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# Seed morphobiometry, morphology of germination and emergence of quinoa seeds 'BRS Piabiru'

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**Abstract:** Quinoa, classified as a pseudocereal, presents greater nutritional value compared to traditional cereals. Considering the potential for cultivation presented by the species and the benefits of studying plant morphology and morphobiometry, this paper describes seed and seedling morphobiometric characteristics of quinoa (*Chenopodium quinoa* Willd.) cultivar BRS Piabiru during germination and emergence. To evaluate seed morphobiometry the 1000-seeds weight, moisture content, seed morphological characterization and the tetrazolium test were performed. The morphological characterization of germination and seedling emergence were performed by periodic observations during the development, allowing the description using pictures and drawings. Quinoa seeds cv. BRS Piabiru present an average diameter of 2.05 mm and 1.07 width. 1000-seeds weight of 2.68 g and moisture content of 11%. Externally, quinoa seeds present the pericarp as testa, the hilum and the raphe and, internally, the embryonic axis (cotyledons, radicle and hypocotyl-radicle), perisperm and endosperm. The germination is characterized as epigeal, phanerocotylar type, with radicle protrusion at 3 hours after sowing and complete formed seedlings at 24 hours after sowing. Emergence occurs at 9 days after sowing and plants are completely formed at 12 days after sowing.

**Key words:** morphological characteristics, *Chenopodium quinoa* Willd., seedlings, pseudocereal, initial development.

## INTRODUCTION

One of the most important fields of study in Botany is Plant Morphology, since this field of knowledge studies shapes and structures, thus enabling the understanding of seed morphological and ecophysiological characteristics, which are extremely relevant to comprehend the life cycle and growth of species (Lorenzi & Gonçalves 2011, Mourão et al. 2002). Therefore, these studies may allow to improve the evaluation of natural regeneration of ecosystems, the analysis of biological cycles, management practices and the definition of strategies for the conservation of species and the development of efficient techniques for production (Batista et al. 2011).

Seedling morphology, specifically as a contribution for research, is important since seedling stages are considered critical phases for the establishment of species in the environment (Ferreira & Barretto 2015). Information about seed morphological characters provides crucial data for the identification and knowledge of the behavior of species in different regions, determination of the variability of the species, in the study of dispersion types and dispersers, in

the interpretation of laboratory tests and in the identification of the species on soil seed banks and in the seedling stage (De Almeida et al. 2010, Melo et al. 2004), specially from lesser known species.

Quinoa (*Chenopodium quinoa* Willd.) has been introduced less than 30 years in Brazil, therefore, there are few morphological studies about this species. This pseudocereal presents a great potential for cultivation, however, production is limited due to some hindrances, such as, the availability of only one cultivar in the market (Spehar 2006).

Quinoa presents greater nutritional value compared to traditional cereals and excellent amino acid, oil and protein contents (Repo-Carrasco et al. 2010). Currently, the species is very requisitioned due to these characteristics. A significant part of the population, for being celiac and/or lactose intolerant, insert the grain in the diet, since quinoa seeds do not contain gluten and possess a protein fraction comparable to milk casein. As a promising alternative for food and feed industries, quinoais being adopted on crop-livestock systems (Spehar & Santos 2002, Strenske et al. 2017).

The research with the species is notoriously incipient, especially regarding seed production and quality evaluations. In this regard, the importance of seed morphobiometry is highlighted since characteristics such as weight and size allow the differentiation of seeds for the formation of more homogeneous lots, enabling greater uniformity and the improvement of seed emergence and vigor (Andrade et al. 1996, Carvalho & Nakagawa 2012).

Considering the potential presented by quinoa and the benefits of plant morphology and morphobiometry, this paper describes seed and seedling morphobiometric characteristics of quinoa (*Chenopodium quinoa* Willd.) cultivar BRS Piabiru during germination and emergence.

