Seed quality of 22 quinoa materials (*Chenopodium quinoa* Willd.) from the department of Boyacá[1]

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ABSTRACT

Chenopodium quinoa Willd. is a pseudocereal with seeds that are a rich source of vitamins and minerals. However, there are few studies on quinoa seed quality, especially for the Colombian germplasm. So, the objective of this research was to determine the quality of 22 quinoa materials from the Department of Boyacá by evaluating the physical (color, shape and diameter) and physiological (tetrazolium test) quality of the seeds. It was found that 36% of the materials had a white grain color, 80% cylindrical shape, 65% smooth edges and diameters smaller than 2mm, desirable characteristics for post-harvest processes. The evaluated physical characteristics presented high variability between the evaluated materials, which is desirable for elite breeding processes. The imbibition rate showed that germination was rapid (at 4 hours, the weight of the seeds doubled), that is, the materials were not dormant. Finally, it was determined that storage conditions, such as temperature and relative humidity, are essential for preventing deterioration in quinoa seeds; these factors can also affect germination and long-term vigor.

Keywords: cereal; germination; vigor; viability.

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is an Andean ancestral crop that belongs to the Amarantaceae family and has great nutritional value and broad agroecological adaptation (Razzaghi *et al.*, 2015), with resistance to biotic and abiotic factors (Hussin *et al.*, 2017). Its seeds are rich in minerals, including phosphorus, calcium, sodium, potassium, iron, zinc, manganese and copper, fiber and vitamins, along with a high antioxidant capacity (Valencia *et al.*, 2017). Therefore, quinoa has been considered in the search for solutions to food shortages worldwide (Bazile *et al.*, 2015).

Quinoa seeds have been described as flat and spherical with an average diameter between 1.4-1.6 mm (Abugoch, 2009), where the endosperm appears as a cap that surrounds the radicle. Starch is found exclusively in the perisperm, while proteins and lipids are in the embryo (Prego *et al.*, 1998). The same structure can be observed in the sugar beet (Abbasi *et al.*, 2018) and amaranth seeds (Ye and Wen, 2017). The high protein content of quinoa is due to the fact that 60% of the seed weight corresponds to the embryo that has a hypocotyl-radicle axis and two cotyledons. In the micropillary region of the seed is present the endosperm with one or two layers of tissue that surround the tip of the radicle (Gomaa *et al.*, 2014). Once the saponins are removed from the pericarp of the seeds, quinoa can be used for different products, such as flour production (Abderrahim *et al.*, 2015). Quinoa is suitable for people on a gluten-free diet, which is beneficial for human health (Vilcacundo *et al.*, 2017).

Due to its composition of starches and lipids, quinoa seed has greater storage stability than other oilseeds (Filho, 2015). Because its seeds have pores in the integument, which facilitate the gain or loss of moisture, they lose viability faster (Spehar, 2007). The quality of the quinoa seed is influenced by the low percentage of germination and the reduction of vigor (Kappes *et al.*, 2012), affecting its longevity. The quality of the seed is determined by its

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genetic, physical, physiological and health attributes that determine its yield potential (Santos *et al.,* 2007). On the other hand, germination is influenced by intrinsic and extrinsic factors such as temperature, relative humidity, oxygen, and the action of fungi and bacteria (Filho, 2015). Seed germination is regulated by physiological factors and environmental conditions, since each species has specific germination requirements such as water availability, temperature, light, and planting depth (Filho, 2015).

In seed analysis, vigor tests have been used primarily to identify differences in seed batch yield during storage and after planting in order to highlight more effective batches for establishment under a wide range of environmental conditions. Germination tests evaluate physiological quality, but the results are not always correlated with emergence in the field, as well as germination rate indices and emergence potential as parameters of vigor; in order to determine the seed yield and the speed of development of normal seedlings under various environmental conditions (Filho, 2015).

Seed quality has a direct impact on productivity, but research on quinoa seeds is just beginning. Gomaa *et al.* (2014) conducted studies on the micro-macromorphological and anatomical characteristics of quinoa (*C. quinoa*), where he described the structural and functional aspects of the seeds. Mäkinen *et al.* (2014) studied the development of proteolytic activities and protein profiles in *C. quinoa* seeds during germination and early growth, where the endosperm has 60 times more proteolytic activity than the embryo, weakening the endosperm during germination. On the other hand, Hager *et al.* (2014) researched amylolytic activities and mobilization of starch reserves during germination in quinoa. In general, the activity levels remained very low, suggesting that these seeds are not a source of amylases.

