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BASIC AREAS
Article

Interaction between germination, physical and nutritional quality parameters of quinoa cultivars from Colombia

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ABSTRACT: The aim of this study was to evaluate the quality, germination, storage conditions and nutritional composition of the seeds quinoa cultivars from the department of Boyacá, Amarilla de Maranganí, Quinua Semi amarga, Quinua Primavera, and Quinua Real, by determining the color of the pericarp, perigonium and the edge of the seed, germination capacity of the cultivars stored at -20 and -4°C, the imbibition rate, the grain diameter, fresh and dry weight of 100 seeds and the moisture percentage and proximal analyses. The cylindrical grain shape was common among cultivars. Amarilla Maranganí was outstanding, with weights greater than 0.40 g. The diameter of the seed was higher in Amarilla Maranganí (2.67 mm), while the smallest diameter (2.25 mm) was presented by Quinoa Real. Moisture contents were similar between materials. Quinoa Real presented the highest protein content (12.25%). Amarilla de Maranganí had the highest ash content (3.77/100 g of sample), and Quinoa Real presented the highest number of calories (366.97 g·100 g^{-1} of fresh weight), but the lowest amount of carbohydrates (66.96 g·100 g^{-1} of fresh weight) and the least amount of lipids, with 3.69 g·100 g^{-1} fw. We found high significant and positive correlations (r = 0.94) between calories and fat, and high significant and negative correlations (r = -093) between protein and ash. Cluster analysis grouped cultivars according to moisture, carbohydrate, protein, lipid, and ash content. It was determined that storage conditions (temperature and moisture) are fundamental for the nutritional quality of the seeds.

Key words: germination, nutritional composition, seeds, storage conditions.

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is a dicotyledonous annual plant native to the Andean highland region of northwestern South America (Hinojosa et al. 2021), being Bolivia, Peru, Chile, and Colombia the largest producers. In the latter, it is cultivated in the departments of Boyacá, Cauca, Cundinamarca and Nariño (Morillo et al. 2022). Quinoa has been considered a pseudocereal, but it does not belong to the Gramineae family. However, it has a starch content similar to other cereals (Villacrés et al. 2022). This species has an excellent nutritional composition that includes proteins of high biological value (13–16%) with an amino acid profile close to that recommended by the Food and Agriculture Organization (Pedrali et al. 2023).

Quinoa is rich in functional ingredients, and different studies have shown that this possesses a great quantity and diversity of bioactive compounds, such as proteins, polysaccharides, saponins, and flavonoids (Pereira et al. 2020, Ren et al. 2023). In addition, it has micronutrients such as calcium, copper, manganese, zinc, iron, vitamins (thiamine, riboflavin, folic acid, niacin, or retinol), among others (Villacrés et al. 2022). Its seeds do not contain gluten, which is why it can be consumed by people with celiac disease (Villacrés et al. 2022). Due to the quality of fatty acids, it is considered an alternative oilseed crop (Pereira et al. 2020). It presents bioactive components such as flavonoids, phytosterols, carotenoids and polyphenols, with beneficial effects for health (Granado-Rodríguez et al. 2021). Given its high genetic variability, adaptation to different agroecological conditions, and its nutritional, pharmaceutical, and industrial potential, it has attracted worldwide attention, expanding its planting area to countries such as Europe, North America, Canada, North Africa, and China (Pellegrini et al. 2018).

On the other hand, the quality of each seed depends on the genetic, physical, physiological, and sanitary characteristics of the seed (Santos et al. 2007). Additionally, environmental conditions, such as temperature and relative moisture, affect the germination rate. The porosity of quinoa seeds causes a loss of viability more quickly than cereals (Spehar and Santos 2007). The poor germination rate affects the quality and vitality of the quinoa seed, which is reflected in the germination percentage (Namrata and Haripriya 2022). Germination and seedling emergence are affected not only by seed properties, but also by various external factors, such as temperature, salinity, pH, soil moisture, burial depth, and agronomic management practices (Le et al. 2021). Currently, the germination process is being evaluated for the generation of nutritionally improved grains and seeds.

Germination occurs in four fundamental stages: imbibition of water; synthesis and activation of enzyme systems; degradation of reserve substances; and elongation of embryo cells and emergence of the radicle (Guardianelli et al. 2022). Germination is an important method to increase the nutritional and functional value of seeds. During germination, several enzymes are activated, improving protein digestibility and mineral bioavailability (D'Ambrosio et al. 2017), since the nutritional composition of quinoa is altered by activation of complex processes involved in protein synthesis and storage (Hao et al. 2022).

The effect of germination has been studied in cereals and pseudocereals (Nkhata et al. 2018). Pilco-Quesada et al. (2020) demonstrated the effects of sprouting and baking on the nutritional components of quinoa and its metabolites. Results similar to those reported by Xu et al. (2009), who found that during germination a large number of biochemical processes produce changes in the composition of primary and secondary metabolites, thus impacting the phenolic profile and antioxidant activity. Therefore, germination seems to have the property of improving the nutritional and phytonutrients profile of some seeds (Namrata and Haripriya 2022).

