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Physiological quality of quinoa seeds submitted to different storage conditions

Flívia Fernandes de Jesus Souza[1]*, Ivano Alessandro Devilla[1], Raniele Tadeu Guimarães de Souza[1], Itamar Rosa Teixeira[1] and Carlos Roberto Spehar[2]

[1]Department of Agricultural Engineering, State University of Goiás, 75132-400, Anápolis-GO, Brazil.
[2]Faculty of Agronomy and Veterinary Medicine, University of Brasília, 70910-970, Brasília-DF, Brazil.

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Quinoa has grown importance in the world due to the nutritional quality of its grains and crop adaptability to diverse climatic conditions. One problem that limits its cultivation is the reduced viability of seeds during storage and the information is rather scarce. This work aimed at evaluating the physiological quality of quinoa seeds along time when submitted at storage conditions and packaging. An entirely randomized experiment was conducted on factorial scheme 2 x 3 x 6 with four repetitions. The treatments consisted of 2 storage conditions: lab environment and Biochemical Oxygen Demand (B.O.D.) chamber set at 4±2°C and 90% relative humidity (RH); 3 package types: permeable, semi-permeable and impermeable; and 6 evaluations: before storage (0), 60, 120, 180, 240 and 300 days after storage. Seed viability was determined by the standard germination test while vigor by accelerated aging test, emergence in sand and emergence speed index. The use of impermeable packaging kept at low temperature maintained the physiological quality of seeds during 300 days of storage. The seeds kept in permeable or semi-permeable packaging under uncontrolled temperature and humidity conditions were viable only for 180 days. The permeable package using kraft paper was the least efficient to conserve physiological quality of quinoa seeds. It was demonstrated that quinoa seeds are rather sensitive to high temperature, loosing viability in short time.

Key words: *Chenopodium quinoa*, seed vigor, seed viability, seed conservation, packaging.

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willdenow) is a pseudocereal of the Amaranthaceae family originated from the Andes of South America where it has been cultivated since more than 5,000 BC (Abugoch, 2009). The protein of its grains has a balanced amino acid composition, with higher quantities of lysine (5 to 8%) and methionine (2.4 to 5.1%) than most cereals (Stikic et al., 2012). The grains are also rich in minerals and vitamins being gluten free and most utilized by celiac patients (Nascimento et al., 2014). Moreover, the content of fiber is 25% higher than the one found in wheat and maize (Lamothe et al., 2015).

*Corresponding author. E-mail: fliviafdejesus@gmail.com. Tel: +55 61 98724616.

The largest world producers are Peru and Bolivia reaching respective 52,129 and 50,489 metric tons in 2013 (FAOSTAT, 2014). This represents only a small fraction of the world's demand. For this reason there has been growing interest to adapt and cultivate quinoa in North America, Europe, Asia, Africa and Australia (FAO, 2011). It was first introduced in Brazil in the 1990's aiming at diversifying cropping systems in the savannahs. Studies have been undertaken to select genotypes adapted to the growing conditions of the Brazilian grain cropping areas, culminating with the release of BRS Syetetuba. It has shown favorable characteristics as grain yield of 2.3 Mt ha^{-1} phenotypic homogeneity and relatively large grains, with the 1,000 seed weight varied between 2.5 and 3.3 g (Spehar et al., 2011).

The ample adaptation and commercial production of quinoa in Brazil depends, however, of seed quality studies. One of the major problems restricting the quinoa crop in sub-tropical and tropical regions of the world is the seed quality. The end products of quinoa are aquene type fruits, with the shape of flat cylinders. They have a layer of dead cells surrounding the seeds. They are highly hygroscopic, presenting root protrusion in short time, 6 to 10 h after imbibing (Parsons, 2012). Therefore seeds can deteriorate rapidly in wet and high temperature environments (Ceccato et al., 2011).

The essential practice common to grain crops is the storage of seeds until next crop season. Their deterioration can be prevented by suitable storage to keep seed viability (Krohn and Malavasi, 2004; Lins et al., 2014). In the storage environment air relative humidity followed by temperature are the factors affecting physiological quality of seeds, interfering directly with their metabolic processes (Sravanthi et al., 2013).

Relative air humidity affects directly the water content in seeds and, when combined to high temperatures, intensifies seed respiration (Marcos, 2005). The consequences of higher respiration are the humidification and the warming up of seed mass, aggravated by the action of micro-organisms and insects (Baudet and Vilella, 2006). Seeds consume internal reserves, causing weight loss and drastic decline of germination (Carvalho and Nakagawa, 2012).

The packaging of seeds during storage could be valuable in maintaining their physiological maturity, depending on their intrinsic characteristics as permeability. The types of packages used in storage could have direct effect on the quality by preventing or not humidity exchange between seeds and the environment (Medeiros and Zanon, 2000). The main function of packaging seeds is to retard their deterioration by reducing respiration (Hong and Ellis, 2003; Tonin and Perez, 2006). The storage conditions and onion (*Allium cepa* L.) seed viability was studied utilizing cloth and paper, rigid polyethylene and paper, rigid polyethylene, flexible polyethylene, aluminum foiled flexible polyethylene and tin. Seed vigor at 20°C and 50% RH was not affected by package type, while at uncontrolled room temperature cloth, polyethylene and rigid polyethylene seeds reduced vigor (Caneppele et al., 1995). Crambe (*Crambe abyssinica* H.) seeds stored in tin at room temperature had better performance than in polyethylene terephthalate (PET) bottles, polystyrene box, and polyethylene bags (Cardoso et al., 2012). In *Cajanus cajan* L., PET bottles and polyethylene bags were more efficient than Kraft paper turning evident that this was associated with low temperature (Lisboa et al., 2014).