## MATERIALS AND METHODS

### Location

The study was carried out at the Didactic Laboratory of Seed Analysis of the College of Agronomy "Eliseu Maciel", at the Universidade Federal de Pelotas, Capão do Leão, RS. Quinoa seeds of the cultivar BRS Piabiru were used. Seeds were subjected to the following analysis:

### Seed morphobiometry

1000-seeds weight: eight subsamples of 100 seeds derived from the pure seed portion were used, weighting each subsample individually. Then, the variance, standard deviation and the coefficient of variation of the data obtained were calculated. When the coefficient of variation presented values less than or equal to 4%, the average weight obtained from the eight subsamples was multiplied by 10, obtaining the 1000-seeds weight, in grams (Brasil 2009).

Moisture content: was performed by the standard oven-dry method of 105°C $\pm$ 3°C, where two subsamples of 5g, obtained from the submitted sample, were accommodated in metal containers and placed into a kiln at 105°C for 24 hours. Moisture content was calculated using the wet basis method. The result was obtained through the arithmetic mean of the percentages of each subsample withdrawn from the submitted sample and expressed with one decimal place, according to the Rules for Seed Analysis (Brasil 2009).

Seed morphological characterization: to characterize seed morphology the analysis of seed biometric measures was performed. Eight samples, containing 25 seeds each, were selected performing measurements, individually, for diameter (from apex to base) and width (dorsal to ventral) using a caliper and expressing the measurements in mm, with a precision of two decimal places. For each variable studied, the

arithmetic mean and measures of dispersion were calculated.

To identify the constituent parts of quinoa seeds (living and dead tissues), an imbibition in water for 40 minutes followed by imbibition in tetrazolium solution (0.5%) for 90 minutes were carried out. After these procedures, seeds were sectioned using steel blades and, with the aid of a table magnifying glass and a stereomicroscope, the external and internal characteristics of seeds were observed in greater detail. Transversal and longitudinal sections were performed using steel blades after seed hydration and softening. The constituent parts of seeds were manually drawn and properly classified.

### Morphology of germination and seedling emergence

#### *Morphologic characterization of germination*

To evaluate the germination process 200 seeds were sown, distributed in four repetitions of 50 seeds disposed into plastic boxes (gerbox). Blotter paper moistened with a volume of distilled water equivalent to 2.5 times the weight of the dry paper was used as substrate. The germination boxes were placed into a germinator, at 20°C, in the presence of constant light until radicle protrusion (Borges 2017).

The criteria to define the germination process was the identification of emission of the first radicle. Therefore, the illustrations of the initial phases of germination were performed manually, and with the aid of a binocular loupe, the record of the details was possible. The characters analyzed in the descriptions and the terminology applied are in accordance to Brasil (2009), Cunha & Ferreira (2003), Feliciano et al. (2008), Ferreira et al. (2001) and Vidal & Vidal (2003).

#### *Morphological characterization of emergence*

To describe seedling development, 10 quinoa seeds were sown in a rhizotron (62 x 53 x 10 cm), previously filled with commercial substrate (S10 Beifort®), maintained under laboratory conditions, with average temperature of 25°C. At the first day after sowing (DAS) the characterization of the emergence was performed for the first seed that presented radicle protrusion, posteriorly, every three days the structures were drawn until the formation of a plant. The drawings were performed from the beginning to the end using the same seeds, characterizing every structure during development.

## RESULTS AND DISCUSSION

Table I presents the average values for diameter and width of quinoa seeds. Quinoa seeds presented an average diameter of 2.05 mm and average width of 1.07 mm, characterized as a round and flat seed. Spehar & Santos (2002) observed that quinoa seeds have a cylindric, flattened shape and size varying from 2 to 2.5 mm diameter and 1.2 to 1.6 mm width, corroborating with the results of this study.

Seeds may present an intense biometric variability between each other. Differences in size for quinoa seeds may be a consequence of the maturation process, since quinoa seeds present different maturation stages in the plant and within the raceme. The racemes of the quinoa plant, that sustains the seeds, resembles those of the sorghum plant (*Sorghumbicolor* (L.) Moench) which, likewise, present different levels of maturation within the raceme, with seeds of different shapes and colors, from yellow to purple (Spehar & Cabezas 2000).

