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Germinative performance of lettuce (*Lactuca sativa* L.) landrace seeds for organic production

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Abstract

Currently, it is observed a gap in the supply of seeds from organic farming production systems to meet the demand for seeds of vegetables. In this sense, landrace seeds may stand as an alternative to commercial seeds, reconciling organic farming practices with the genetic diversity of landrace seeds and family farming. This work aimed to evaluate the germinative performance of landrace lettuce seeds obtained directly from the farmer and produced with an organic system as an alternative seed source for use in organic farming. A batch of commercial seeds and two batches of landrace seeds from different places were evaluated. The following tests were carried out: germination, first germination count, germination speed index, accelerated aging test, resistance to stress induced by cold, and tetrazolium test. The obtained results underwent ANOVA and Tukey's multiple range test at 5 % error probability. The results showed that both the conventional and the landrace seeds had germination percentages above 90 %. The vigor of the seeds produced with the conventional system was greater in the tetrazolium test. The three batches had a similar performance in the tests of resistance to stress induced by cold at 5 °C.

Keywords: Germination speed index; germination test; sustainable farming; vigor.

Resumo

Desempenho germinativo de sementes crioulas de alface (*Lactuca sativa* L.) para produção orgânica

Atualmente, observa-se uma lacuna na oferta de sementes oriundas de sistemas de produção de agricultura orgânica para atender a demanda por sementes de hortaliças. Nesse sentido, sementes crioulas podem ser uma alternativa às sementes comerciais, conciliando as práticas de agricultura orgânica com a diversidade genética das sementes crioulas e da agricultura familiar. Este trabalho teve como objetivo avaliar o desempenho germinativo de sementes de alface crioula obtidas diretamente com o produtor e produzidas sob sistema orgânico como fonte alternativa de sementes para uso na agricultura orgânica. Foram avaliados um lote de sementes comerciais e dois lotes de sementes crioulas de diferentes localidades. Foram realizados os seguintes testes: germinação, primeira contagem de germinação, índice de velocidade de germinação, teste de envelhecimento acelerado, resistência ao estresse induzido pelo frio e teste de tetrazólio. Os resultados obtidos foram submetidos a ANOVA e teste de Tukey a 5 % de probabilidade de erro. Os resultados mostraram que tanto as sementes convencionais quanto as crioulas tiveram porcentagem de germinação acima de

90 %. O vigor das sementes produzidas com o sistema convencional foi maior no teste de tetrazólio. Os três lotes tiveram o mesmo desempenho nos testes de resistência ao estresse induzido por frio a 5 °C.

Palavras-chave: Agricultura sustentável; índice de velocidade de germinação; teste de germinação; vigor.

Resumen

Comportamiento germinativo de semillas criollas de lechugas (*Lactuca sativa* L.) para producción orgánica

Actualmente, existe un vacío en la oferta de semillas provenientes de sistemas de producción de agricultura orgánica para satisfacer la demanda de semillas de hortalizas. En ese sentido, las semillas criollas pueden ser una alternativa a las semillas comerciales, conciliando las prácticas de la agricultura orgánica con la diversidad genética de las semillas criollas y la agricultura familiar. Este trabajo tuvo como objetivo evaluar el comportamiento germinativo de semillas de lechuga criolla obtenidas directamente del agricultor y producidas con un sistema orgánico como fuente alternativa de semillas para uso en agricultura orgánica. Se evaluó un lote de semillas comerciales y dos lotes de semillas nativas de diferentes localidades. Se realizaron las siguientes pruebas: germinación, conteo de primeras germinaciones, índice de velocidad de germinación, prueba de envejecimiento acelerado, resistencia al estrés por frío y prueba de tetrazolio. Los resultados obtenidos se sometieron a ANOVA y prueba de rangos múltiples de Tukey al 5 % de probabilidad de error. Los resultados mostraron que tanto las semillas convencionales como las variedades criollas tuvieron porcentaje de germinación superior al 90 %. El vigor de las semillas producidas con el sistema convencional fue mayor en la prueba de tetrazolio. Los tres lotes tuvieron el mismo desempeño en las pruebas de resistencia al estrés inducido por frío a 5 °C.

