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Responses of quinoa (*Chenopodium quinoa* Willd.) seeds stored under different germination temperatures

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ABSTRACT. In this experiment, we assessed the germination and vigor of quinoa seeds packed in paper bags and stored at room temperature for 36, 85, 119, 146, 177 and 270 days. The seeds were harvested under experimental conditions in Marechal Candido Rondon, Paraná, during the 2012/13 growing seasons. Four replicates of 100 seeds each were established for each storage time, and the seeds were evaluated, on paper, based on the BOD under the following experimental temperature conditions: alternating temperatures of 20 and 30°C and a constant temperature of 25°C. The seeds from both treatments were subject to seven-hour photoperiods and 25°C under continuous darkness. The germinated seeds were counted daily for eight days after sowing, and we evaluated the percentages of normal and abnormal seedlings and the germination index. The experimental design was completely randomized using a split-plot design. Increasing the storage time decreased the percentage of germinated seeds and seed vigor due to the increased number of abnormal seedlings. Over the 430-day study period, quinoa seed germination completely declined under the experimental conditions. The final number of germinating seeds should be evaluated 7 days after the beginning of the germination test.

Keywords: Germination test, temperature, photoperiod, seed vigor.

Resposta de sementes de quinoa (*Chenopodium quinoa* Willd.) estocadas em diferentes temperaturas de germinação

RESUMO. Neste ensaio, avaliou-se a germinação e o vigor de sementes de quinoa, acondicionadas em sacos de papel e armazenadas à temperatura ambiente durante 36, 85, 119, 146, 177 e 270 dias. Semearam-se quatro repetições de 100 sementes, de cada período de armazenamento, sendo as mesmas avaliadas, sob papel, em BOD, sob as seguintes condições experimentais de temperatura: alternância de temperaturas de 20 e 30ºC e temperatura constante de 25ºC. As sementes de ambos os tratamentos foram dispostas em fotoperíodo de sete horas, adicionalmente, outras em 25ºC e escuro contínuo. A contagem de sementes germinadas foi realizada diariamente, durante oito dias após a semeadura, sendo avaliados a porcentagem de plântulas normais, anormais e o índice de velocidade de germinação. O delineamento experimental empregado foi inteiramente casualizado no esquema de parcela subdividida. Com o aumento do período de armazenamento, ocorreu diminuição do percentual de sementes germinadas e vigor das sementes, devido ao aumento do número de plântulas anormais. Durante o período de 430 dias, as sementes de quinoa perderem todo seu potencial de germinação sob as condições experimentais. A germinação total de sementes de quinoa deve ser avaliada 7 dias após o início do teste de germinação.

Palavras-chave: teste de germinação, temperatura, fotoperíodo, vigor de semente.

Introduction

Quinoa (*Chenopodium quinoa* Willd.) produces edible grains with high nutritional value for humans and animals. This grain is highly important in the human diet because it is free of gluten and used by people with allergies (Spehar, 2007), but it also contains protein, amino acids, iron, zinc and other nutrients (Gewehr, Danelli, Melo, Flôres, & Jong, 2012). The composition of the seed allows for greater storage potential than that of other oil seeds due to the greater chemical stability of the starches and lipids (Marcos Filho, 2015).

However, quinoa seeds lose viability more rapidly than cereals because of the porosity in the integument, which allows a seed to easily gain or lose moisture and may initiate germination in the panicle (Spehar, 2007).

Quinoa seed quality is influenced by the low germination rate and reduced vigor (Kappes et al., 2012), which consequently reduce longevity; i.e.,

lower seed quality reduces the germination percentage.

Seed quality is characterized by the sum of the genetic, physical, physiological and sanitary attributes that determine seed performance when sown or stored (Santos, Póla, Barros, & Prete, 2007). The degree of germination is influenced by intrinsic and extrinsic factors, the latter of which include temperature, relative humidity, oxygen, and the action of fungi and bacteria (Marcos Filho, 2015).

Seed germination is regulated by the interaction between physiological qualities and environmental conditions in that every plant species has specific germination requirements in terms of the availability of water, temperature, and light as well as sowing depth (Carvalho & Nakagawa, 2012).