The variable seed size (dimensions) is an important characteristic, from the point

**Table I. Arithmetic mean,standard deviation (SD) and coefficient of variation (CV) of the biometric measures of quinoa seeds (*Chenopodium quinoa* Willd.).**

| Quinoa | Arithmetic Mean (mm) | SD | CV (%) |
|---|---|---|---|
| Diameter | 2.05 | 0.059 | 2.9 |
| Width | 1.07 | 0.004 | 0.4 |

of view of classification, for determination of physiological quality and is commonly used on seed production of different plant species (Frazão et al. 2011). Carvalho & Nakagawa (2012) affirm that greater seeds, for example, are generally well-nourished during development, presenting well-formed embryos and greater quantities of reserve substances being, consequently, more vigorous.

These data allow the optimization of seed processing, which is an important step of production as well as posterior seed commercialization as seed processing is necessary to remove contaminants such as: foreign materials (straw, branches, clods and insects), seeds from other crops or weeds. Moreover, seed processing has the purpose of separating seeds by size (Peske et al. 2012).

During measurements, quinoa seeds presented a moisture content of 11% (Table II). One of the most important aspects that interfere grains and seeds is the moisture content, exercising pronounced effects on seed physical and chemical properties and has great relevance with respect to quality maintenance during various stages, such as harvest, storage and commercialization. Therefore, frequent determinations of the moisture content are indispensable for determining adequate procedures to reduce seed damage, either due to the deterioration process or disease occurrence (Carvalho & Nakagawa 2012, Pedrosa et al. 2014, Rodrigues et al. 2016).

Quinoa seeds presented a 1000-seeds weight of 2.68 g (Table II), similar value to the observed by Spehar et al. (2011) that evaluated the variable for the same species; using the lineage BRS Syetetuba (2.5 -3.3g).

Figure 1 presents the characterization of seed internal and external structures. It is possible to observe that quinoa seeds are involved by the pericarp covering the seed, this structure may also be called testa. The pericarp was also identified in a study performed by Prego et al. (1998). Externally, quinoa seeds present the micropyle, a structure derived from the intern layers of the ovule (primine and secundine) that do not merge, and the raphe, which is a “scar” caused by the disruption of the funiculus and by the position of the ovary in the flower.

Quinoa seeds are, actually, fruits of the achene type, disposed in layers as pericarp and integument, respectively, from the external to the internal region with the shape classified as rounded (cylindrical). The morphological characterization of quinoa seeds illustrated are in accordance with those presented by Risi & Galwey (1989) for the same genus.

Internally (Figure 1), lies the perisperm, the main reserve tissue of the seed, correspondent to the endosperm in the grains of cereals and consists, mainly, of starch granules (Mujica et al. 2001, Prego et al. 1998). The perisperm is a dead tissue, derived from the non-consumption of the nucellus, a layer of highly nutritive cells, during the development of seed structures and the embryo (Carvalho & Nakagawa 2012).

**Table II.** Moisture content (%) and 1000-seeds weight (g) of quinoa seeds (*Chenopodium quinoa* Willd.).

| Species | Moisturecontent (%) | 1000-seeds weight (g) |
|---|---|---|
| Quinoa | 11 | 2.68 |