A comprehensive knowledge of seed germination traits is essential to develop strategies for successful management and/or suppression of crops germination. Seed germination is an important process that leads plants to adapt to their natural or agricultural environment. This process has attracted attention not only for showing the transition of the life cycle, but also for its direct impact on the production and quality of the crops (Finch-Savage 2020). However, in Colombia, and especially in the department of Boyacá, there is a deficit of scientific literature about the physiological and nutritional quality of quinoa seeds (Morillo et al. 2022), which is important to ensure high standards of quality and yield, and to take advantage of the productive potential of quinoa in the region. Thus, the current study aimed to evaluate the quality, germination, storage conditions and nutritional composition of the seeds of four quinoa cultivars from the Department of Boyacá, Colombia.

MATERIALS AND METHODS

The study was carried out at the Laboratory of Plant Cellular and Molecular Biology of the Pedagogical and Technological University of Colombia. The color of the pericarp, perigonium and border was evaluated in 25 seeds of each cultivar (Amarilla de Maranganí, Quinua Semi amarga, Quinua Primavera, Quinua Real) using a Leica EZ4 stereoscope. The grain diameter (DG) and the weight of 100 seeds (P) were carried out in triplicate for each cultivar. The dry weight (PS) was found by drying 100 seeds in an oven at 80°C for 8 hours. The moisture content was established with the fresh and dry weight of 100 seeds. The % moisture (H) was calculated using Eq. 1.

$$\% \text{ Moisture} = \frac{m^1 - m^2}{m^2} \times 100 \quad (1)$$

where: m^1: fresh weight of 100 seeds (g); m^2: dry weight of 100 seeds.

Ten g of seeds with two replicates per cultivar were weighed to determine the imbibition rate.

The germination capacity of the seeds was carried out by selecting 25 seeds per cultivar, with three repetitions for a total of 75 seeds, which were disinfected with 15% hypochlorite and distilled water. The seeds were placed in Petri dishes, with filter paper moistened with distilled water. The germinated seeds were recorded at 12, 24, 48 and 72 h, considering as germinated those seeds from which the radicle of about 2 mm length. The germination percentage (PG) was determined by the Eq. 2:

$$G = \frac{N° \ seeds \ germinated}{Total \ seeds \ sown} \tag{2}$$

For the storage tests, the seeds were kept 15 days prior to the germination tests at temperatures of 4 and -20°C. After this time, the germination capacity of the cultivars was determined by selecting 25 seeds per cultivar, with three repetitions for a total of 75 seeds. All seeds evaluated in this study were initially stored with 8% humidity. All the evaluated cultivars were previously characterized, and their initial quality was measured, to guarantee each of the tests carried out in this study.

Nutritional analysis

The proximal analyses of the seeds of the four cultivars were carried out after conditioning the sample. For the ash content, the AOAC 923.03 (1990) method was carried out, in which a complete incineration of the organic matter of the sample was carried out in a muffle furnace at 525°C, leaving only the residue of inorganic matter. The total fat content was estimated using the AOAC 920.39 (1990) method, using a Tecator Soxhlet-type extractor. The determination of the crude protein content (AOAC 2001.11, 1990) was done by Kjeldahl distillation, of carbohydrates and calories through the procedures of INVIMA technical regulation 810 of 2021.

Information analysis

The data was analyzed in Microsoft Excel 2010, Infostat (Version 2017), and R version 1.0.136. A descriptive analysis of the quantitative variables and a frequency analysis for the qualitative ones were performed. Normality was tested by the Shapiro–Wilk's test for the variables weight of the 100 seeds, moisture, germination percentages, and seed diameter. Subsequently, an analysis of variance (ANOVA) was applied to establish the existence or not of significant differences between the cultivars. Additionally, a Tukey's multiple comparison test ($p \leq 0.05$) was performed. A principal component analysis was performed for the proximal analyses of the cultivars, as well as a cluster analysis with the Infostat program (Version 2017).

RESULTS AND DISCUSSION

Characterization of quinoa seeds

In the nutritional and quality evaluation of the four quinoa cultivars, it could be observed that the cylindrical grain shape was common among the cultivars, while the color of the pericarp, the perigonium, and the edge of the seed presented variation. Quinoa Real and Quinoa Semi amarga were characterized by presenting beige pericarp color, light yellow perigonium color, and intermediate seed edge. Maranganí yellow presented orange pericarp color, with a dark yellow perigonium, and wavy seed edge (Table 1). In the Colombian market, quinoas with beige pericarp color are the most desired for consumption, this being an important parameter for the acceptance of the food product by consumers (Morillo et al. 2022).

The characterization of quinoa seeds is essential for the processes of conservation and commercial management of the grain. Since the quality of the seeds can decrease over time, and factors such as the way of storage can affect qualities such as viability and germination, it can also influence the weight, moisture, size, shape, among others (Gómez et al. 2022). The quinoa grain is covered by layers, and the perigonium is the one that covers the seed and comes off easily when rubbed, while the pericarp is attached to the seed (Meyhuay 2013). The colors of both perigonium and pericarp are essential for the commercialization of quinoa grain.