Every plant species has its particularities of seed viability and conservation, mostly related to the environment it was domesticated and adapted. Such is the case of quinoa, originated in the Andean Mountains at 3,800 m above the sea level. Therefore, in adapting its cultivation to the low altitude high temperature in tropics seed quality is a setback. This study aimed at evaluating the effect of packaging and environments in maintaining the physiological quality of *C. quinoa* seeds.

MATERIALS AND METHODS

The work was conducted in the Laboratory of drying and storage plant products of the Agricultural Engineering Couse, State University of Goiás, Anapolis, GO, Brazil, between February and November 2012.

Quinoa fruits

The quinoa fruits, and treated here as seeds, were of cultivar BRS Syetetuba, grown in the 2011 summer cropping at Emater Extension Service farming and experimentation area in Anápolis Goiás, Brazil. It is located at an altitude of 980 m above sea level, 48°18'23"WM and 16°19'44"SL. At physiological maturity, seeds had 20% wet basis (w.b.) moisture. After harvest, the seeds were dried down in forced ventilation greenhouse at approximately 60 m^3 min^{-1} m^{-2} and temperature of 35°C until moisture level reached 13.5% w.b.

Experimental design and treatments

The experimental design was entirely randomized on 2 x 3 x 6 factorial scheme with 4 repetitions. The treatments were: natural laboratory conditions and Biochemical oxygen demand (B.O.D.) chamber set at 4±2°C e 90% R.H. Three packaging types were used: impermeable – 250 ml, 0.126 mm PET bottles sealed with paraffin; semipermeable – 0.125 mm aluminum foil sealed with permeable sticking tape; and permeable – double-foiled Kraft paper bags sealed with sticking tape. The six evaluations were made at 0, 60, 120, 180, 240 and 300 days after beginning of experiment.

The respective maximum, (Tmax), mean (Tmean) and minimum (Tmin) temperatures and relative humidity in the storage environment during the experiment are presented (Figures 1 and 2).

Seed physiological quality

From the beginning of experiment up to 300 days seeds were evaluated by the following tests: i) water content – seed samples

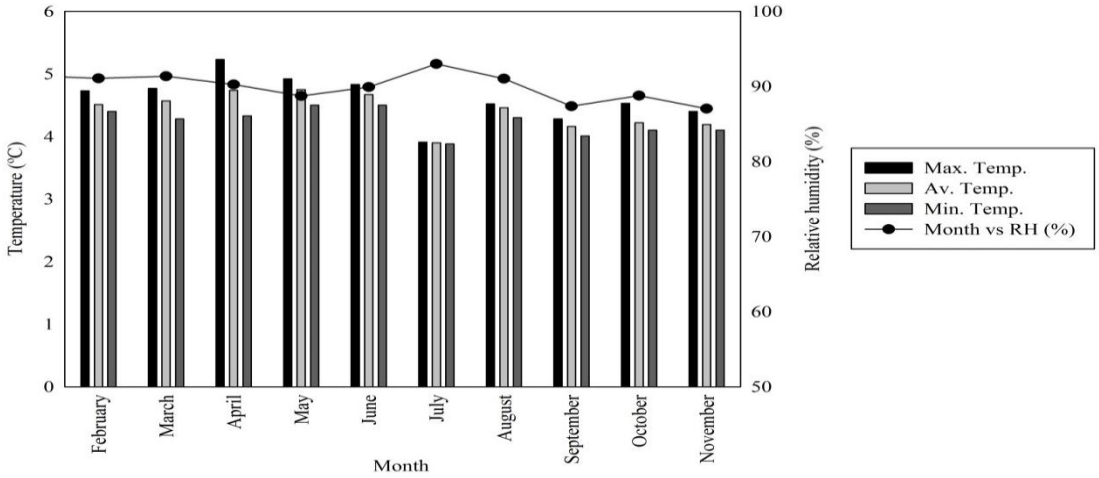

Figure 1. Maximum, average and minimum temperature and relative humidity in the B.O.D. chamber during the storage of quinoa seeds.