In seed analysis, vigor tests have mainly been used to identify differences associated with the performance of batches of seed during storage and after sowing with the goal of highlighting more effective batches for stand establishment under a wide range of environmental conditions (Marcos Filho, 2015).

The germination test evaluates physiological quality, but the results do not always correspond to emergence in the field. Vigor parameters, such as germination speed indexes and emergence potential, are used to complement the information obtained in the germination test to evaluate seed performance and the development speed of normal seedlings under a wide diversity of environment conditions (Franzin, Menezes, Garcia, & Wrasse, 2004).

Considering the expansion of the area under quinoa cultivation, the growing popularity of the grain in domestic and foreign markets, and the deficit of scientific information about the physiological quality of stored seeds, the current study aimed to evaluate the influence of storage period and different temperature conditions on quinoa seed germination.

Material and methods

This experiment was carried out in the Seed Technology Laboratory located at the Marechal Candido Rondon campus of the State University of Western Paraná (UNIOESTE). Seeds were harvested during the 2012/2013 growing season on the experimental farm of Doctor Antonio Carlos dos Santos Pessoa in the western region of Paraná State, Brazil, at 24º 33' SL and 54º 31' WL and an altitude of 235 m (Instituto Agronômico do Paraná [IAPAR], 2004).

Seeds at physiological maturity were manually collected and arranged in a single layer to achieve a moisture content of approximately 14%. After processing, they were stored in Kraft paper bags under different environmental conditions for periods of 85, 119, 146, 177, and 270 days.

For the germination tests, gerbox boxes were cleaned and rinsed with distilled water, and the filter paper was autoclaved at 120°C to minimize contamination. Seeds were selected and manually graded to eliminate any that were damaged or deformed. We evaluated four replicates of 100 seeds for each storage time. The substrate in the germination boxes (11 cm x 11 cm x 3.5 cm) was moistened with water prior to the test to 2.5 times the weight of the dry paper and placed in twin BOD (Biochemical Oxygen Demand) chambers. The experimental treatments included alternating temperatures of 20 and 30°C and a constant temperature of 25°C under a photoperiod of seven hours for both thermal conditions. Furthermore, one of the BOD chambers was under a controlled temperature of 25°C under darkness.

The germinated seeds were counted from the third day after sowing, and the experiment was finished when no further radical protrusions were observed after an average of six days. In the germination test, we evaluated the percentage of normal seedlings as recommended by the Rules of Seed Germination, and we considered germinated seeds to be those that developed a primary root and a normal shoot.

The germination index was calculated as the sum of the number of seeds germinated each day divided by the number of days between sowing and germination, following the equation by Maguire (1962): $GI = (G1/N1) + (G2/N2) + (G3/N3) + ... + (Gn/Nn)$, where:

GI was the germination index;

G1, G2, G3, ..., Gn were the number of seedlings that germinated on the first, second, third, etc. until the nth day of counting;

N1, N2, N3, ..., Nn were the first, second, third, etc. to the nth day after sowing.

The data were submitted to a normality test, analysis of variance and a response surface using program Genes (Cruz, 2013).

Results and discussion

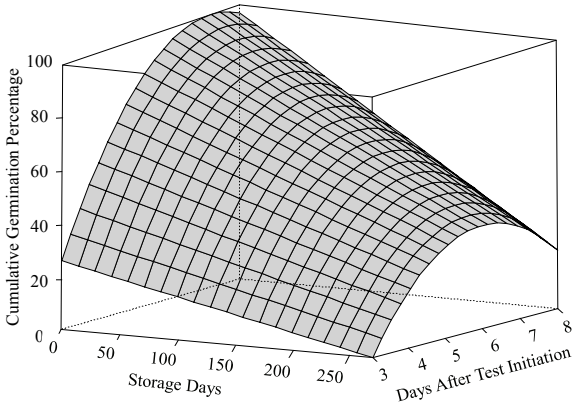
In the germination test with alternating temperatures between 20 and 30°C and a photoperiod of 7 hours, there was a linear decrease in the germination of quinoa seeds in relation to storage period over all of the counting days (Figure 1).