Table 1. Color of the pericarp, perigonium, and edge of the seed of the four quinoa cultivars evaluated.

Cultivar	Pericarp color	Perigonium color	Seed edge
Quinua Real	Beige	Light yellow	Intermediate
Amarilla de Maranganí	Orange	Dark yellow	Wavy
Quinua Semi amarga	Beige	Light yellow	Intermediate
Quinua Primavera	Beige	Ochre	Smooth

On the other hand, in parameters such as the weight of 100 seeds, Amarilla de Maranganí was outstanding, with weights greater than 0.40 g, while Quinoa Semi amarga and Quinua Primavera did not exceed 0.35 g. The seed diameter was Amarilla de Maranganí (2.67 mm), while the cultivar with the smallest diameter was presented by Quinoa Real with values lower than 2.25 mm. Regarding moisture, Quinua Semi amarga presented significant differences with respect to the other three cultivars with a percentage of moisture of 11.6% (Fig. 1). According to Meyhuay (2013), the seeds should not contain values higher than 14% moisture to be stored, among other things, because too wet grains can be affected by fungi. Therefore, the moisture of the grains must be controlled since this factor is important for the handling, processing, and storage of quinoa (Guardianelli et al. 2022). Characteristics such as the size, as well as the percentage of moisture of the seeds, are factors that directly affect the viability of the seeds over time. They are also indicators of the appropriate moment for the renewal of the seed in the germplasm banks (Morillo et al. 2022).

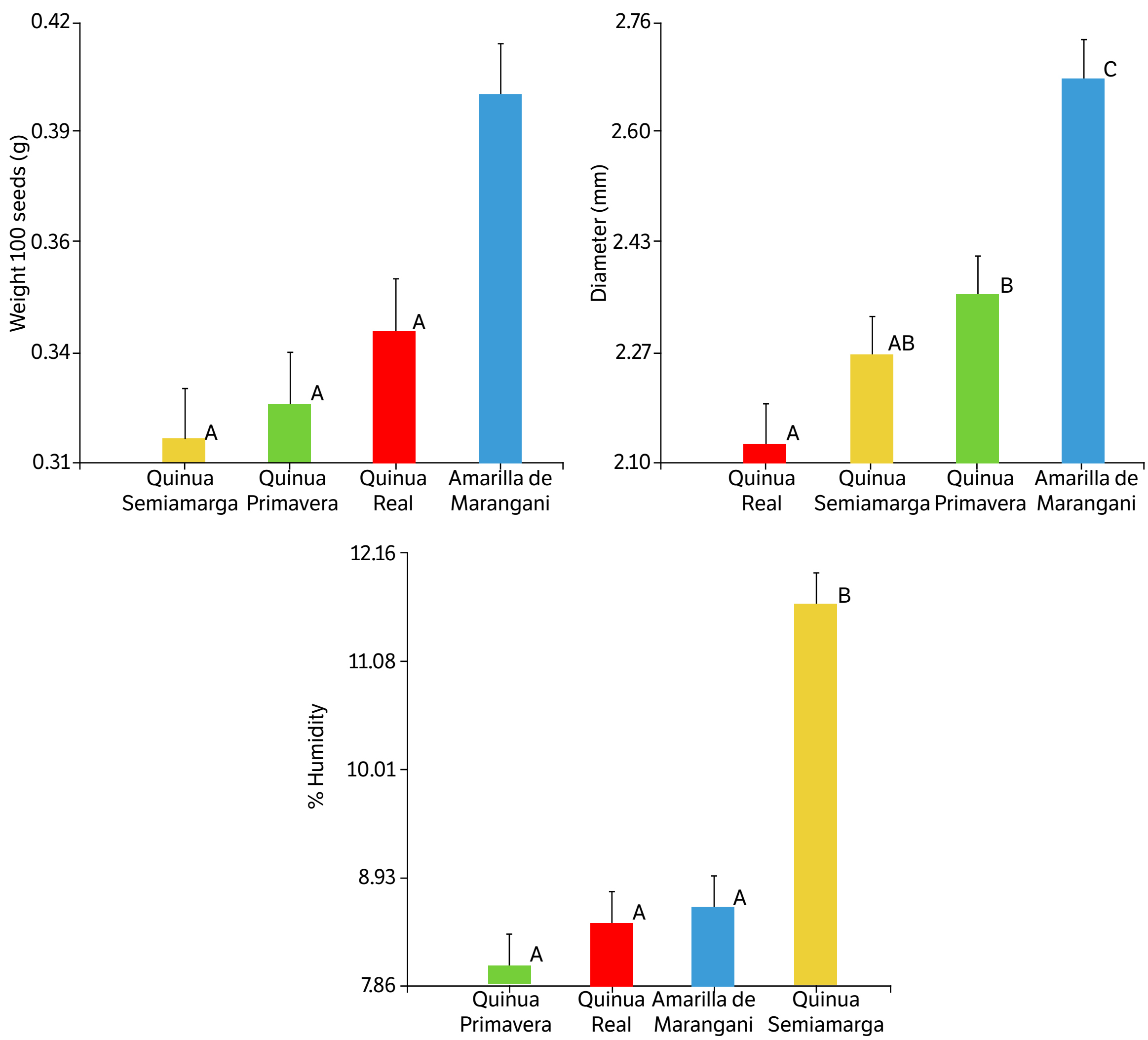

Figure 1. Means and Tukey's test of the weight of 100 seeds, diameter and % moisture of the seeds evaluated in four quinoa cultivars: Quinoa Real, Amarilla de Maranganí, Quinoa Semimarga, and Quinoa Primavera.