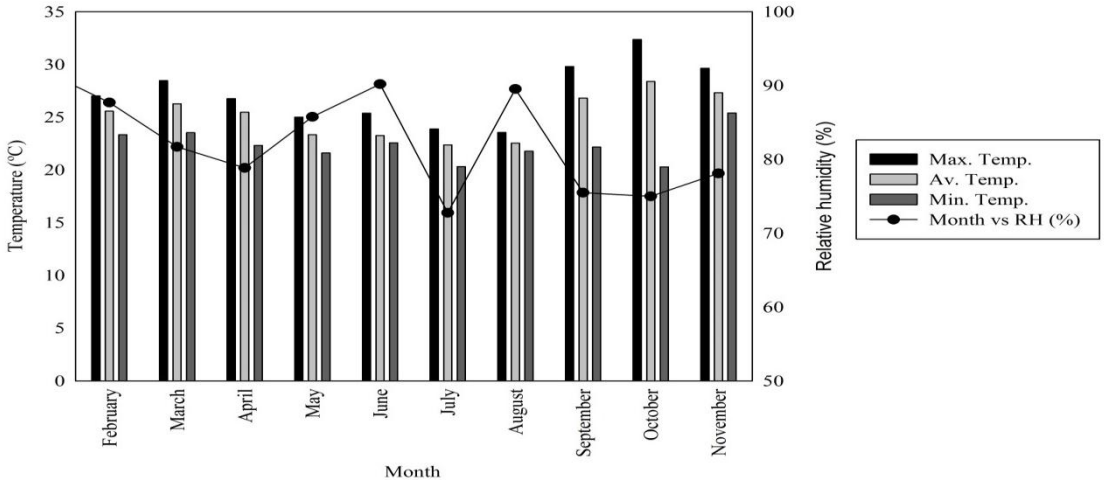

Figure 2. Maximum, average and minimum temperature and average relative humidity in the laboratory environment, in Anápolis, Goiás, Brazil, during the storage of quinoa seeds.

were placed on greenhouse at 105 ± 3°C for 24 h, on three replicates following the standard procedure test (BRASIL, 2009); ii) germination – a sample of 200 seeds from each storage package was divided into 4 replicates and sown on transparent plastic boxes (11 x 11 x 3.5 cm) containing distilled water soaked filter paper 2.5 times the weight. The boxes were placed in the dark with alternated temperatures of 25 to 30°C for 8 to 16 h. (Dias et al., 2003). Normal seedlings were scored on the 10th day following the standard procedure test (BRASIL, 2009); iii) first germination count – it was conducted simultaneously with the germination test with evaluation of normal seedlings rate on the 7th day (BRASIL, 2009); iv) accelerated aging: 12 g seeds were uniformly distributed on wire mesh placed in transparent plastic boxes (11 x 11 x 3.5 cm) containing 40 ml NaCl saturated solution (40 g .100 ml^{-1} in distilled water). The boxes were covered and kept in B.O.D. a 45°C for 48 h, being subsequently submitted to germination test, followed by evaluation of normal plants on the 7th day; v) emergence – from each storage treatment four replicates of 50 seeds were sown in autoclaved washed sand at 120°C, in 10 cm long furrows 1.5 cm deep. The trays were kept in the laboratory and daily irrigated by micro-sprayers to keep the substrate highly moist. Evaluation was conducted on the 10th day, expressing the results in percent of normal plants (Krzyzanowski et al., 1999); vi) emergence speed index (ESI): the test was conducted simultaneously with emergence, by scoring daily and at the same time the number of emerged plants. At the end of test, ESI was calculated by the Maguire (1962) formula - IVE = $E_1+E_2+E_3+...+E_n/N_1+N_2+...+N_n$, where E1, E2,. En = number of emerged plants at each day and N1, N2,... Nn = number of days from sowing until last count.

Statistical analysis

Analysis of variance was conducted for the observations and the values expressed in percentage were transformed in arcsen $\sqrt{x/100}$. Means were compared by Tukey test at the 0.05

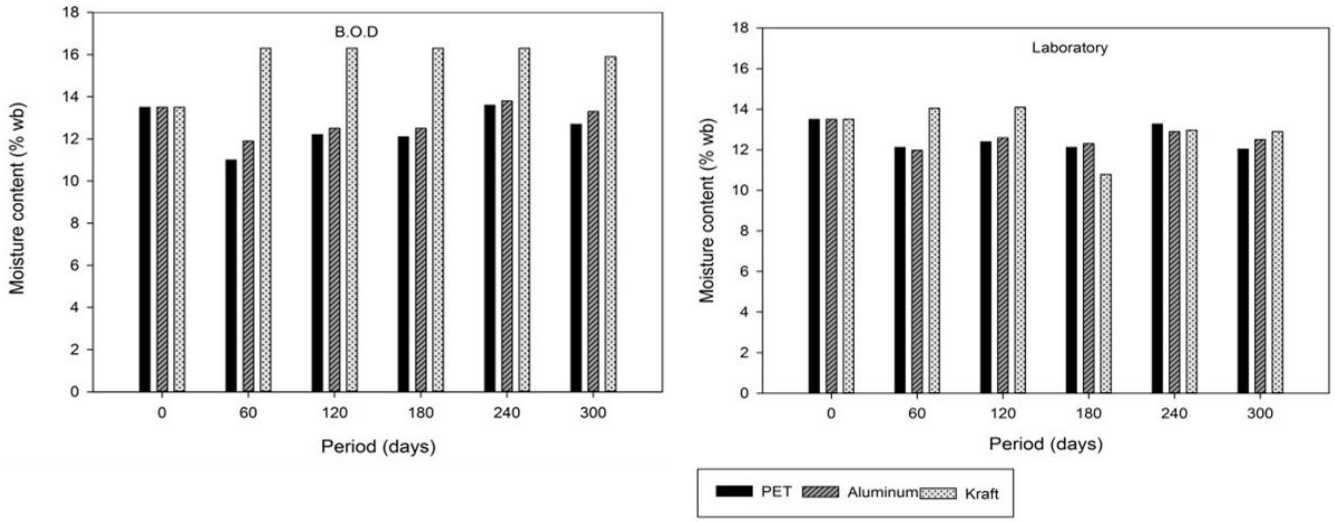

Figure 3. Moisture content of the quinoa seeds (% w.b.) stored in different environments and packages during 300 days.