Several studies have been carried out to determine the physical characteristics, phenolic components and antioxidant capacity of quinoa seeds because of their high nutritional value and their effect on human health (Valencia *et al.,* 2017; Vilcacundo *et al.,* 2017), and it has also been determined that, in the *Chenopodium* genus, there is embryonic latency, for which the seed covers are largely responsible, that is greatly affected by the environmental conditions of seed development (Ceccato *et al.,* 2015; Strenske *et al.,* 2017). Recent studies have evaluated the effect of different environments, such as saline conditions, and phytosanitary quality on germination, vigor, and number of normal plants, among other parameters (Al-Barakah and Sohaig, 2019; El-Assiuty *et al.,* 2019), showing that the physical, genetic, physiological and sanitary quality of seeds are a fundamental requirement for maintaining productivity and that quinoa seeds, like other seeds, need specific conditions to germinate and maintain vigor. Taking into account the expanding cultivation area for quinoa, the growing popularity of grain in domestic and foreign markets, and the lack of scientific information on seed quality, this study aimed to assess the influence of the storage conditions of the Boyacá quinoa germplasm bank on the viability and vigor of its quinoa seeds.

MATERIAL AND METHODS

The 22 materials stored in the quinoa seed collection of the Laboratory de Biotechnology Vegetal of Secretary of Agricultural Development of the Government of Boyacá from municipalities in the Department of Boyacá were evaluated: Duitama, Tibasosa, Siachoque, Tunja, Soracá, Tuta, Cerinza, Combita, Beteitiva and Sotaquirá. Three materials were collected directly from farmers in the municipalities of Tunja, Tibasosa and Siachoque (Table 1). The physical and physiological characterization of the seeds was carried out in the Plant Physiology Laboratory and the Plant Molecular Biology Laboratory of the Pedagogical and Technological University of Colombia, in Tunja, with the geographical coordinates 5°332 163 N and 73°212 093 W.

To determine the physical quality of the seeds, 20 seeds of each material were evaluated with the help of a stereoscope. The studied parameters included color, shape and diameter of the seeds. The weight was determined with 100 seeds for each material in triplicate, the moisture content was established with the fresh weight of 100 seeds and, after drying in an oven at 80 °C for 8 hours, the dry weight was measured.

$$\%Moisture = \frac{m^1 - m^2}{m^2} \times 100$$

Where: m^1 is the fresh weight of 100 seeds (g). m^2 is the dry weight of 100 seeds.

For the imbibition rate, an initial weight of 10g of seeds and two repetitions per sample were taken. Each sample was placed in distilled water, and, at intervals of one hour, the weight gain of the seeds was recorded. The readings were taken until the weight of the samples stabilized or the variation between the samples had narrow values (Moreno *et al.,* 2006).

The viability of the seeds was evaluated with a biochemical tetrazolium test, for which 100 seeds were taken from each sample and placed in the solution at a concentration of 1%. The intensity of coloration (red) was then read.

The germination tests were carried out *in vitro* with petri dishes and filter paper; for this, 25 seeds were taken from each material, with three repetitions. After 12 hours, the germinated seed data were recorded for 120 hours, with the germinated criterion being seeds that have a radicle of about 2 mm emerged.