Palabras clave: Agricultura sostenible; índice de velocidad de germinación; prueba de germinación; vigor.

Introduction

Lettuce is one of the most important leafy vegetables and has a market share with a growing trend through *in natura* or minimally processed consumption. The productive chain of lettuce seeds had a revenue of US$ 17.07 million in 2016, being 70 % of the production destined for nurseries (ABCSEM, 2017).

Brazilian market of organic vegetables had a growth of 18.5 % between 2011 and 2016. The projected average growth trend for this sector was 4.4 % per year until 2021 (ABCSEM, 2017). In 2021, Brazilian lettuce production, including both organic and conventional crops, was 66,430 t (CONAB, 2022). The production of organic seeds is regulated by Law 10.831/2003 and Normative Instruction n° 38/2011, establishing that their production must follow the standards of organic farming and the Brazilian regulation of seeds and seedlings (BRASIL, 2003, 2011).

The greatest difficulty observed in vegetable production is associated with the low supply of organic seeds to meet the certification process of the productive chains. This fact implies higher investments in techniques of organic farming, enabling the use of germplasm adapted to local conditions and the recovery of traditional and landrace cultivars which were produced under agroecological principles (NASCIMENTO; VIDAL; RESENDE, 2012).

However, organic seeds are so-called because of the farming system used in their production. The seeds used can be landrace seeds, seeds from enhanced cultivars, or commercial seeds adapted to organic farming conditions. Nonetheless, landrace seeds are associated with genetic variability, rusticity, and adaptability, considering these seeds are produced and kept by family farmers throughout the years in the same community (FERNANDES, 2017).

Normative Instruction 42/2019 establishes the following germination percentages for lettuce seeds destined for marketing, considering batches of 10,000 kg of seeds: 70 % for basic seeds and 80 % for certified seeds of first (C1) and second (C2) generation, and seeds of first (S1) and second (S2) generation (BRASIL, 2019). For lettuce, considering seeds stored under anaerobic conditions, with 5 % relative humidity and temperature of 20 °C, the estimated seed longevity is 11 years (FLOSS, 2006). In addition, Fleming, Hill, and

Walters (2019), studying the seed viability of several vegetable species, reported that lettuce seeds stored at 5 °C and relative humidity between 35 % and 50 % had a negligible decline in seed vigor and germination performance even after 10 years of storage.

Regarding lettuce seed storage conditions, studies in the literature report temperatures below 20 °C as desirable to avoid the loss of vigor and deterioration of tissues. This was observed by Catão *et al.* (2018), studying the short-term (up to 120 days) impact of different storage temperatures on lettuce seed vigor and germination, and by Fleming, Hill, and Walters (2019), in the long-term storage (more than ten years) of lettuce seeds at 5 °C.

Lettuce adapts itself to temperatures in the range of 18 – 25 °C, however, genetic enhancement techniques allowed the development of cultivars with tolerance to wider temperature ranges, providing production cycles throughout the year in several regions of the country (AZEVEDO FILHO, 2017). Lettuce seeds have an optimum germination temperature of 20 °C. Most cultivars do not germinate at temperatures above 30 °C (NASCIMENTO; CANTLIFFE, 2002), and below 2 °C (MARCOS FILHO, 2005). Thus, depending on the cultivation place and planting season, the seeds may not germinate at all (NASCIMENTO; CANTLIFFE, 2002).

The search for organic seeds with germination quality proves to be a concern for the establishment of a large-scale organic production system for this vegetable. The present work aimed to evaluate the germinative performance of lettuce seeds for potential use in organic production, from conventional untreated seeds and landrace organic seeds from commercial and family production sources.