probability level. The interaction of storage period x packaging type was submitted to regression analysis, at 5% de probability by F test. All statistical analysis utilized Sisvar 5.3 programme (Ferreira, 2011).

RESULTS AND DISCUSSION

The seed water content for packaging and environments are presented in Figure 3. In the laboratory environment, as would be expected, showed higher oscillation in temperature and relative humidity as related to seasonal variations along the year. In B.O.D. the relative humidity was constantly high and related to low temperature (4 ±2°C). Association of low temperature in storage and increase in relative humidity has been demonstrated by Regalo and Brena (2006).

The higher water content in B.O.D. stored seeds was related to increased relative humidity. Therefore, seeds packed in permeable Kraft paper had the equilibrium reached at 60 days of beginning, presenting average value of 16% w.b. The hygroscopic equilibrium has occurred when the water vapour pressure in seeds equals to the air water vapor pressure, after they were exposed to a long storage period (Amaral and Baudet, 1983; Silva et al., 2008). In environments with constant variations in humidity seeds are exposed to fluctuations in water content. This was verified in seeds maintained in Kraft paper packages at laboratory condition. At 180 days from beginning of experiment, in July when relative humidity was 73%, 10.8% w.b. was obtained. It should be worth emphasizing quinoa seeds are aquene fruits that possess a permeable outer layer of dead cells turning them prone to exchange moisture (Spehar et al., 2015).

In both laboratory and B.O.D. environments the seed water content of impermeable package (PET bottle) altered during storage periods. This could be related to increased respiration rate and intrinsic biological factors proper to each seed type (Almeida et al., 1999; Carvalho and Nakagawa, 2012). Experiments with seeds of *Copaifera multijuga* and *Caesalpinia pyramidalis*, kept in impermeable packages showed similar pattern in storage (Silva et al., 2011; Oliveira et al., 2011). The analysis of variance for physiological seed quality of quinoa during storage showed that environment (E), packaging (P), period (PE) and the interactions ExP and ExPE had an effect on all observations ($p<0.05$). Except for germination, the interaction ExP influenced significantly the results with other seeds tests ($p<0.05$). The interaction ExPxPE influenced significantly only the emergence speed index and accelerated aging ($p<0.05$).

The high relative humidity in B.O.D., independently of package type, did not affect germination rate of seeds during storage. In the laboratory, the germination decreased steadily turning the seeds unviable at 300 days (Figure 4). Physiological quality of seeds could be maintained for some time under controlled condition storage, but what was lost cannot be recovered unless there is dormancy (Carvalho and Nakagawa, 2012), which is not the case.

The rate of normal plants decreased along time in all packages tested. However, this was more evident for seeds kept in Kraft paper which had reduced seed viability as soon as 60 days comparatively lower than PET bottles and aluminum foil with decrease viability at 120 days (Figure 5). Seeds of sunflower and pigeon pea also decreased germination when stored in Kraft paper, compared the seeds stored in semi-permeable and impermeable (Lins et al., 2014; Lisboa et al., 2014).

The first count and emergence tests for seeds kept in B.O.D. showed seed viability during all the storage

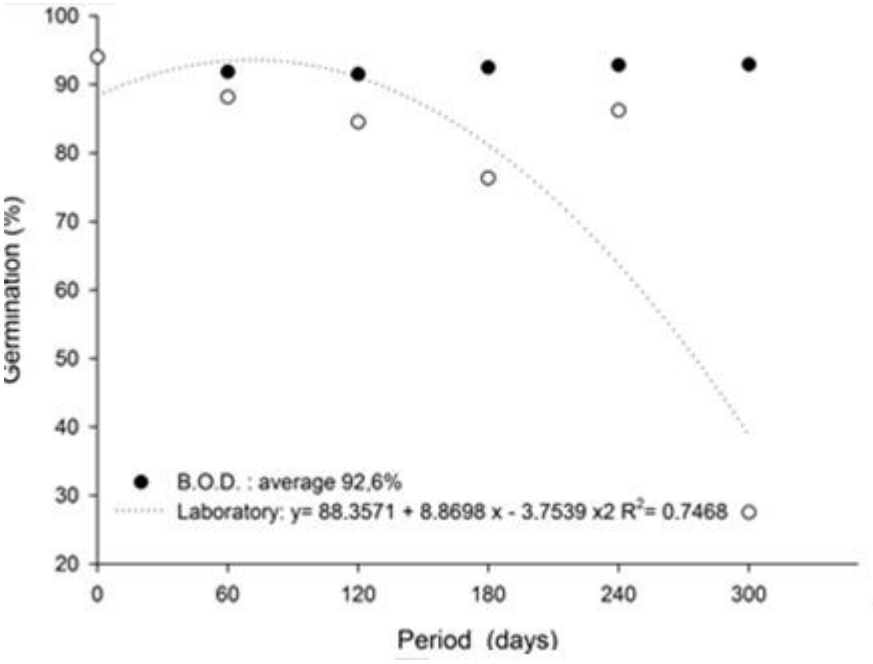

Figure 4. Quinoa seed germination from storage in different environments during 300 days. ** Significant at 0.01 probability.