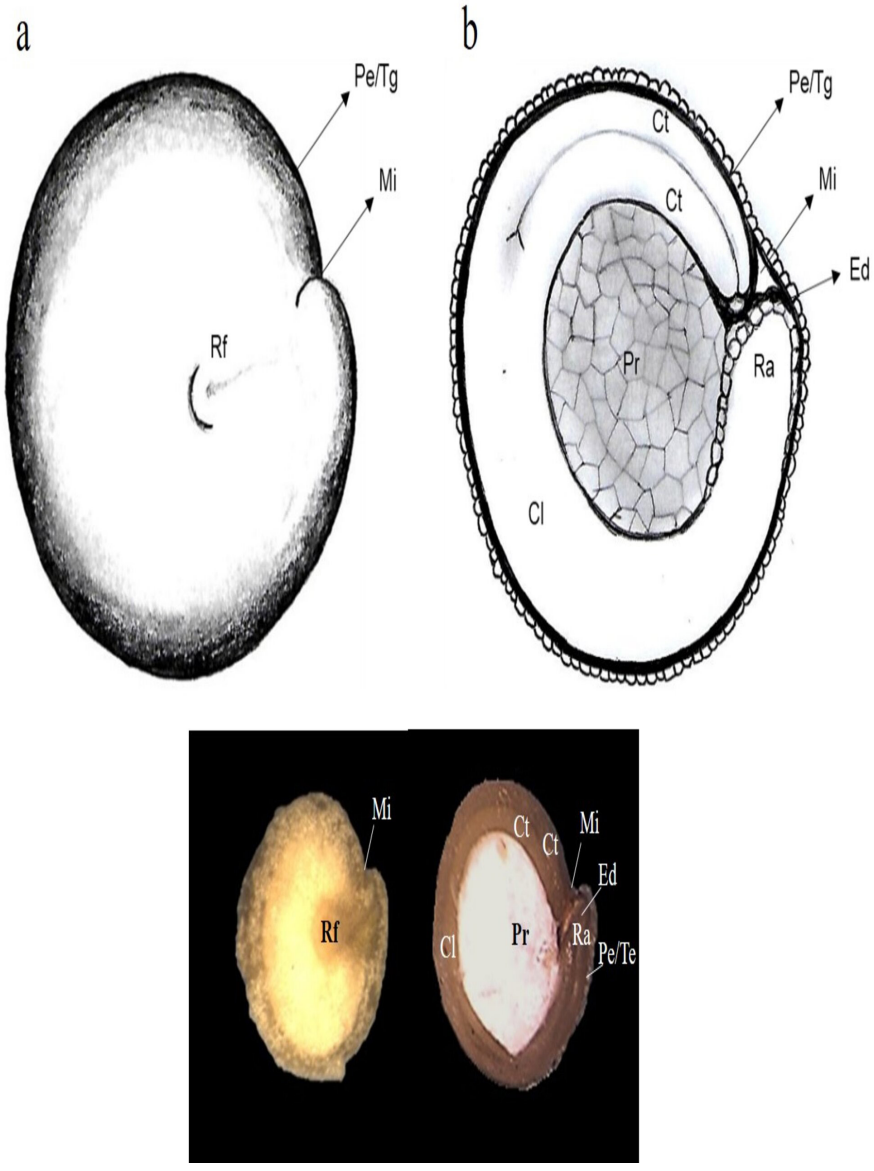


**Figure 1.** Characterization of external (a) and internal (b) structures of quinoa seeds. Mi: micropyle; Pe: pericarp; Te: testa; Rf: raphe; Hr: hypocotyl-radicle; Ct:cotyledon; Ed: endosperm; Pr: perisperm; Ra: radicle.

Besides the perisperm, quinoa seeds also present other two reserve tissues. The first is composed of two cotyledons, living tissue that characterizes the species as an angiosperm of the dicotyledonous type. The other, which is the smallest, the endosperm (Figure 1), is originated from the fertilization of the two polar nuclei by one sperm nucleus (Marcos Filho 2015).

According to Prego et al. (1998), quinoa seeds are classified as *campylotropous*, which means that the embryo is peripherical and the basal body functions as a reserve tissue or perisperm.