Materials and methods

Seed source and characterization

The seeds studied in this work were from three batches. Batch 1: conventional seeds produced in 2018, without treatment, of the ‘crispa’ variety (leaf lettuce), classified as S1 and stored at 11 – 13 °C and 41 – 42 % RH, produced in the town of Nova Porteirinha (MG), Southeast Brazil. Batch 2: Landrace seeds from commercial production, from 2017 harvest, of the ‘crispa’ variety, and stored at approx. 10 °C and 40 – 50 % RH, produced in the town of Candiota (RS), South Brazil. Batch 3: landrace seeds from organic family production, of the ‘crispa’ variety, produced in 2018 in the town of Farroupilha (RS), South Brazil, and stored in paper envelopes at room temperature and relative humidity. This landrace cultivar was reproduced on the property for approximately 40 years.

Seed germinative tests

The following tests were carried out: germination percentage, first germination count, germination speed index, accelerated aging test, resistance to cold, and tetrazolium test.

The germination test was performed following the procedures described in the Rules for Seed Analysis - RAS (BRASIL, 2009), at the temperature of 20±2 °C, photoperiod of 8 h of light and 16 h of dark, and relative humidity of 90 %, being evaluated daily at 15:00 h, for seven days. The germination percentage was calculated using equation 1.

$$G(\%) = 100 \times \frac{N}{A} \qquad (1)$$

Being ‘G’ the germination percentage, ‘N’ the number of germinated seeds, and ‘A’ the number of seeds in the sample. The seed was considered germinated when radicle length was equal to or greater than 2.0 mm.

The first germination count was carried out concomitant with the germination test, based on the number of normal seedlings, with radicle length equal to or greater than 2.0 mm, on the fourth day of the germination test (FRANZIN, 2004b).

Germination speed index (GSI) was also carried out concomitant with the germination test. Seedlings were evaluated at the same time as the germination test. The GSI was calculated using equation 2, proposed by Maguire (1962).

$$IVG = \sum_{x=1}^{n} \frac{G_x}{N_x} \qquad (2)$$

Being 'GSI' the germination speed index, 'Gx' the number of normal seedlings counted in the evaluation 'x', and 'Nx' the number of days between sowing and evaluation 'x'.

The accelerated aging test was carried out using a 'gerbox' type plastic box, 40 mL of distilled water was added, and an aluminum screen covered with blotting paper was put inside the box (FRANZIN *et al.*, 2004a). A uniform layer of seeds with approx. mass of 0.5 g was put on the paper and dispersed homogeneously (MARCOS FILHO, 2005). The boxes were capped and transferred to an accelerated aging chamber at 41±1 °C for 24 h, 48 h, and 72 h. After, the seeds underwent the germination test immediately, as previously described (FRANZIN *et al.*, 2004a).

To carry out the test of resistance to cold, the seeds of each replicate were distributed on a blotting paper moistened with distilled water and kept at 5±1 °C and 10±1 °C in a cold chamber for seven days. After, the seeds underwent germination test immediately, as previously described (GODOY *et al.*, 2012). Seed viability was evaluated by the tetrazolium test, following the RAS procedures (BRASIL, 2009).

Experimental design and statistical analysis

The experiment was carried out with a completely randomized design, with three treatments (each seed batches) and four replicates of 100 seeds, totaling 400 seeds per treatment in the tests of germination, accelerated aging test, and resistance to cold. Tetrazolium test was carried out using four replicates of 50 seeds, totaling 200 seeds per treatment.

The obtained results underwent Levene's test (homoscedasticity) and Shapiro-Wilk test (normality of residuals), followed by Analysis of Variance (ANOVA) and Tukey's multiple range test at a 5 % error probability ($\alpha = 0.05$). The statistical analyses were carried out using the AgroEstat® software.

Results and Discussion

Germination tests and germination speed index

Despite the intrinsic and extrinsic factors that differentiate the lettuce seeds studied in this work, the percentages of first count and germination showed the characteristic of quick seedling emergence and highlight the differences in seed longevity, as observed by Marcos Filho (2005) when analyzing the physiology of lettuce and other crop plants.