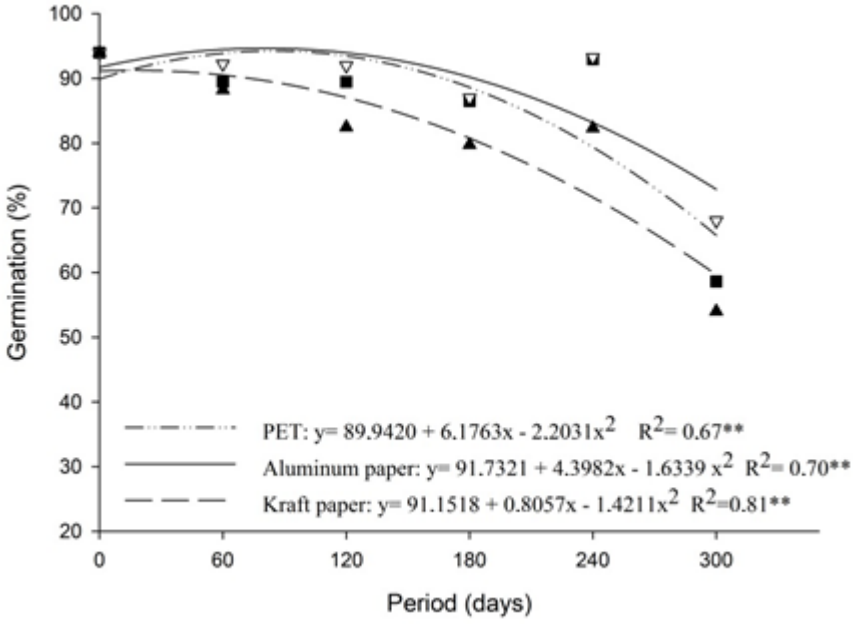

Figure 5. Seed germination of quinoa kept in different packages during 300 days. **Significant at 0.01 probability.

period, contrary to what was verified for the seeds kept in laboratory, which presented sharp decrease in vigor (Figures 6 and 7). The reduction in seed vigor in this case may have been related to variations in temperature and relative moisture during storage (Marcos, 2005). Seeds kept in impermeable PET bottle and semi-permeable aluminum foil had similar performance in the first count and emergence test (Figure 8), being superior to permeable Kraft paper package. The result could be associated to the thickness of semi-permeable aluminum foil, 0.25 mm maximum value for this type of packaging material (Baudet, 2003). Seed moisture had the same

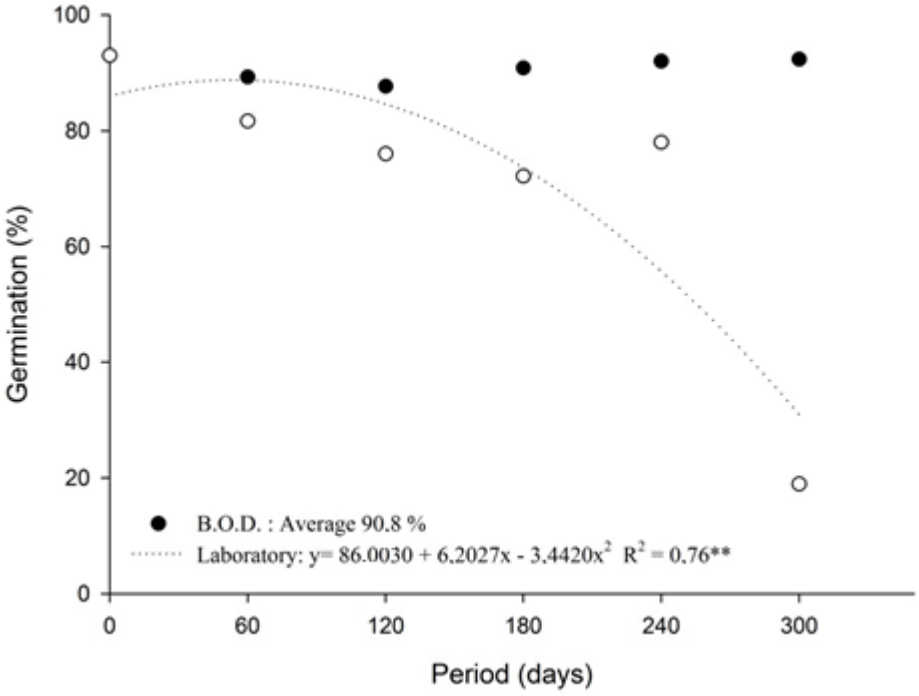

Figure 6. First count germination of quinoa seeds stored at laboratory and B.O.D. condition during 300 days. ** Significant at 0.01 probability.