The analysis of variance showed statistically significant differences between the four evaluated cultivars, for the weight of the 100 seeds, diameter, and moisture (Table 2). Amarilla de Maranganí was the one that presented the largest size of the seeds, with the highest values for weight of 100 seeds and grain diameter, while the Quinua Semi amarga cultivar had the highest moisture content.

Table 2. Analysis of variance of grain diameter, weight of 100 seeds, moisture percentage evaluated in the four quinoa cultivars for type I error.

Source of variation	Mean squares			
	Degrees of freedom	Grain diameter	Weight of 100 seeds	Humidity (%)
Cultivar	3	0.48*	0.01*	24.43*
Error	8	0.08	4.2E-03	2.32
Total	11			

*Statistically significant value ($p < 0.05$).

Germination capacity of quinoa cultivars

Figure 2 shows the imbibition curves of the seeds of the four cultivars, and a similar behavior was observed between them, since at 24 h the highest absorption capacity of the seeds was presented. Amarilla de Maranganí had a greater absorption of water with approximately 25 g of absorption, while the other cultivars absorbed less than 22 g of water. This may be due to the high protein content that quinoa has, since the seeds with a protein endosperm have a higher degree of hydration than those with a higher starch content (El-Hakim et al. 2022). In quinoa seeds, the embryo can represent 60% of the weight of the seed. This high percentage explains the high protein content of the quinoa grain compared to other cereals (Pathan and Siddiqui 2022). Quinua Semi amarga y Primavera are the cultivars that presented a higher rate of water absorption after 12 h of imbibition, probably due to their high protein content.

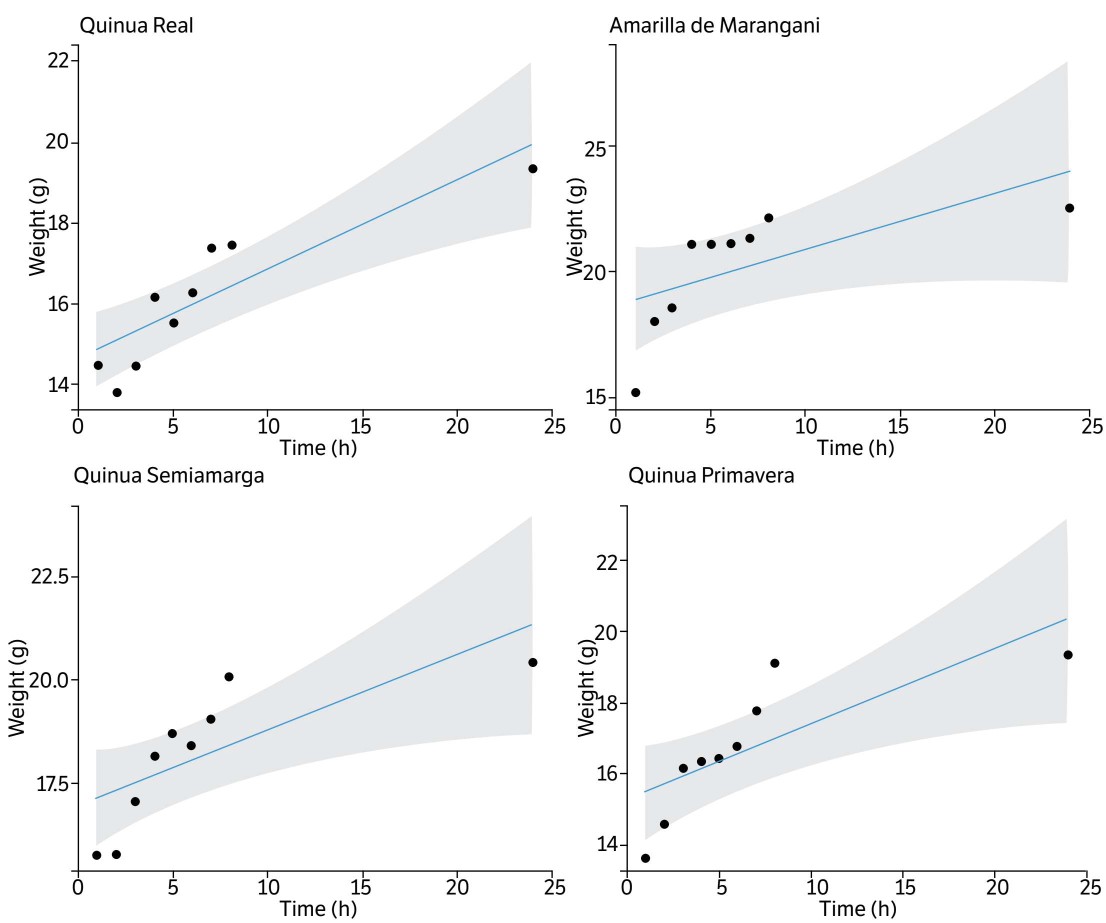

Figure 2. Imbibition curves of the seeds of the four quinoa cultivars, using water as a hydration medium.

