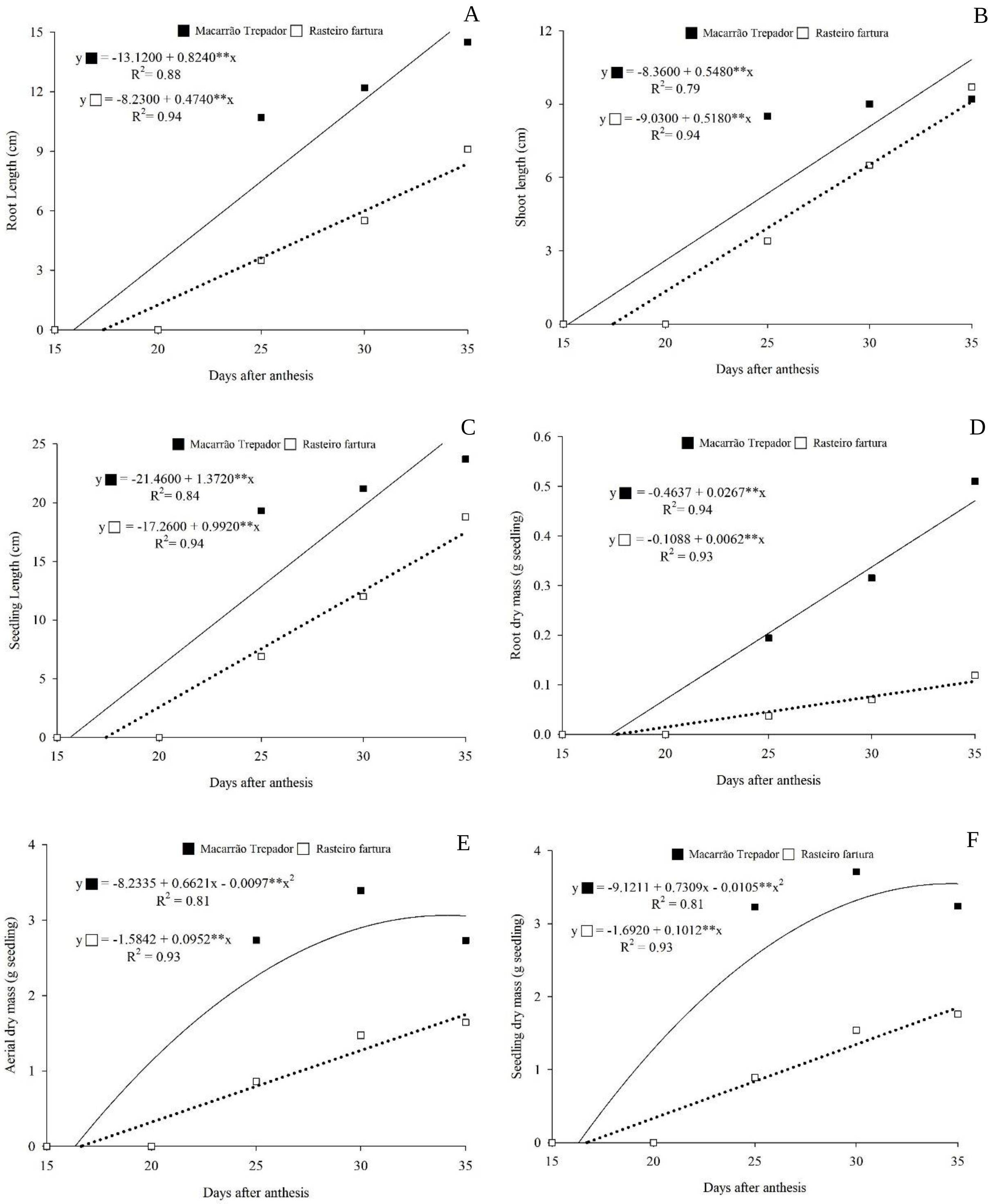

Figure 6. Root (A), shoot (B), seedling (C) length, root (D), shoot (E) and total (E) dry matter of *Phaseolus vulgaris* L., at different harvest times

The crude protein did not fit any statistical model and kept its values constant over the days of physiological maturity, whose values were in the order of 20 and 21%, for the "Rasteiro Fartura" and "Macarrão Trepador" cultivars, respectively.

The lignin content was higher in the "Rasteiro Fartura" cultivar whose highest increase (1.66%) occurred at 25 DAA, followed by decreases with the maturation of the pods. For the "Macarrão Trepador" cultivar, a higher lignin content (1.23%) was found in fruits harvested at 15 DAA, followed by a reduction with increasing maturity stage (Figure 7A).