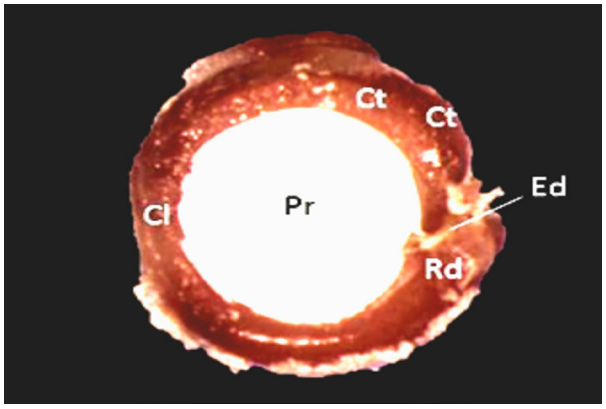


**Figure 2. Characterization of tissues of quinoa seeds subjected to the tetrazolium salt. Ct: cotyledon; Ed: endosperm; Pr: perisperm; Rd: radicle.**

On Figure 2, the longitudinal section of quinoa seeds previously subjected to immersion in the tetrazolium solution is presented. Seed structures that presented carmine red coloring, due to the reaction of the tetrazolium salt in response to respiratory activity, were classified as living tissue, which is the case of the structures that compose the embryonic axis (cotyledons, hypocotyl-radicle and radicle). Besides the characterization of the living tissues, those tissues that did not color, were classified as dead, which is the case of the perisperm and endosperm.

The tetrazolium test consists of an evaluation of seed viability and vigor based on the alterations of the living tissues, using a solution of 2, 3, 5 - triphenyl-tetrazolium chloride, which detects the activity of dehydrogenases. The coloring occurs because the diffusion of the tetrazolium salt on seed tissues results in the formation of a reddish compound which is stable, insoluble and not diffusible, named formazan. This reaction translates the significative respiratory activity on the mitochondria, allowing to delimitate, punctually, tissues that respire, that is, living tissues and tissues that present low or inexistent physiological activity, since those tissues remains discolored or exhibit abnormal coloring (Marcos Filho 2015).

The characterization of the internal structures of quinoa seeds using tetrazolium may be of utmost importance for improving the test for the species, since one of the major challenges for the standardization of the tetrazolium test, on less studied species, is the identification of the seed constituent parts, to prevent erroneous decision making by seed analysts when assessing viability.

The germinative process initiated with radicle protrusion followed by the emergence of the hypocotyl and root hair (Figure 3). The main root is expressive, long and has a whitish color.

Radicle protrusion occurred in the first few hours of germination. At 6 hours after sowing (HAS), the emergence of the hypocotyl was observed and, posteriorly, at 9 HAS the emergence of the first root hairs was verified. At 12 HAS the appearance of the cotyledons was observed and at the 24 HAS the rupture of the cotyledons from the tegument was verified.

According to Beltrati & Paoli (1989) the cotyledon is the first or every of the first leaves that are formed in the embryo. Cotyledons may have the appearance of a leave (foliaceous) and, after germination, act on photosynthesis, or accumulate nutritional reserves, functioning as reserve organ (fleshy).

In the soil, under uncontrolled conditions, quinoa seeds presented radicle protrusion at the first day after sowing. At 3 DAS, the emergence of roots hairs and the formation of the hypocotyl hook were observed. The rupture of the soil surface was observed at 6 DAS, with the emergence of the hypocotyl hook. Furthermore, the appearance of the first secondary roots was observed (Figure 4).

At 9 DAS, the appearance of the cotyledonary leaves was observed, sustained by a well-developed hypocotyl, and the emergence of