Table 1: Passport data of the quinoa materials studied

Material code	Bank code	Place of collection	Storage time (years)	Georeferencing	Variety or common name
1	BGQ0009	Ventaquemada	7	5°22’00.4"N 73°31’16.9"W	Quinua real
2	BGQ0011	Soracá	1	5°30’06.9"N 73°20’00.5"W	Aurora
3	BGQ0012	La colorada Tunja	3	5°34’44.7"N 73°20’36.0"W	Ceniza
4	BGQ0013	Beteitiva	4	5°54’39.1"N 72°48’31.2"W	
5	BGQ0015	Sotaquira. Vereda Bociga	5	5°45’57.6"N 73°14’52.2"W	
6	BGQ0017	La colorada Tunja	1	5°34’44.7"N 73°20’36.0"W	Quinua negra
7	BGQ0018	ICA Surbata	21	5°47’45.5"N 73°04’20.2"W	Tunkahuan
8	BGQ0019	Tuta	3	5°41’26.6"N 73°13’39.1"W	Blanca de Jericó
9	BGQ0020	Pasca	9	4°18’32.8"N 74°17’59.6"W	Amarilla de Marangani
10	BGQ0022	La colorada Tunja	2	5°34’44.7"N 73°20’36.0"W	
11	BGQ0024	Soracá	4	5°30’06.9"N 73°20’00.5"W	Blanca dulce de Soracá
12	BGQ0025	Choconta	4	5°08’44.3"N 73°41’07.0"W	Piartal
13	BGQ0027	Centro de Diagnóstico Agropecuario	5	5°33’13.8"N 73°21’37.1"W	
14	BGQ0028	Tuta	3	5°41’26.6"N 73°13’39.1"W	Quinua dulce de Tuta
15	BGQ0029	Duitama	6	5°49’36.3"N 73°02’03.9"W	Quinua semiamarga
16	BGQ0032	Combita	3	5°38’01.9"N 73°19’28.4"W	Peruana
17	BGQ0034	Siachoque	5	5°30’45.3"N 73°14’44.3"W	
18	BGQ0035	Pasca	9	4°18’32.8"N 74°17’59.6"W	Posible Piartal
19	BGQ00101	Centro de Diagnóstico Agropecuario	1	5°33’13.8"N 73°21’37.1"W	Dorada
20	CQ001	Tuta	1	5°41’26.6"N 73°13’39.1"W	Blanca de Jericó
21	CQ002	Tibasosa	1	5°44’40"N 73°14’16"W	Piartal
22	CQ003	Tunja	1	5°31’4"N 73°23’48"W	Blanca de Jericó

For the germination speed, daily germination percentage readings were taken for five days to determine the average number of seeds germinated per day, using the formula proposed by Maguire (1962):

$$VelG = \sum_{i=1}^{n} \frac{xi}{n}$$

Where: *xi* = Number of germinated per day. *n* =Number of days that passed after sowing.

RESULTS AND DISCUSSION

The results showed that, for the variable grain color in the evaluated materials, the color white predominated, followed by a light brown color (Figure 1), which favors quality in this parameter. In addition, the commercialization of quinoa prefers grain that is white, large and clean, with low saponin contents. Determining seed quality is essential to conservation and commercial management, ensuring germination capacity, rapid emergence, uniformity and, thus, good crop establishment (Ceccato *et al.*, 2015). Therefore, factors, such as the physical, chemical and physiological quality of seeds, must be controlled. Within quinoa, two large groups are recognized: cultivated quinoa, which is characterized by seeds that are clear with thin and translucent testae, and wild quinoa with dark seeds and dense testae (Fuentes and Bhargava, 2011). However, although the processes of domestication of quinoa crops reduced their genetic diversity, the phenotypic diversity of cultivated quinoa at the seed level remains broad.

The descriptors proposed by International Biodiversity for quinoa and its wild relatives include seed shape: lenticular, cylindrical, ellipsoidal and conical; however, in this study, it was found that, in addition to these shapes, there are different types of seed edges, as can be seen in Figure 1, where some evaluated materials presented smooth, sinuous or wavy edges. Likewise, variation in seed color was observed, ranging from black, yellow, white and beige, 36% being white; 80% of the materials were cylindrical and 65% were smooth (Table 2).

Figure 1: Photographs of quinoa seeds of the materials present in the Department of Boyacá.

In Colombia, there are no certified or selected quinoa seed, meaning crops have high variability in color and size in seeds and plants in general (Morillo *et al.*, 2017). On the other hand, for the commercialization of quinoa, the physical characterization of the grains is vital because it is necessary to adapt the technologies associated with post-harvest, cleaning, separation and classification of the seeds, leading to acceptance in the market when used in conjunction with existing quality standards and consumer preference, such as: grains that are ≥ 2mm, clean, white and free of saponins.

This study showed that the diameters of the seeds of the evaluated materials were less than 2mm and that there was a correlation between the weight of the seeds and their diameter, which are important in postharvest processes because they aid the design of threshing machinery and the sorting and deponification of quinoa materials from the Department of Boyacá (Figure 2).

The germination of the seeds has three stages that can vary or overlap according to each species. The first stage is imbibition, a process in which seeds absorb water as a result of differences between the water potential (matrix) in the seeds and the imbibition solution, thereby hydrating the reserve tissue (Moreno *et al.*, 2006). In the second stage, water absorption is decreased, initiating the metabolic reactions necessary for seedling growth. In the last stage, there is growth that is associated with the emergence of the radicle, along with an increase in metabolic activity and new water absorption activity.