The percentages of germination, germination in the first count, and GSI values for the evaluated lettuce seeds are presented in Table 1, in which it is possible to observe significant differences between the batches.

Table 1 - Germination percentage obtained in the first count, germination test, and germination speed index (GSI) of lettuce seeds from different sources, tested at 20±2 °C and a photoperiod of 8 h of light and 16 h of dark.

Source	First germination count (%)	Germination (%)	GSI
Batch 1	94.75 a	95.50 a	54.62 a
Batch 2	65.00 c	72.00 b	32.36 b
Batch 3	83.75 b	92.75 a	39.27 b
CV[1] (%)	6.46	6.47	18.24
MSD[2]	10.35	11.08	15.16

Batch 1: conventional seeds without treatment. Batch 2: commercial organic seeds. Batch 3: organic seeds from family farming. Means in column followed by the same letter do not differ statistically by Tukey's multiple range test at a 5 % error probability (α = 0.05). [1] – Coefficient of variation. [2] – Minimum significant difference.
Source: authors (2022).

In the first germination count, Batch 2 had the lowest germination percentage (65 %), being necessary to stress that these seeds were produced in 2017, whereas the seeds from batches 1 and 3 were produced in 2018, these ones had germination percentages of 94.75 % and 83.75 %, respectively. According to Marcos Filho (2005), the seeds from Batch 1 may be considered as having more vigor since seeds that present a higher germination percentage in the first count are regarded as more vigorous.

The results of the germination test showed that only Batch 2 differed statistically, with a germination percentage below 80 %. However, high germination percentages may not characterize seeds with greater field fitness, considering that the conditions in which the test was carried out are regarded as the ones that allow for the maximum expression of seed potential (WEBER *et al.*, 2010).

According to Nascimento, Croda, and Lopes (2012), commercial seeds have a high germination percentage when exposed to 20 °C, which is favorable to this species. This was observed in the present work; however, it was noted that the seeds from family farming are also capable of reaching high germination percentages. This was the case of Batch 3, which had a germination percentage of 92.75 %, having a similar performance to Batch 1, which had 95.50 %.

Germination percentages above 90 % were also observed by Nascimento, Croda, and Lopes (2012) when analyzing the thermotolerance of 20 lettuce cultivars, tested with the same conditions used in this work. According to the authors, only one cultivar had a germination percentage below 90 %. Paiva, Lopes, and Costa (2017), evaluating the germination of organic coriander seeds, reported germination percentages above 90 %, a behavior like those observed for Batch 3 in the present work.

Regarding GSI values, Batch 1 had the highest GSI, 54.62, being the most vigorous. Batches 2 and 3, both composed of landrace seeds, had similar GSI values (32.36 and 39.27, respectively). According to Oliveira *et al.* (2009), the higher the GSI, the more vigorous the seeds.

Bufalo *et al.* (2012), evaluating stratification times for lettuce seeds exposed to different light and temperature conditions, reported a GSI of 59.85, similar to the GSI observed for Batch 1, considering seeds without physiological conditioning for the germination test.

Catão *et al.* (2014), verifying the physiological and biochemical aspects in lettuce seeds exposed to different temperatures, have observed for some commercial cultivars GSI values ranging between 31.7 and 37.2, results similar to the ones observed for batches 2 and 3, composed of landrace seeds, considering the temperature of 20 °C. Thus, it is possible to observe that the production system has not affected the GSI in the seeds evaluated in the present work.

According to Marcos Filho (2005), the genotype and aging process may influence seed vigor and germination potential, increasing or reducing emergence speed. The same author commented that the physiological characteristics related to seed vigor, obtained by the GSI test, may be associated with tissue deterioration. This way, the smaller GSI values observed for Batch 2 regarding Batch 1 may be related, among other factors, to tissue deterioration caused by aging since Batch 2 was produced one year before the other two batches. However, Batch 3 also had lower GSI and vigor, as could be seen in the tetrazolium test (Table 2), despite the higher first germination count and similar performance to Batch 1 in the germination test (Table 1), indicating that this may not be a storage or harvest issue.