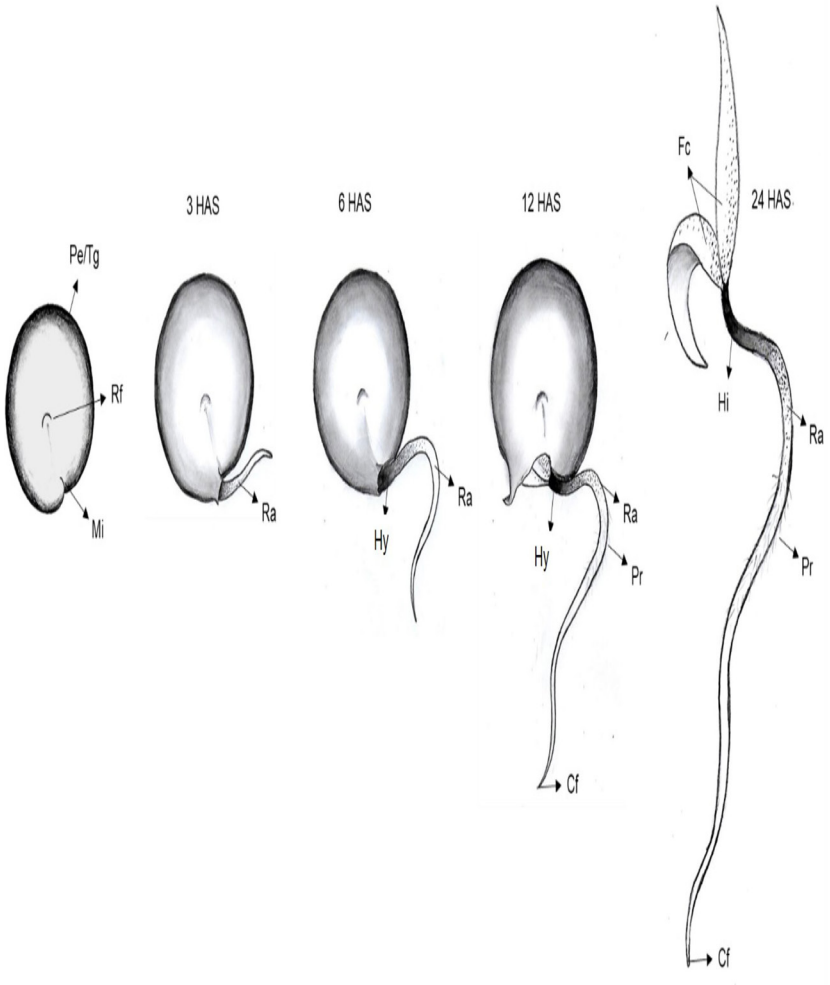


**Figure 3. Morphological characterization during the germination of quinoa seeds. Mi: micropyle; Te: testa; Rp: raphe; Cf: coif; Cl: cotyledonary leaf; Hy: hypocotyl; Rh: root hair; Ra: radicle.**

new secondary roots and the coif were verified, however, still in seedling stage. Seedlings are composed by the hypocotyl-radicle axis, one or more cotyledons and the primordial stem. The cotyledons are structures of fundamental importance for the initial development of the embryo (Beltrati & Paoli 1989, Raven et al. 2001). According to Damião Filho (1993), seedlings are immature plants which still rely on nutritional reserves and, therefore, are not completely autotrophic.

At 12 DAS, the formation of all the essential structures which characterize the passage from the seedling to the plant stage were observed, since the primary leaves, able to perform photosynthesis and sustained by the epicotyl, were formed (Figure 4). At this moment, newly-formed secondary roots, a distinct tap root, the root collar and leafy cotyledons could be verified (Figure 4).

The emergence of quinoa seeds is characterized as epigeal (Figure 4). On plants of epigeal germination, the cotyledons are raised aboveground due to the growth of the hypocotyl (Ressel et al. 1989).

According to the classification proposed by Duke (1965), which considers the relationship cotyledon/testa, quinoa seedlings may be classified asphanerocotylar with foliaceous cotyledons. On phanerocotylar seedlings, the cotyledons completely emerge from the seed coat, whereas on cryptocotylar seeds the cotyledons remain within the seed coat.