The relationship cumulative germination percentage rate with the day of counting was

0.00141 to 0.3616* counting days, according to the derived relationship between cumulative germination and the number of counting days. This variation increased when the number of counting days during the germination test increased and decreased when the seeds were stored for a longer period of time.

Seven days after sowing, the seeds stored for a 270-day period had limited cumulative germination, while the batch composed of seeds stored for shorter periods exhibited germination until the eighth day after the initiation of the germination test. This result is related to seed vigor because greater vigor increases the probability of successful germination. A similar result was observed by Nobre et al. (2013), who noted that amaranth seeds showed maximum germination and vigor to the point of maturity and then began to decline.

The storage temperature and relative humidity are the main factors affecting the reduction of physiological seed quality (Goldfarb & Queiroga, 2013). Strenske, Vasconcelos, Herzog, and Malavasi (2015) observed that the germination of stored quinoa seeds was maintained under controlled environmental conditions. The lack of controlled temperature and humidity during the storage period in this assay may explain the decline in the cumulative germination of quinoa seeds.

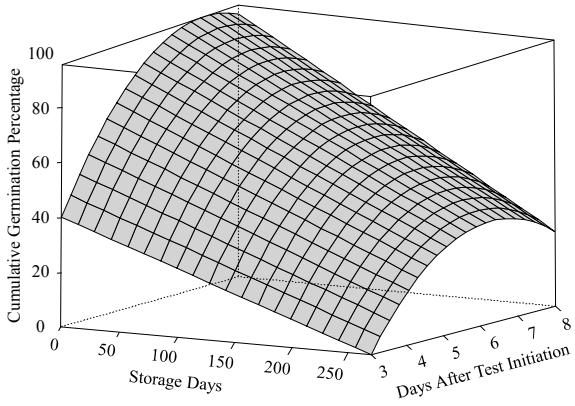


$Z = -108.51935 - 0.00141x + 60.64499y - 4.22099y^2 - 0.03616xy$ $R^2=83.4\%$

Figure 1. Cumulative germination of quinoa seeds under alternating temperature conditions of 20 and 30°C and a photoperiod of 7 hours in relation to the storage time (days after harvest) and the number of days for germination after the initiation of the germination test at Marechal Candido Rondon, Paraná State, 2013/2014.

When the quinoa seed germination test was conducted at a constant temperature of 25°C and a photoperiod of seven hours, a linear reduction in cumulative germination with the storage period was also observed, but it was less dependent on the number of counting days (Figure 2). Therefore, the rate of variation in the cumulative germination was -0.09014 -0.01901* counting days. Under these experimental conditions the value multiplied by the number of counting days is less than the length of the germination test, resulting in a lower effect of the number of counting days.

Regardless of the length of the storage period, germination occurred seven days after sowing, which is relatively fast; the protrusion of the radicle is observed within the first few days. The data obtained during the implementation of this test are similar to the observations of Oliveira and Gomes-Filho (2009) for sorghum and Bonacin, Rodrigues, Fernandes, and Rodrigues (2006) for alfalfa.



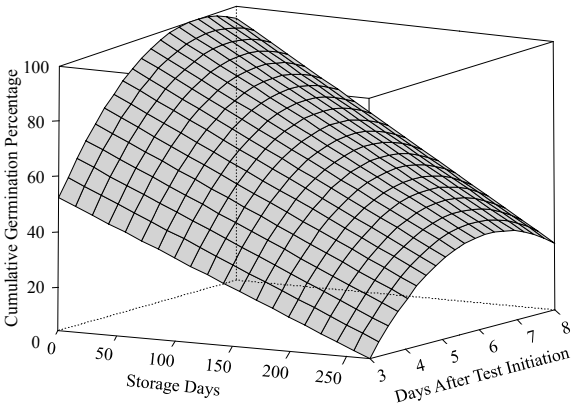
$Z = -67.04085 - 0.09014x + 47.94891y - 3.407y^2 - 0.01901xy$ $R^2 = 82\%$

Figure 2. Cumulative germination of quinoa seeds under a constant temperature of 25°C and a photoperiod of 7 hours relative to the storage time (days after harvest) and number of days for germination after the initiation of the germination test at Marechal Candido Rondon, Paraná State, 2013/2014.