It should be noted that imbibition is the first stage for seed germination. This water absorption is given by the water potential between the seed and the imbibition solution (Namrata and Haripriya 2022). Thus, the reserve tissue of the seed absorbs water until its hydration is complete. After the hydration of the reserve tissue, the enzymatic activation occurs and initiates the metabolic reactions for the development of the seedling (Mansouri et al. 2022).

The germination of quinoa seeds begins a few hours after having moisture, first elongating the radicle that continues to grow and gives rise to a tap root (Gómez et al. 2022). In the four cultivars, the germination capacity showed significant differences between cultivars at 12 h of germination; the Amarilla de Maranganí cultivar was superior, with 100% of the germinated seeds, while Quinua Real and Quinoa Semi amarga germinated 90% at 12 h. However, after 24 h, all cultivars germinated 100% (Figs. 3a and 3b). These results were consistent with those reported by Mäkinen et al. (2014), who observed that quinoa seeds completed their germination at 6 and 7 h.

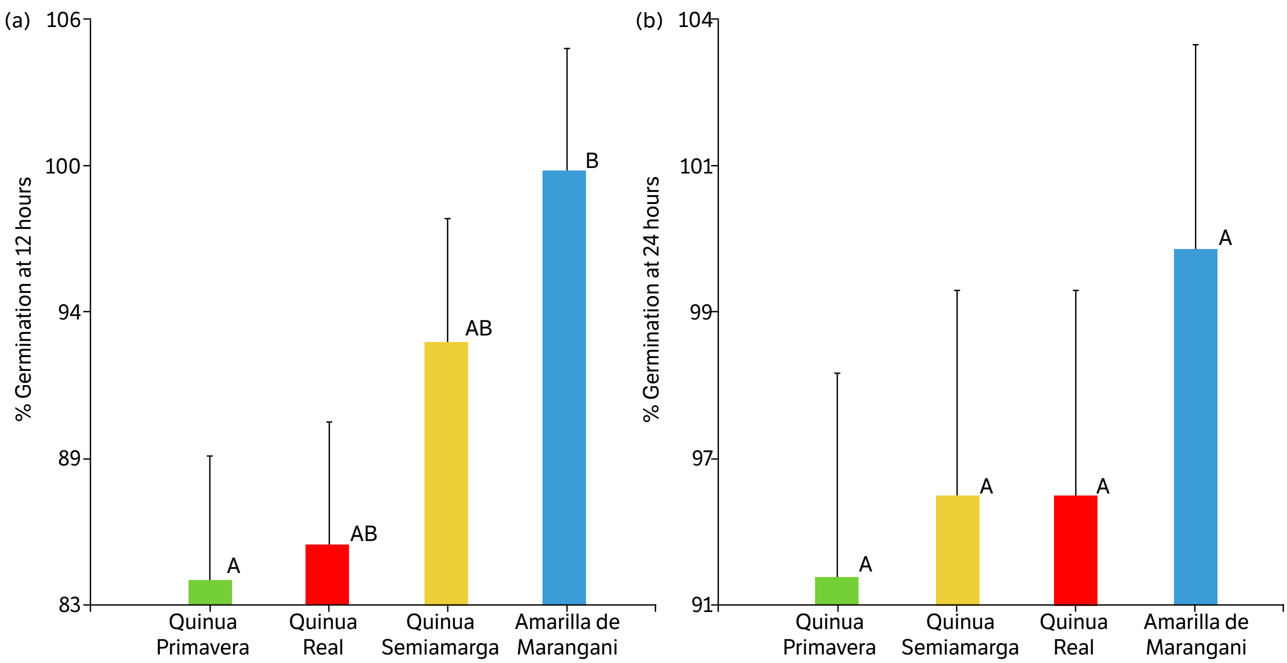

Figure 3. *In-vitro* germination of the four quinoa cultivars under different storage conditions. (a) Germination at 12 hours in the four quinoa cultivars. (b) Germination of the four quinoa cultivars at 24 hours.

On the other hand, studies on the physical dormancy of quinoa seeds showed that the thickness and color of the seed coat affect its dormancy as it is associated with the abscisic acid signaling mechanisms (Hao et al. 2022). It should be noted that Amarilla de Maranganí is the cultivar that presents an orange coloration of the seed coat and that this characteristic, as in cereals such as barley, can alter the connection between grain color and the regulation of dormancy (El-Hakim et al. 2022). Studies on quinoa's close relatives, such as *Chenopodium album*, *Chenopodium berlandieri* and *Chenopodium bonus-henricus*, reported that darker seed coat color was associated with stronger dormancy (Namrata and Haripriya 2022).