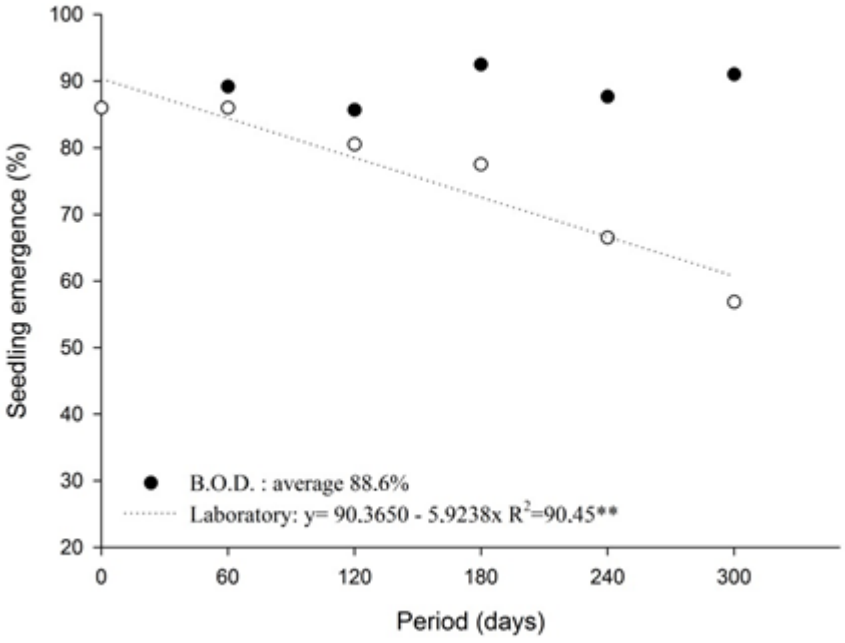

Figure 7. Seedling emergence of quinoa seeds stored at laboratory and B.O.D. condition during 300 days. ** Significant at 0.01 probability.

trend as in PET bottle, turning evident vigor is directly influenced by water content (Rao and Singh, 2006).

First count and emergence tests were also sensitive to detect significant differences for E x P interaction (Tables 1 and 2, respectively). Vigor was superior for all packaging types when stored in B.O.D. chamber. When

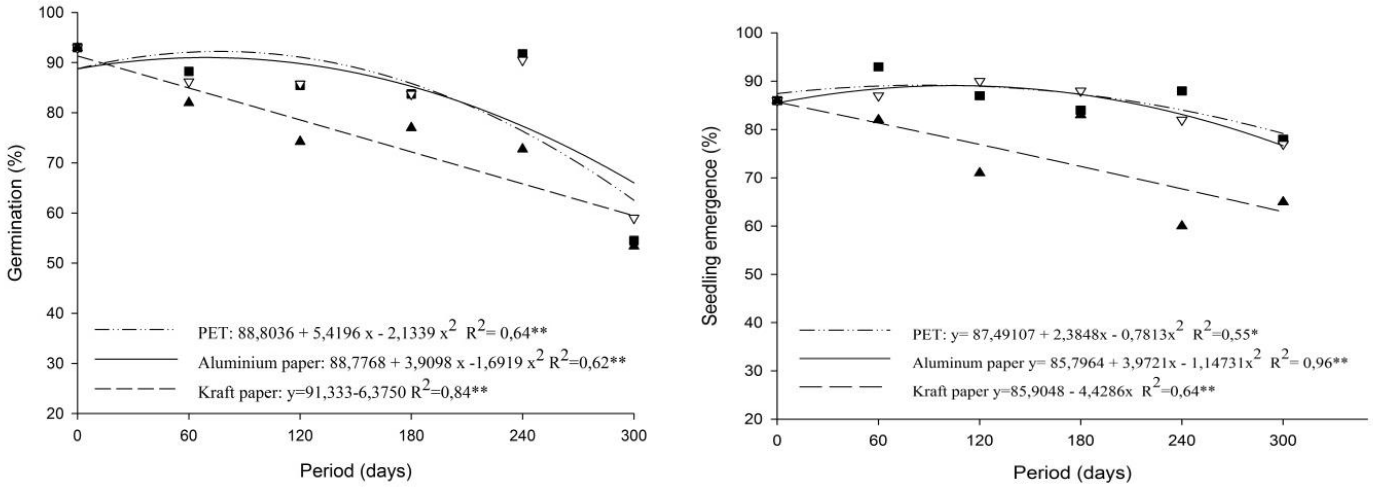

Figure 8. Germination at first count and emergence of seedlings from quinoa seeds kept in different packages during 300 days storage. Significant at *0.05 and **0.01 probability.

Table 1. Germination at first count of quinoa seeds kept in different environments and packages.