When the seed germination test was conducted at a constant temperature of 25°C without a photoperiod, there was a linear reduction in the cumulative germination of quinoa seeds during the storage period that was also less dependent on the number of counting days (Figure 3) compared to the test with alternating temperatures and a seven-hour photoperiod. Thus, the rate of variation in cumulative germination percentage in relation to the number of counting days was -0.13211 - 0.01483* counting days.

Seven days after the start of the germination test, the cumulative seed germination was already limited at any storage period. In contrast, under the germination test condition of a constant temperature

of 25°C without a photoperiod, the overall germination of the quinoa seeds could be assessed seven days after start of the test.



$Z = - 49.22657 - 0.13211x + 43.9303y - 3.20239y^2 - 0.01483xy$
R^2=84.8%

Figure 3. Cumulative germination of quinoa seeds under a constant temperature of 25°C without a photoperiod in relation to the storage time (days after harvest) and the number of days for germination after the initiation of the germination test at Marechal Candido Rondon, Paraná State, 2013/2014.

Starting from the maximum value obtained for the first three counting days of seeds stored for 36 days, which appeared to be the best condition for quinoa germination, 51% germination was observed in the test with a constant temperature of 25°C without a photoperiod (Figure 3) followed 42% germination of seeds placed at a constant temperature of 25°C with a seven-hour photoperiod (Figure 2). The germination test with alternating temperatures of 25 and 30°C with a seven-hour photoperiod produced the lowest germination, 34%, three days after the initiation of the test (Figure 1).

The number of abnormal plants increased by 5.8% after 100 days of storage when the quinoa seeds were subjected to alternating temperatures of 20 and 30°C and a photoperiod of seven days, and 7.6% abnormal plants were observed after 100 days of storage when the germination test consisted of a constant temperature of 25°C without a photoperiod. When the seeds were subjected to germination at a constant temperature of 25°C and a seven-hour photoperiod, the number of abnormal seedlings had a cubic relationship with the number of days of storage (Figure 4). The increase in the number of abnormal seedlings with the increase in storage period has also been observed by Nakagawa, Cavariani, and Toledo (2009) in pigeon pea seeds.

Marcos Filho (2015) found that seeds deteriorate, which is considered to be any negative change after the seed has reached its maximum quality, when exposed to long periods of storage in uncontrolled conditions. The mechanisms responsible for these changes have not yet been fully elucidated, and the sensitivity of seeds to decay in a particular environment has been attributed to genetic constitution. In addition, the temperature and relative humidity during this period are of great importance to the process of deterioration, which cannot be avoided but can be minimized through storage under appropriate conditions.

The causes of deterioration are most likely related to the loss of the integrity of the membrane system, reduced selective capacity, lipid peroxidation, solute leaching, changes in the respiratory activity of the seeds, changes in the enzymatic activity and synthesis of proteins, changes in the inability to maintain the electrochemical gradient, the loss of cellular compartmentalization and the accumulation of toxic substances (Marcos Filho, 2015). Physiological changes were also observed in this study, which included delay in germination, decreased tolerance to sub-optimal environmental conditions during germination, reduced growth and/or seedling vigor, an increased number of abnormal seedlings, increased susceptibility to pathogens attacks, uneven emergence, reduced productivity, altered seed color, decreased storage potential, complete loss of germination capacity and death.

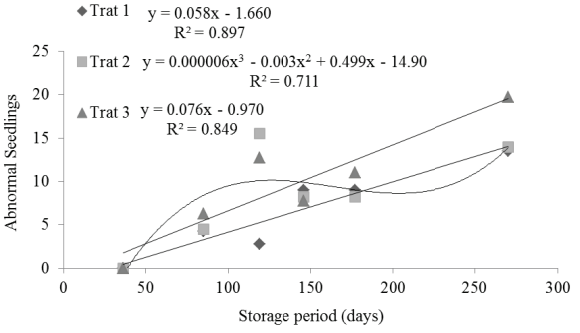


Figure 4. Relationship between the storage period and the number of seeds that produced abnormal seedlings under different germination conditions1 at Marechal Candido Rondon, Paraná State, 2013/2014. Treatment 1 – Alternating temperature between 20 and 30ºC and a photoperiod of seven hours; Treatment 2 – constant temperature of 25ºC and a photoperiod of seven hours; Treatment 3 – constant temperature of 25ºC under darkness.