Germination is a process that is influenced by several factors. The rapid germination of quinoa is a characteristic of seeds exposed to high levels of stress, because this trait would help the survival of the species (Manjarres et al. 2020). Studies on the dormancy of quinoa seeds suggest that its varieties present a combination between primary and physiological dormancy, or do not have dormancy (McGinty et al. 2021). However, it is necessary to continue evaluating the dormancy of the seeds between the different varieties associating it with the agronomic characteristics and thus achieve the selection of resistant genotypes in this species (Morillo et al. 2022). The absence of dormancy or the loss of primary dormancy increase the probability of seed germination on the same plant, if there is a rainy season before harvest, which is very common in the tropics. Therefore, it is necessary to continue evaluating the molecular mechanisms that explain these alterations in seed dormancy (Hao et al. 2022).

When evaluating the effect of temperature on the germination capacity of the four cultivars, it was observed that there is no significant effect at temperatures of 4 and -20°C on the germination percentage of the four cultivars (Fig. 4). The influence of temperatures may be associated with the concept of slight physiological dormancy that would have been released by the post-ripening of the seed (Namrata and Haripriya 2022). McGinty et al. (2021), evaluating the germination capacity and dormancy of the seeds of two quinoa varieties in different photoperiods and temperatures, showed that high temperatures and long photoperiods increase seed dormancy. Additionally, they concluded that modern quinoa varieties they have adapted to germinate in cooler temperatures and environmental conditions affect seed dormancy.

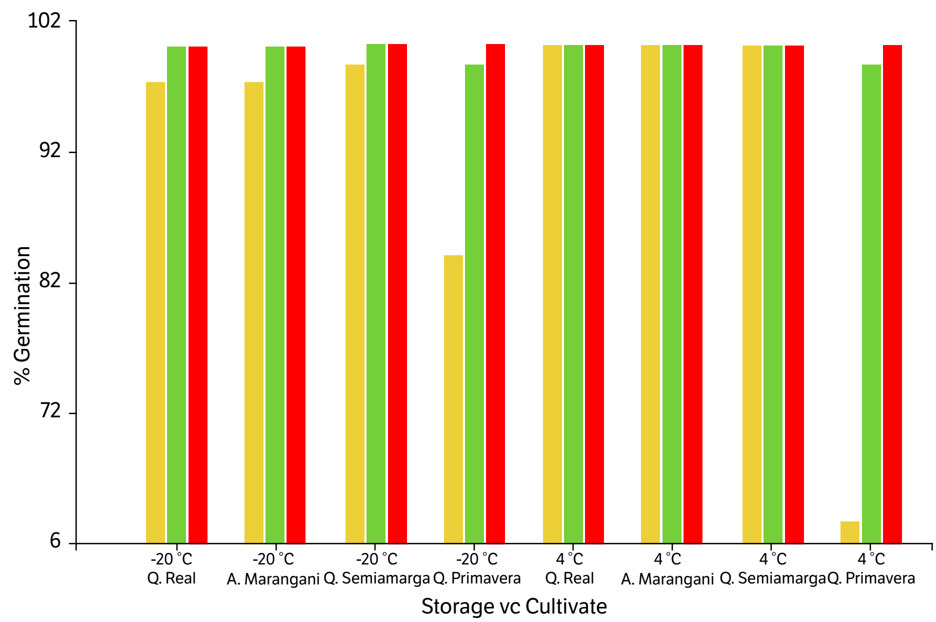

Figure 4. In-vitro germination of the three replicates for four quinoa cultivars stored at 4 and -20°C.

Proximal analyses

It is well known that quinoa seeds have a high protein content among other nutritional compounds. The proximal analyses of this study showed that Quinoa Real presented the highest protein content (12.25%), followed by Quinua Primavera (12.10%). This agrees with the behavior in the speed of imbibition of these two cultivars (Table 3). Although these protein values are lower than those obtained by Granado-Rodríguez (2021), when evaluating the Regalona, Puno, Titicaca and Vikinga quinoa cultivars, the authors found a protein range between 14.1 to 18.6%, being higher than the contents reported in cereals such as barley (11%), wheat (10%), or rice (8%) (Pathan and Siddiqui 2022). Studies have reported that the protein content of quinoa seeds depends on environmental conditions and genotype (Granado-Rodríguez et al. 2021). Regardless of the protein content in quinoa, its quality stands out, since it has been reported that most genotypes present the nine essential amino acids (Craine and Murphy 2020). However, studies have shown that factors such as genotype, environment, management, and their interactions influence the quality and quantity of protein in quinoa cultivars (Tang et al. 2022), but more research is still needed to elucidate the effect on the protein composition of quinoa.

Table 3. Proximate analysis of the four cultivars of quinoa that were tested.