Storage environment	Package		
	PET	Aluminum foil	Kraft paper
B.O.D.	90^{Aa}	93^{Aa}	90^{Aa}
Natural (laboratory)	75^{Ba}	74^{Ba}	61^{Bb}

Means followed by the same capital letter in column and low case letter in the line are not statistically different (Tukey's test, p <0.0 5).

Table 2. Emergence of quinoa seedlings from seeds stored in different environments and packages.

Storage environment	Package		
	PET	Aluminum foil	Kraft paper
B.O.D.	90^{Aa}	90^{Aa}	86^{Ab}
Natural (laboratory)	82^{Ba}	80^{Ba}	64^{Bb}

Means followed by the same capital letter in column and low case letter in the line are not statistically different (Tukey's test, p<0.0 5).

emergence is observed, there was no significant difference for packaging, while the Kraft paper had significant effect in reducing it. Under uncontrolled laboratory environment, PET bottle and aluminum foil, showed no significant differences, although superior to Kraft paper package. The latter are permeable to water vapor, justifying the fluctuations in seed water content and reduction in vigor (Marcos, 2005).

The emergence speed index was more sensitive to detect significant difference in the environment x packaging x period. From 180 days after beginning of storage, there appeared a significant difference between laboratory and B.O.D. chamber conditions. In the latter, vigor reductions were lower than room conditions for the three package types during the time evaluations were made (Figure 9). This can be attributed to gradual deterioration in B.O.D. related to lower seed respiration and metabolic processes under reduced and constant temperature (Das et al., 1998).

In the first 120 days of storage, seeds for all packaging types kept in B.O.D. conditions and kept in PET bottle and aluminum foil in laboratory, had similar results (Figure 9), except the ones kept in Kraft paper package, which declined rapidly from 60 days, turning unviable at 180 days after beginning of experiment, similar to seeds of *Adenanthera pavonina* L. and *Sebastiania*