Quinoa seeds do not present germination problems when they are properly stored: 4 °C and humidity percentages lower than 10%. In this study, the imbibition curves showed that the germination rates were fast because, at 4 hours, the weight of the seeds doubled, promoting the metabolic processes for seedling growth (Figure 3). In studies conducted by Parsons (2012), it was also observed that most of the seeds formed a radicle 6-10 hours after imbibition. Very fast germination (< 24 h) is a characteristic found in some seeds in high stress habitats, most of which belong to the Chenopodiaceae family, which can be an important survival characteristic (Parsons, 2012).

In grains, such as quinoa, moisture percentage is essential to prolonging shelf-life for conservation or commercialization. Grains that are subjected to drying can achieve a humidity of approximately 12%. In this case, the materials conserved in the germplasm bank have between 8 and 13% moisture, while some commercialized grains have between 10 and 16% moisture. Studies have shown that quinoa seeds dried to 5-10% moisture remain viable (Ceccato *et al.*, 2015). Because the studied materials were in storage for conservation purposes, the moisture percentages were between 8 and 13%, which are adequate because quinoa grains are hygroscopic, making them susceptible to increased humidity. Therefore, it is vital to control humidity during storage (Figure 4).

Table 2: Shape, color and border of the seeds of quinoa materials

Material	Shape	Color	Border
1	Lenticular	Cream	Smooth
2	Cylindrical	White	Sinuate
3	Cylindrical	Light-dark brown blend	Smooth
4	Cylindrical	Cream	Smooth
5	Cylindrical	Light brown	Smooth
6	Ellipsoidal	Black	Smooth
7	Cylindrical	White	Smooth
8	Cylindrical	Cream	Smooth
9	Ellipsoidal	Orange	Sinuate
10	Cylindrical	White	Smooth
11	Cylindrical	White	Sinuate
12	Cylindrical	White	Smooth
13	Cylindrical	Light brown	Sinuate
14	Cylindrical	Cream	Smooth
15	Lenticular	Cream	Smooth
16	Ellipsoidal	Orange-Yellow	Sinuate
17	Cylindrical	Cream	Smooth
18	Cylindrical	Cream	Smooth
19	Cylindrical	Yellow	Sinuate
20	Cylindrical	White	Smooth
21	Cylindrical	White	Sinuate
22	Cylindrical	White	Sinuate

The quinoa seeds preserved at 4 °C and 10 to 12% relative humidity presented germination percentages of 90 to 100% from 24 to 96 hours; however, the materials that were not kept at low temperatures, as farmers do, lost germination capacity, as seen in materials 6, 13, 18 and 20 (Table 3). Studies conducted by Souza *et al.* (2017) found that more than 80% of the evaluated quinoa seeds germinated at 20 to 30 °C with a 7-hour photoperiod and only 60% of the water retention capacity. Other factors, such as saline environments, also affect germination; however, they can contribute to increased concentration of antioxidants in seeds (Al-Barakah and Sohaib, 2019). Identifying the morphological and physiological processes that control dormancy in quinoa seeds and how they are affected by the environment is of great importance for the genetic improvement of this species. In this sense, Ceccato *et al.* (2015) suggested that embryonic latency is present in the Chenopodium genus and that the seed cover is largely responsible for this process. In addition, high temperatures and long photoperiods increase the cover and decrease the embryonic latency.

Germination is a process that is influenced by internal and external factors. The internal factors include viability, which can be measured with the biochemical tetrazolium test, which consists of the differentiation of living tissues. In this case, materials 6, 13, 18 and 19, which did not germinate, were tested, evidencing that the storage and conservation conditions directly generated damage on the

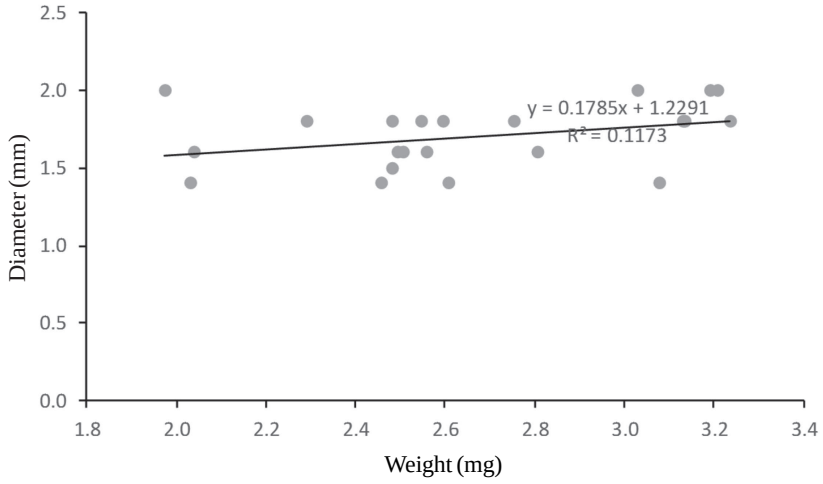

Figure 2: Relationship between the diameter (mm) and the weight of the 1000 seeds of quinoa.