Cultivar	Ash	Lipids	Proteins	Carbohydrates	Calories
Quinoa Real	2.45	5.57	12.25	66.96	366.97
Quinoa Semi amarga	3.08	6.09	11.34	66.61	366.61
Amarilla Marangani	3.77	3.69	11.17	67.72	348.77
Quinoa Primavera	2.25	4.26	12.10	68.05	358.94

The analysis of the ash, which represents the total content of minerals in the food, showed that Amarilla de Maranganí was the cultivar with the highest ash content (3.77/100 g of sample) (Table 3), which agrees with the results obtained by Craine and Murphy (2020), who obtained mean values of 3.11/100 g of sample. Likewise, the values obtained are comparable with those reported by Pathan and Siddiqui (2022) (3.4%).

Regarding calories, Quinoa Real presented the highest number of calories, with 366.97 g·100 g^{-1} fresh weight, but less carbohydrates (66.96 g·100 g^{-1} fresh weight) (Table 3). The amount of carbohydrates in quinoa has shown to be a variable characteristic depending on the cultivar and the planting season. Studies carried out by Stoleru et al. (2022) in quinoa leaves reported that the Puno cultivar presented 5.10 g·100 g^{-1} fw of carbohydrates, followed by Titicaca, with 4.27 g·100 g^{-1} fw, and Vikinga, with 2.90 g·100 g^{-1} fw. Likewise, it has been observed that the carbohydrate content in quinoa leaves is lower compared to other vegetables or with the content of the seeds (Maldonado-Alvarado et al. 2023). In quinoa seeds, the main carbohydrates are starch, that is between 58 and 68%, and 5% sugars. It also presents high amounts of soluble fiber, that is reported at 12% of dry weight in quinoa seeds (Gómez et al. 2022).

On the other hand, quinoa has a low lipid level and does not have cholesterol. Polyunsaturated fatty acids have been reported, such as linoleic acid (omega 6), present in a 50.2% proportion, oleic acid (omega 9), with 26%, followed by palmitic acids, 9.5%, and linolenic acids (omega 3), 4.8% (Gómez et al. 2022). In this study, the cultivar that presented the lowest amount of lipids, with 3.69 g·100 g^{-1} pf (Table 3), and also that agrees with what was obtained by Vera et al. (2019), was Amarilla de Maranganí, which also presented the lowest number of calories (348.77 ·100 g^{-1} pf) and the highest ash content (3.77/100 g of sample). Studies such as that one by Namrata and Haripriya (2022) showed that quinoa germination can improve the nutritional value of the seeds, since germination has been reported to affect the fatty acid profile of the seeds. Vera et al. (2019) showed that Amarilla de Maranganí has a better relationship between omega 6, three fatty acids and tocopherol 48 h after germination, which showed that germination increased the concentration of lipids in this cultivar. However, it is necessary to study the effect of storage on fatty acid profiles to optimize the management of quinoa accessions in germplasm banks.

In the Pearson's correlation analysis between the nutritional parameters of the four cultivars, a significant and positive high correlation was observed between calories and fats, with r = 0.94, while parameters such as proteins and ashes presented significant but high negative correlations, with r = -0.93. This same behavior was observed between carbohydrates and fats, with r = -0.92 (Fig. 5a). The main components agreed with the Pearson's correlation analysis; it was evidenced that 98.2% of the total variance is explained in the first two components, PC1 (61.6%) and PC2 (36.6%). Amarilla de Maranganí was characterized by its high moisture and ash content, while the Quinoa Primavera was characterized by its high carbohydrate and protein content, as well as the Quinoa Real, which also has the highest amount of calories. Quinua Semi amarga showed intermediate amounts of fat and ash (Fig. 5b).