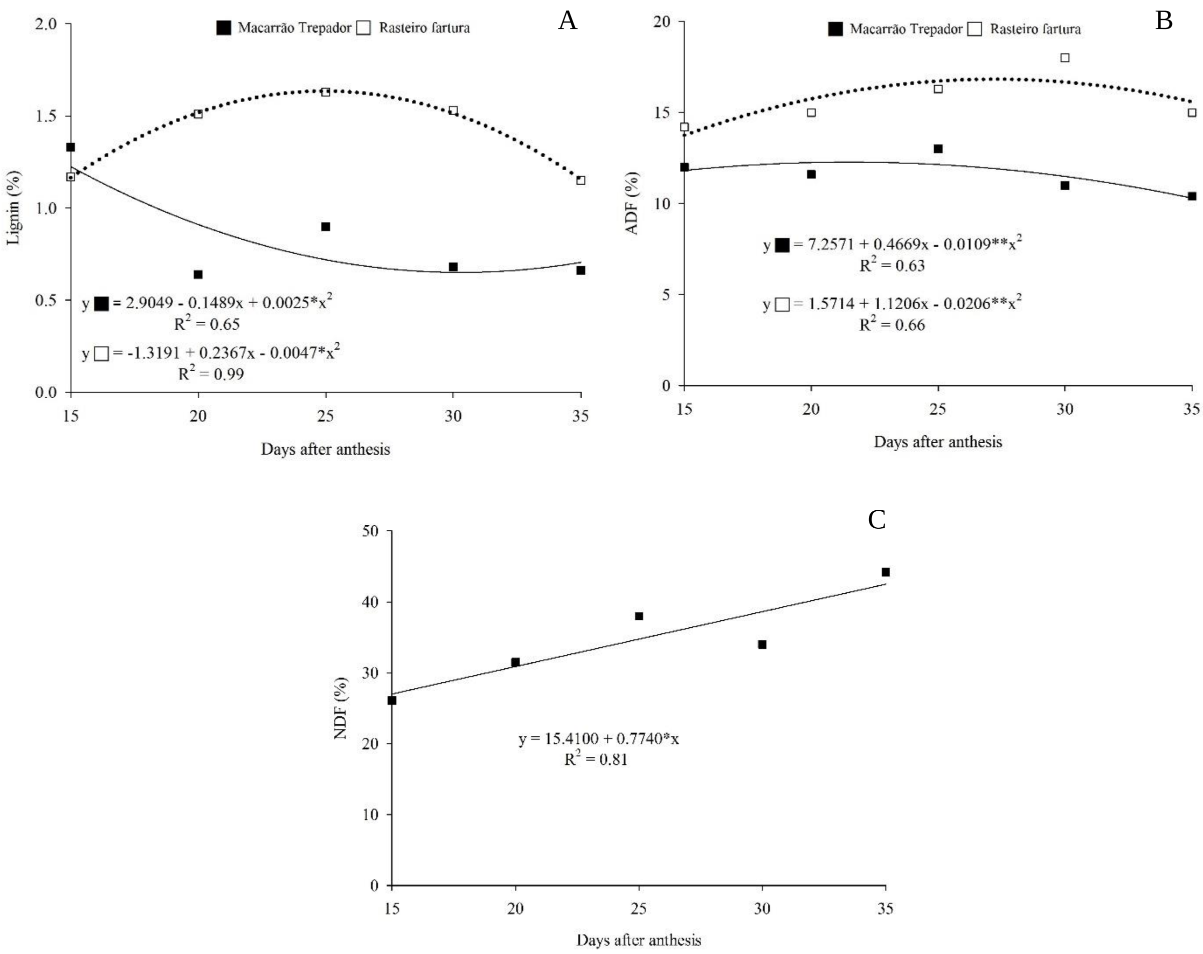


Figure 7. Quantification of lignin (A), acid detergent fiber (B) and neutral detergent fiber (C) in fruits of *Phaseolus vulgaris* L., harvested at different times

The acid detergent fiber (ADF) content was also higher in "Rasteiro Fartura" cultivar with 16.8% at 27 DAA, while for "Macarrão Trepador" cultivar the maximum increase (12.2%) in the contents of this fiber occurred at 21 DAA, these values being reduced with advancing maturation stage (Figure 7B). Regarding the neutral detergent fiber content, there was no difference between pod bean cultivars, with increasing linear effect as a function of maturity stage, with the maximum increase (42.5%) at 35 DAA (Figure. 7C).