Regarding seed resistance to high temperatures, it was observed in the accelerated aging test that no seed germinated after exposure to the accelerated aging chamber for 24 h, 48 h, and 72 h, regardless of seed batch. Similar results were reported by Fiordalisi (2012), who compared lettuce cultivars; the seeds were susceptible to stress caused by high temperature and relative humidity, even at short exposition times (e.g., 24 h). Nascimento and Cantliffe (2002), evaluating the effects of high temperatures on lettuce seed germination, reported that the seeds failed to germinate after exposition to high temperatures (30 °C).

Ramos *et al.* (2004), evaluating the accelerated aging of arugula seeds, reported that vegetable species with small seeds may have some limitations in the accelerated aging test. This happens because of the reduced seed size, with prompts the seed to absorb water quickly and unevenly. This may cause behavior variations within the tested sample and a drastic reduction of germination, resulting in a more intense deterioration.

This was also commented by Frandoloso *et al.* (2017), stating that the sudden and massive sorption of water by the seeds prevented an internal organization of cell membranes during first imbibition, hindering cell development. Moreover, metabolic changes (DNA transcription, protein synthesis, among others) associated with a higher temperature may also have prevented seed germination (ADETUNJI *et al.*, 2020; FRANDO-

LOSO *et al*., 2017). Thus, the lack of germination in the lettuce seeds observed in the accelerated aging test observed in this work may also be related to small seed size and/or metabolic changes caused by high temperature and humidity.

Tests of resistance to cold and tetrazolium

The results of resistance to cold at 5 °C and 10 °C and tetrazolium test are presented in Table 2, in which significant differences can be seen among the three seed batches.

Table 2 - Germination percentages of lettuce seeds from different sources after the tests of resistance to cold at 5 °C and 10 °C and percentage of viable seeds obtained by tetrazolium test.

Source	Cold test at 5 °C	Cold test at 10 °C	Tetrazolium test
Batch 1	86.00 a	98.75 a	83.00 a
Batch 2	94.00 a	66.75 b	66.00 b
Batch 3	85.00 a	95.00 a	76.50 b
CV[1] (%)	10.75	6.84	6.38
MSD[2] (%)	18.75	11.73	9.46

Batch 1: conventional seeds without treatment. Batch 2: commercial organic seeds. Batch 3: organic seeds from family farming. Means in column followed by the same letter do not differ statistically by Tukey's multiple range test at a 5 % error probability ($\alpha = 0.05$). [1] – Coefficient of variation. [2] – Minimum significant difference.
Source: authors (2022).

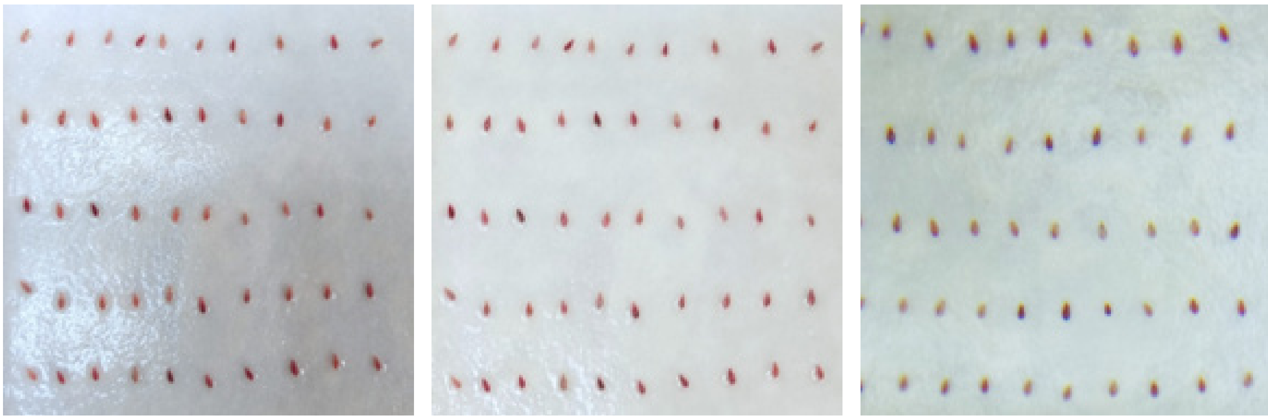
When exposed to the temperature of 5 °C, the three seed batches performed similarly with an average germination percentage of 88.33 %. It can be observed a possible greater resistance to cold in Batch 2 when comparing the germination percentages of the cold test at 5 °C and the germination test at 20 °C (Table 1). However, this resistance at 5 °C was not observed when the temperature was increased to 10 °C because Batch 2 had a germination percentage of 66.75 %, a value smaller than the one of batches 1 and 3, with germination percentages of 98.75 % and 95.00 %, respectively.