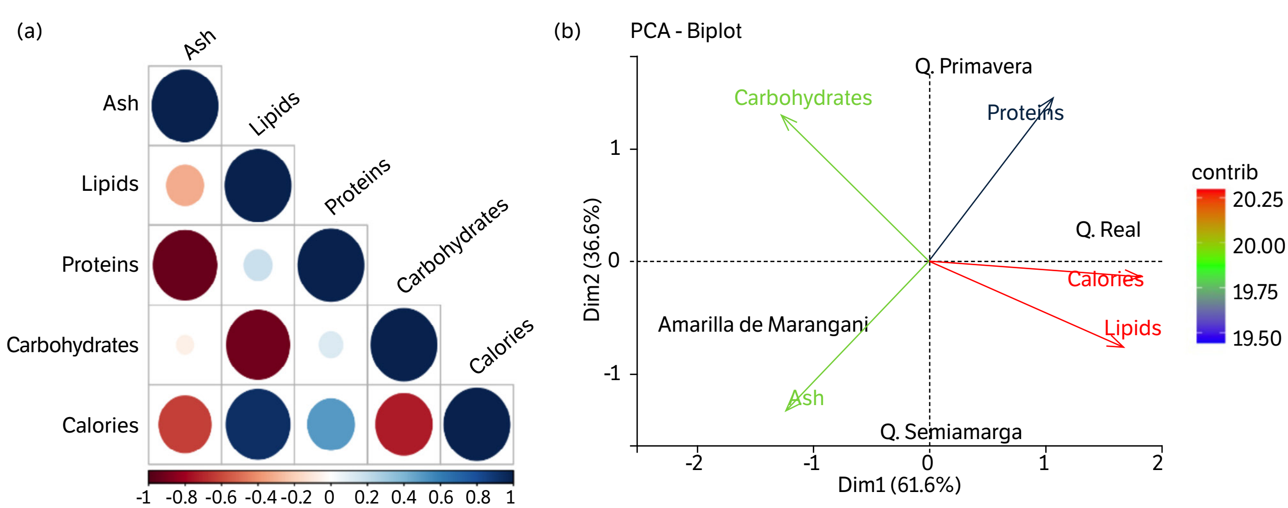

Figure 5. Correlation and principal component analysis that shows the distribution of genotypes according to their nutritional quality. (a) Pearson's correlation analysis between the nutritional components of the four quinoa cultivars. (b) Principal component analysis of the four quinoa cultivars considering their nutritional characteristics.

The cluster analysis grouped Quinua Semi-amarga with Quinoa Primavera by an average moisture content of 12.82%, carbohydrates (66.78 g·100 g^{-1} fresh weight) and calories (366.79 g·100 g^{-1} fresh weight) (Fig. 6), while Amarilla de Maranganí was characterized by its low calorie, protein and lipid content and high ash content. However, compared to other cereals, quinoa stands out due to its nutritional content of high biological value, also because it presents a great versatility of alternative uses for human consumption, since there are derivatives such as flakes, breads, cookies, energy bars, flour, noodles, granolas, drinks, among others (Gómez et al. 2022).

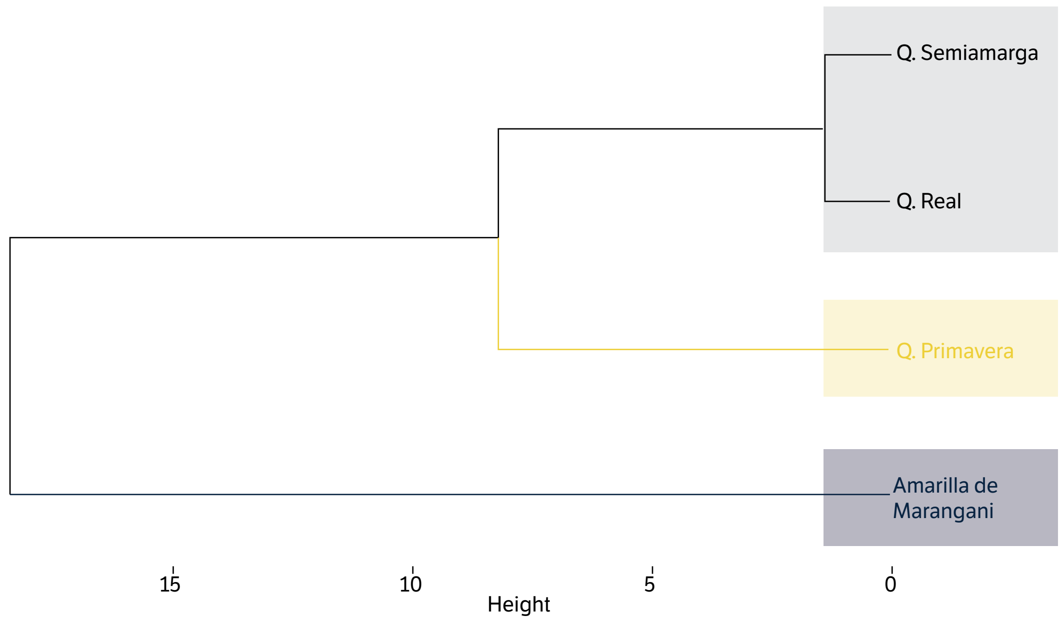

Figure 6. Cluster analysis of the four quinoa cultivars considering their nutritional characteristics.

CONCLUSION

The cultivars evaluated in this study, Amarilla de Maranganí, Quinua Semi amarga, Quinua Primavera, and Quinua Real, presented high variability in terms of quality and nutritional composition of the seeds. It was observed that environmental conditions play an important role on seed quality parameters such as germination. In addition, it is of great importance to control seed storage conditions such as temperature and moisture to avoid physiological deterioration of quinoa seeds. The storage conditions of the seeds allow them to maintain their physical, physiological, and phytosanitary quality and, thus, ensure good germination.

CONFLICT OF INTEREST

Nothing to declare.

AUTHORS' CONTRIBUTION

Conceptualization: Morillo-Coronado, A. and Manjarres-Hernández, E. H.; **Methodology:** Morillo-Coronado, A., Manjarres-Hernández, E. H. and Pedreros-Benavides, M. C.; **Investigation:** Morillo-Coronado, A., Manjarres-Hernández, E. H. and Pedreros-Benavides, M. C.; **Writing–Original Draft:** Morillo-Coronado, A. and Manjarres-Hernández, E. H.;

Writing–Review and Editing: Morillo-Coronado, A. and Manjarres-Hernández, E. H.; **Funding Acquisition:** Morillo-Coronado, A.; **Supervision:** Morillo-Coronado, A.; **Final aprovall:** Morillo-Coronado.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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