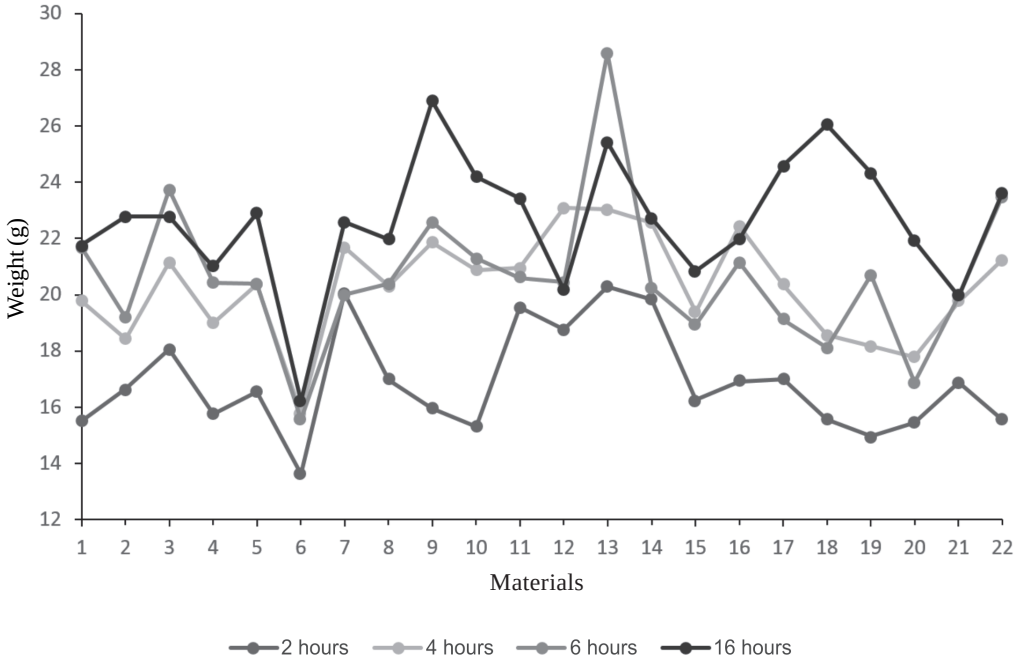

Figure 3: Relationship between the weight and time evaluated in the 22 quinoa materials studied.

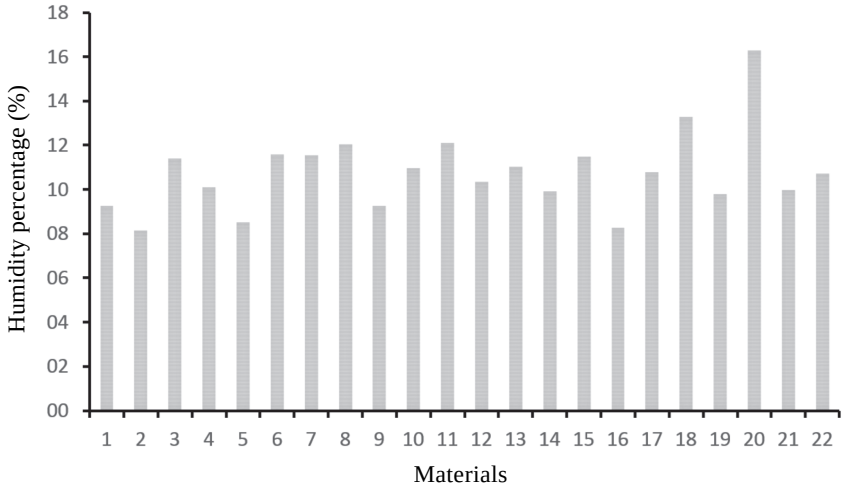

Figure 4: Moisture percentage in quinoa materials evaluated.

viability of the embryos for initiating germination processes (Figure 5).