The germination values of quinoa seeds approached 90% at 36 days after harvest, indicating that the genotype does not present numbness (Figure 5), a condition presented by quinoa genotypes 2-Want and Chadmo (Ceccato, Bertero, Batlla, & Galati, 2015).

The germination of quinoa seeds decreased linearly regardless of the germination test condition, either alternating temperatures of 20 and 30°C with a photoperiod of 7 hours or a constant temperature of 25°C with or without a photoperiod of 7 hours (Figure 5). The percentage of germinated seeds decreased as the storage period increased, so it is possible to conclude that longer quinoa seed storage periods under ambient conditions result in greater deterioration. These results are supported by Strenske et al. (2015), who reported a decrease in quinoa seed germination with storage time. Under the storage conditions used in this study, it is expected that quinoa seeds completely lose their germination potential between 430 and 456 days after the seed is harvested.

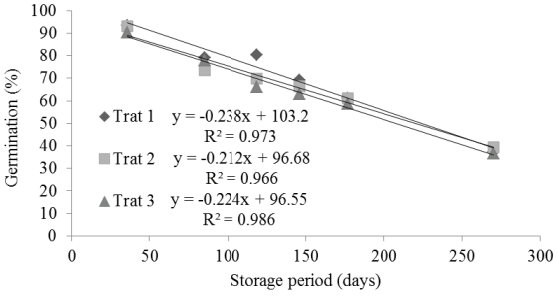


Figure 5. Relationship between the storage period and quinoa seed germination under different germination conditions at Marechal Candido Rondon, Paraná State, 2013/2014. Treatment 1 – Alternating temperature between 20 and 30ºC and a photoperiod of seven hours; Treatment 2 – constant temperature of 25ºC and a photoperiod of seven hours; Treatment 3 – constant temperature of 25ºC under darkness.

As observed for seed germination, there was also a reduction in the germination rate (Figure 6) throughout the storage period. Under the three conditions for seed germination, alternating temperatures between 20 and 30°C with a seven-hour photoperiod or a constant temperature of 25°C with or without a seven-hour photoperiod, the loss of quinoa seed vigor was similar, so the different conditions yield similar results.

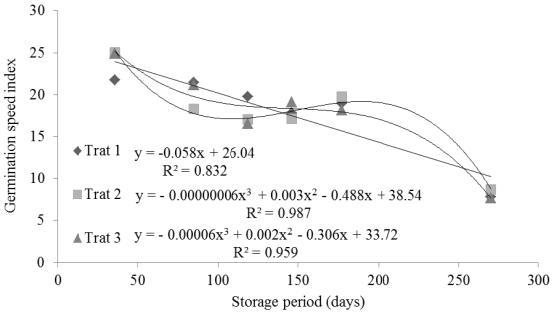


Figure 6. Germination rate of quinoa seeds with storage period under different germination conditions at Marechal Candido Rondon, 2013/2014. Treatment 1 – Alternating temperature between 20 and 30ºC and a photoperiod of seven hours; Treatment 2 – temperature of 25ºC and a photoperiod of seven hours; Treatment 3 – constant temperature of 25ºC under darkness.

The increase in the number of abnormal seedlings and the reduction in the number of germinated seeds as well as the germination rate were expected because storage under uncontrolled environmental conditions may result in seed loss (Castellión, Mantiacevich, Buera, and Maldonado, 2010; Strenske et al., 2015), so the results for the quinoa genotype evaluated in this study were not different from previous findings. However, after 430 days, all seeds lose their germination potential, so the above values for seeds stored under uncontrolled environmental conditions were above expectations.

Conclusion

With longer storage periods under ambient conditions, the quality of quinoa seeds deteriorated as indicated by the increasing number of abnormal seedlings and the decreasing number of normal seedlings.

The final count of germinated quinoa seeds should be evaluated at 7 days after the beginning of the test.

Quinoa seeds can be stored up to 430 days under uncontrolled environmental conditions before germination completely declines.

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