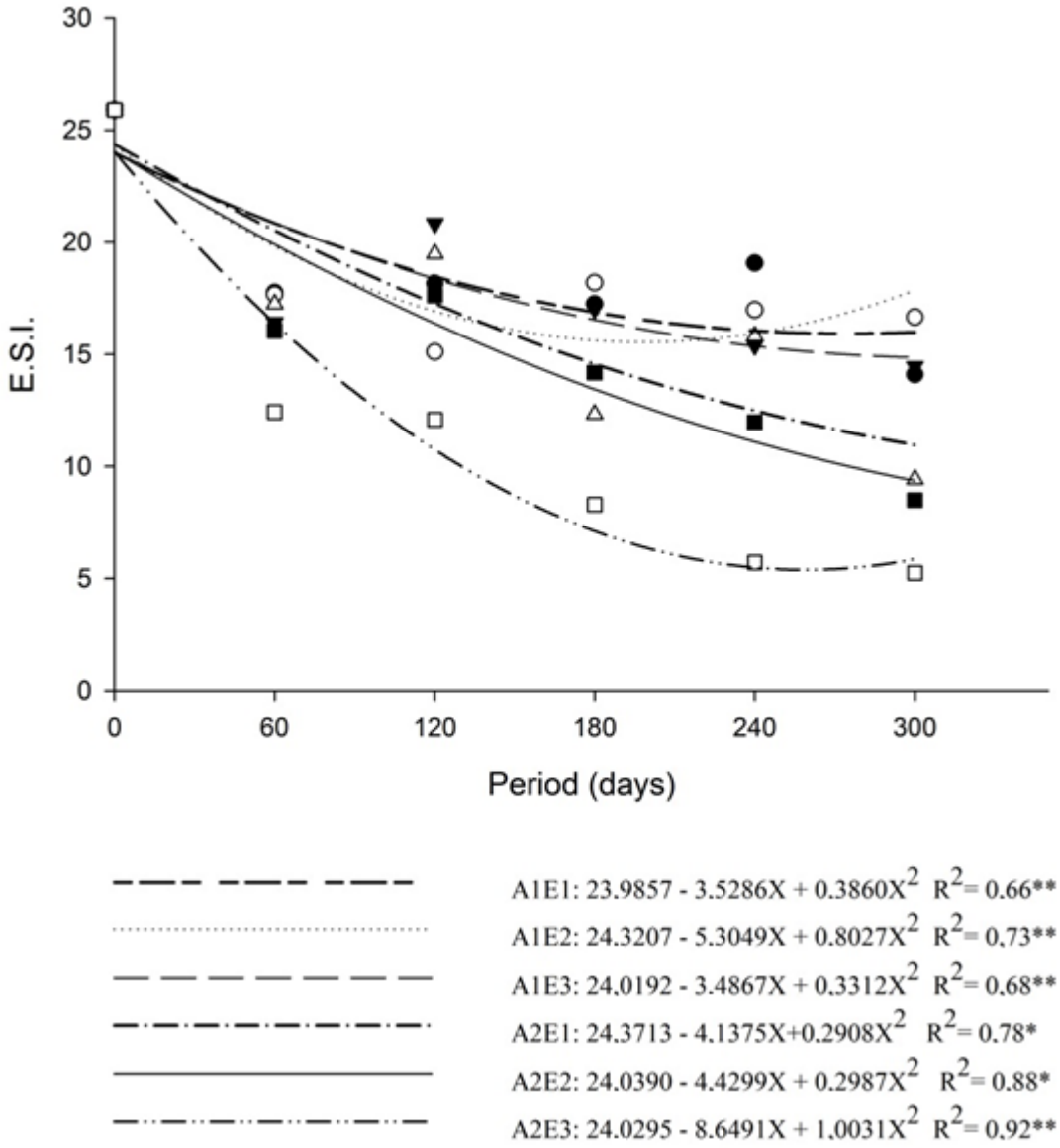

Figure 9. Emergence speed index (E.S.I.) of quinoa seedlings coming from seeds stored in different environments and packages during 300 days. (A1: B.O.D.; A2: laboratory; E1: PET botle, E2: aluminum foil and E3: Kraft paper). Significant at * 0.05 and ** 0.01 of probability according to test F.

commersoniana (Oliveira et al., 2012; Santos and Paula, 2007).

In the accelerated aging test there was also significant interaction for environment x packaging x period (Figure 10). The interactions that maintained the vigor, by the accelerated aging test, for all storage periods, were in lots of the kept seeds in PET bottle and aluminum foil in B.O.D. In the other treatments seed vigor decreased as early as 60 days. As in the other tests, seeds were more vigorous for all types of packages when kept in B.O.D. controlled low temperature, with the exception of the ones kept in Kraft paper reducing to zero at 240-300 days. These were kept viable until 180 days but in the uncontrolled laboratory conditions they became unviable at 120 days.

Conclusion

Quinoa seeds maintain physiological quality for long period (300 days) when kept in impermeable package and low temperature (4±2°C). Under uncontrolled temperature and moisture semi-permeable and impermeable package seeds are viable until 180 days of storage. Permeable package as Kraft paper is the least efficient in conserving physiological quality of quinoa seeds.

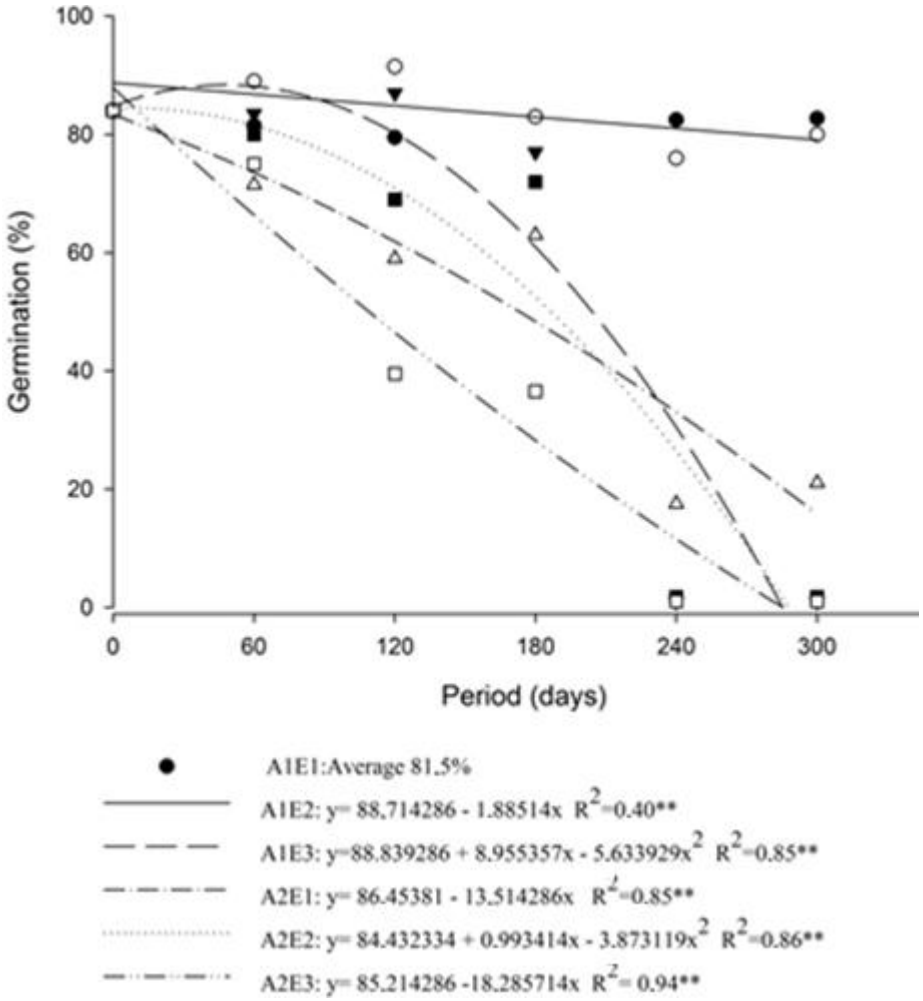

Figure 10. Germination assessed from the accelerated aging test for quinoa seeds stored in different environments and packages during 300 days. (A1: B.O.D.; A2: laboratory; E1: PET bottle, E2: aluminum foil and E3: kraft paper). ** Significant at 0.01 probability.

Conflict of Interests

The authors have not declared any conflict of interests.

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