Strenske *et al*. (2017) evaluated the germination and vigor of quinoa seeds wrapped in paper bags and stored at temperatures of 20, 30 and a constant temperature of 25 °C; the seeds were subjected to seven-hour photoperiods and 25 °C under continuous darkness for 36, 85, 119, 146, 177 and 270 days. The increase in storage time decreased the percentage of germinated seeds and their vigor as a result of the greater number of abnormal seedlings. During the 430 day study period, the germination of the quinoa seeds under the experiment conditions decreased. Therefore, the final number of germinated seeds should be evaluated seven days after the start of the germination test. Similar results were obtained by Nobre *et al*. (2013) who observed that amaranth seeds showed maximum germination and vigor to the point of physiological maturity and then began to decrease.

Storage temperature and relative humidity are the principal factors that affect the physiological quality of seeds. Strenske *et al.*, (2017) observed that the germination of the stored quinoa seeds was maintained under controlled environmental conditions, since if the temperature and humidity were not controlled in storage, the germination of the seeds decreases.

Filho (2015) stated that seeds deteriorate when they are exposed to long storage periods under uncontrolled conditions. However, these mechanisms have not been fully elucidated, so the deterioration has been attributed to the genetic factors of the seed. Furthermore, temperature control such as relative humidity under storage conditions can minimize seed deterioration. At the biological level, deterioration is related to loss of membrane integrity, decreased selective capacity, lipid oxidation, solute leaching, changes in enzyme, transpiration, and protein synthesis in seeds (Filho, 2015).

Finally, it was observed that, during long storage periods under ambient conditions, the quality of quinoa seeds deteriorates and that environmental factors such as temperature and relative humidity must be controlled in order to prevent the germination and vigor of the seeds from rapidly decreasing.

CONCLUSIONS

The evaluated quinoa materials displayed phenotypic variability for the color of the seeds and of the grains. It

Table 3: Germination percentage at 24, 28, 72 and 96 hours in the 22 quinoa materials evaluated

Materials	% G24	%G48	%G72	%G96
1	73	92	97	100
2	95	99	100	
3	93	96	100	
4	36	76	92	96
5	64	75	84	85
6	0	0	0	5
7	83	95	96	97
8	76	91	95	96
9	89	92	99	100
10	8	40	69	81
11	76	96	96	97
12	91	96	100	
13	0	0	0	0
14	63	73	80	88
15	35	65	84	91
16	99	100		
17	77	93	99	100
18	0	0	0	0
19	0	0	0	0
20	100			
21	100			
22	76	91	95	97

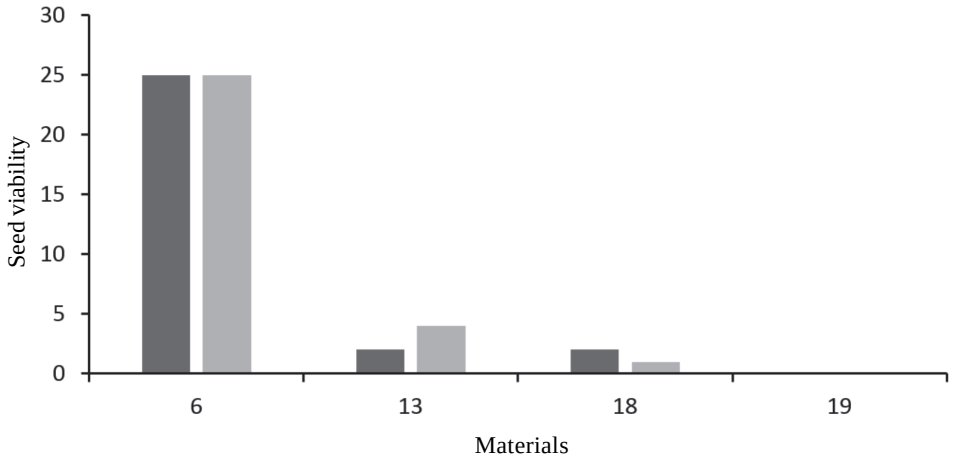

Figure 5: Viability of the seeds of materials 6, 13, 18 and 19.

was determined that environmental conditions play a very important role in seed quality parameters, such as germination and vigor, and that storage temperature and humidity can delay or decrease the physiological deterioration of quinoa seeds.

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CONFLICT OF INTEREST

The authors declare no financial or other competing conflicts of interest.

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