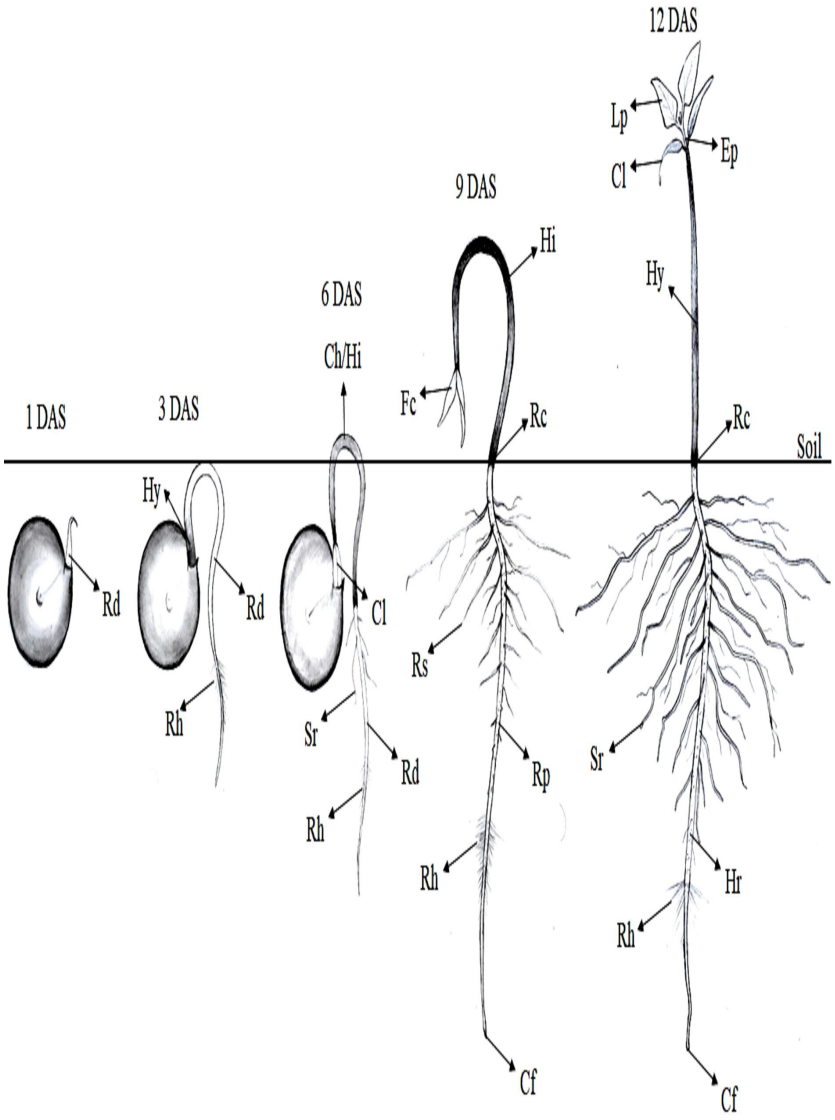


**Figure 4. Morphological characterization of the emergence of quinoa seeds. Cf: coif; Rc: root collar; Ep: epicotyl; Cl: cotyledonary leaf; Hy: hypocotyl; Lp: leaf primordia; Ch: cotyledon handle; Rh: root hair; Rd: radicle; Tr: taproot; Sr: secondary root.**

## CONCLUSIONS

Quinoa seeds of the cultivar BRS Piabiru present an average diameter of 2.05 mm and average width of 1.07 mm, with a 1000-seeds weight of 2.68 g and a moisture content of 11%. Externally, quinoa seeds present the pericarp as testa, the hilum and the raphe and, internally, the embryonic axis (cotyledons, radicle and hypocotyl-radicle), perisperm and endosperm. The germination is characterized as epigeal, phanerocotylar type, with radicle protrusion at 3 HAS and complete formed seedlings at 24 HAS. The emergence occurs at 9 DAS and plants are completely formed at 12 DAS.

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## Author contributions

Daniele Brandstetter Rodrigues: Idealization, execution, evaluation, statistical analysis and writing. Jerffeson Araujo Cavalcante: Idealization, evaluation, making of the drawings and writing. Andreia da Silva Almeida and Lilian Vanussa Madruga de Tunes: Orientation and writing. Camila de Avila Nunes, Ana Flávia Alcantara Serrão and Anna dos Santos Suñé: Execution and evaluation. Luis Henrique Konzen: Illustrations and execution.