The test of resistance to cold at 10 °C of lettuce seeds allowed for a differentiation of the seeds' batches studied in this work, in which only Batch 2 differed statistically from the other two batches, with a smaller germination percentage. Coraspe-Léon, Idiarte, and Minami (1993) reported a germination percentage of 93.75 % for non-pelletized lettuce seeds exposed to 10 °C, a result that was similar to the ones observed for batches 1 and 3.

Nascimento and Cantliffe (2002) stated that seeds produced under conditions of high temperature tend to germinate better in environments with high temperatures than when produced at low temperatures. Thus, it is possible that the results obtained in the tests of resistance to cold at 5 °C were influenced by the environmental conditions in which the seeds were produced. The same authors evaluated the effect of different temperatures on the germination of lettuce seeds, ranging from 5 °C to 35 °C, and concluded that seed susceptibility to temperature depends on the genotype, and the germination temperatures ranged between 5 °C and 33 °C.

Regarding the tetrazolium test (Table 2; Figure 1), Batch 1 had a seed viability percentage of 83.0 %, which indicates a high vigor, whereas Batch 2, with poorer performance, had a viability percentage of 66.0 %, which was statistically similar to Batch 3, with 76.5 %. In this work, even with similar germination percentages (Table 1), batches 1 and 3 differed between themselves regarding seed vigor, in which Batch 1 had a greater vigor in relation to the other two batches.

Figure 1 - Results of the tetrazolium test of seeds from the three batches studied. Source: authors (2022).



Batch 1 (commercial seeds) | Batch 2 (commercial seeds produced under organic practices) | Batch 3 (landrace seeds)

Bhering, Dias, and Barros (2005), reviewing the methods used for germination test in watermelon, stated that seed vigor and germination percentage are complementary parameters. The former refers itself to the seed's physiological potential to germinate and generate normal seedlings, whereas the latter only refers to the emergence potential of the seedlings. Fiordalisi (2012) highlighted the lack of a well-defined methodology for the tetrazolium test in seeds from different botanical families. This issue was also observed for lettuce seeds, especially regarding the coloration time and the coloration pattern of the seeds, although several works carried out tetrazolium tests in vegetable seeds. This issue is in line with what is reported by Bhering, Dias, and Barros (2005), who commented that the obtainment of an adequate and uniform coloration is paramount to carrying out a safe and efficient interpretation of tetrazolium test. However, the tetrazolium test is not widely used in the testing of vegetable seeds, being more used to evaluate grains and the seeds of forage grasses.

The results obtained through the performing of physiological tests on the three seed batches showed advantages related to the germinative potential and seed vigor for Batch 1. However, these advantages have not stood out when the seed resistance to cold at 5 °C and accelerated aging test were carried out, indicating that landrace seeds may be employed successfully in specific environments and without great losses in common field conditions.

Conclusion

It could be observed that the landrace seeds had a germinative performance and resistance to cold similar to the ones of the commercial variety. Thus, landrace seeds may be considered a viable alternative to commercial seeds to be employed in organic production systems.

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