## 4. Discussion

The variation in the color of fruits and seeds is characteristic and is associated with maximum fruit development. Fact justified by Michelangeli et al. (2013), by showing that the coloration dynamics of the pods in *Phaseolus vulgaris* L., corresponding to the two clear phases, being the first predominant green coloration stable in the early stages of development, coinciding with seed development, and the second phase that chlorophyll degrades, where green gives way to yellow and, in even more advanced stages, shades of yellowish brown in bean pods. This characteristic is important for establishing the ideal point of seed physiological maturity, allowing the determination of the best time for harvest. Thus, this change in color as the days of physiological maturity advance is indicative of the onset of pod senescence, the end of the rapid seed growth phase and acceleration of pod and seed desiccation (Michelangeli et al., 2013).

The reduction in the biometric characteristics of the fruits tends to decrease over the days after anthesis and can stabilize or decrease until the physiological maturity (Santos et al., 2020). This result is justified by the increased stage of fruit development, with greater translocation of reserves to the pods, promoting greater expansion. Thus, fruits with a more advanced stage of development tend to have more formed, more vigorous seeds with a higher reserve content, promoting more vigorous seedlings (Santos et al., 2019).

This behavior is associated with the ideal point of physiological maturity, in which there is a gradual reduction in water content and an increase in the concentration of reserves, allowing the formation of seeds with higher quality and greater vigor. Sumathi and Srimathi (2015) verified this fact in *Psoralea corylifolia*, Tavares et al. (2016) in *Vigna angularis* Willd. And Cruz et al. (2019) in *Vigna unguiculate* and where the same authors state that the ideal point of maturity is the moment of greatest expression of the seed's physiological potential.

The oscillation in the biometric variables of pod seeds is common, where there was a maximum increase and a reduction in these characteristics, being associated with the process of dehydration and the point of physiological maturity of the seed. Thus, this rapid growth comes from the cell multiplication that makes up the reserves of the embryonic axis and, when reaching the maximum point of accumulation of dry matter, the seed begins the dehydration process and the end of the maturation process (Silva et al., 2019). This oscillation is linked to the increase in the dry mass of the seeds, due to the accumulation of translocated metabolites of the plant during maturation (Kambhampati et al., 2020). As there is a reduction in the water content of the seeds, the accumulation of dry mass occurs simultaneously in the last days of harvest and to obtain seeds of high physiological quality (Menendez et al., 2019).

The quality of the seeds presented by the percentage of germination and by the vigor tests are indicative of the point of the physiological maturity of the seeds occurred with the maximum accumulation of reserves at 35 DAA, obtaining a direct correlation between percentage and speed of germination and dry mass of the seeds (Figure 4E). Thus, the greater the accumulation of reserves, the greater the vigor and quality of the seeds, reflecting the germination capacity (Pino et al., 2019).

Thus, as the fact that the seeds, in the initial phase of their formation, have a high water content, decreasing with time and allowing better conditions for the complete formation of the embryonic axis. The initial stage of seed development is characterized by its high amount of water, as it plays a fundamental role in the synthesis and metabolization of reserves that occur in an aqueous medium (Liu et al., 2018). Thus, as the development progresses, the seed tends to lose water and accumulate as many reserve substances as will be assimilated and used for the development of the embryonic axis (Jorge et al., 2018).

The increase at 35 DAA of seedling growth can be attributed to the reserve's metabolizing capacity at the end of the physiological seed maturity. Thus, stored reserves are degraded to nurture the development of the embryonic axis, resulting in more vigorous and higher quality seedlings (Moreira et al., 2019).These results are indicative of the maximum physiological quality (vigor) of the pod seeds, since there was a maximum accumulation of reserves, enabling the development of the embryonic axis and obtaining more vigorous seedlings. Thus, after reaching the ideal point of physiological maturity, the seed accumulates reserves that will be mobilized and degraded to provide maximum expression of vigor during the initial growth of the seedlings (Silva et al., 2019).

The biochemical characterization of the seeds, presented levels of proteins obtained are within the limits found by other authors, such as Marquezi et al. (2016) finding values ranging from 21.6 to 27.3%. Reddy et al. (2013) found a variation between 15 and 20% of protein and stated that these quantities occur due to the species that present it in a large part of its constitution.

Likewise, the lignin content obtained is within the limits found in the literature for pod culture, ranging from 1.2 to 1.7% (Ferro et al., 2017). It is important to note that lignin has a protective function, thus providing a barrier to the entry of pathogens, and its quantification varies according to the species, cell type and stage of development (Buchanan et al., 2015).

The fiber content obtained at work showed acceptable values, since they interfere with the qualitative characteristics for commercial acceptance, being related to the flavor of the pods, where the high fiber content is undesirable for consumption, requiring a balance of these contents at the right time of the harvest, because very low values are also not indicated, due to the benefits of the components for the organism (Francelino et al., 2011). The increase in fiber content provides improvements in the proportion of insoluble / soluble fibers during germination, leading to an increase in the total sugar content, oil retention capacity, water retention and absorption (Benitez et al., 2013).

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