Enviromental temperature and age of seeds in tolerance to thermoinhibition in lettuce genotypes[1]

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ABSTRACT

Lettuce seeds usually show germination problems that can be related to dormancy and or thermoinhibition, as well as to the genotype constitution. The knowledge of the temperature at which the thermoinhibition process begins, as well as the influence of the age of the seed on its germination, is extremely important to establish more suitable parameters for the selection of higher genotypes in breeding programs. The objective of this work was to evaluate the seed germination of three lettuce cultivars according to the enviromental temperature and the age of the seeds. The cultivars Everglades (tolerant to thermoinhibition), Luisa (medium tolerant) and Verônica (sensitive) were used. Seeds of each cultivar were evaluated by the standard germination test, first count test, germination speed index and germination test of remaining seeds. The analyses were performed at four environmental temperatures and in seven storage periods after harvest. None of the cultivars showed primary dormancy. The tolerance to thermoinhibition showed by cultivars Everglades and Luisa and the sensitivity of cultivar Verônica were confirmed. The most suitable temperature for differentiating tolerant and sensitive thermoinhibition genotypes occurred from 30.5 ºC and 260 days after harvest.

Keywords: *Lactuca sativa* L; germination; seed storage.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is the principal leafy vegetable produced and consumed in Brazil. Its cultivation is found in all Brazilian regions. Despite the advances achieved by the breeding programs over the years (Sala & Costa, 2012), lettuce seeds are sensitive to variations in the humidity and temperature of the medium in which they germinate. Germination problems result in poor quality and a delay in seedling production, causing a drop in yield and direct losses for the producer (Nascimento *et al.*, 2012; Bufalo *et al.*, 2012).

Environmental temperature is the primary factor that regulates seed germination, acting directly through the germination itself, or indirectly, through dormancy and viability, even when the water availability is enough to enabling seed soaking (Deng & Song, 2012). In general, lettuce seeds show maximum germination at temperatures around 20 °C, while most cultivars do not germinate when exposed to temperatures higher than 30 °C (Villela *et al.*, 2010).

High temperatures during the imbibition and or environmental storage of the seed may lead to the occurrence of two distinct phenomena known as thermoinhibition and

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thermo-dormancy. Both mechanisms promote the reduction of seed germination ability, however, they differ in their natural reversibility or not (Catão *et al.*, 2014). Thermoinhibition is naturally reversible from the moment when all factors promoting inhibition such as temperature, light and humidity return to adequate values for germination. From other hand, thermo-dormancy, when installed in the seed, does not revert naturally, so there is a need to proceed with some type of treatment in the seed for occurrence of germination, even if there are favorable environmental conditions to germination (Yoong *et al.*, 2016).

The correct storage can favor the breakdown of thermodormency naturally during the period. However, there must be controlled by environmental factors, especially temperature, when elevated it can reduce the germination rate (Catão *et al.*, 2016. Once in Brazil the lettuce cultivation is done majority on southeast region, and the temperatures are often found at or higher than 30 °C the thermodorment seeds cannot germinate, but lettuce genotypes that can be resistant to thermoinhibition can germinate even under higher temperature, expanding the regions where the crop can be cultivated and, consequently, reducing the seasonality of lettuce supply in tropical regions. In this sense, there is a need to grow lettuce cultivars that have a higher temperature limit for seed germination (Bufalo *et al.*, 2012; Catão *et al.*, 2014).

Previous researches has been showed that there is a genetic variability associated with thermoinhibition resistance, as well as the physiological mechanisms enrolled in lettuce seeds germination under higher temperatures, such as abscisic acid, gibberellin and ethylene content and the genes involved in the synthesis and degradation of theses hormones, among other compounds (Argyris *et al.*, 2008; Huo *et al.*, 2013; Gonai *et al.*, 2004).

Understand the behavior of thermotolerant genotypes in relation to the thermosensitive ones, and the hypothesis of correspondence with the effect of temperature to the age of the seed, as well as to establish a minimum temperature for which there is a percentage of germination able to different these genotypes, it becomes of great importance to improve the efficiency in selecting higher genotypes in lettuce breeding programs. Thus, the objective of this study was to elucitate the effect of temperature and storage period on the characteristics related to lettuce seed germination, in order to identify more suitable parameters for the selection of thermoinhibition-tolerant genotypes.

MATERIAL AND METHODS

The first step of the experiment was carried out in a protected environment, located at 831 meters above sea level within the coordinates 21º 09'24" S and 44º 55'34" W longitude. Sowing was performed in April and harvest in October. Seeds of the Everglades cultivar considered tolerant to thermoinhibition (Almeida *et al.*, 2019), 'Verônica'', considered sensitive to thermoinhibition (Kano *et al.*, 2011; Villela *et al.*, 2010) and 'Luisa', which shows intermediate tolerance to thermoinhibition (Catão *et al.*, 2014; Villela *et al.*, 2010) were sown in polypropylene trays filled with commercial substrate for vegetables to obtain the seedlings. Then, they were transplanted into six-litter plastic pots containing a mixture of soil, sand and commercial substrate in a 3:3:1 ratio. The pots were arranged in a completely randomized design with fifteen plants from each cultivar that were conduct until maturation and seed harvest. When the seeds of the plants reached the harvest point, approximately 80% of them ripened, six plants of each cultivar were chosen, which showed greater coincidence of flowering and maturation, and seeds were harvested separately, constituting six samples for each cultivar.

The maximum and minimum room temperatures during the seed formation and maturation phases were 27.3 °C and 14.1 °C from August, beginning of anthesis, to October, harvest phase of the seeds of the three cultivars.

After harvesting, the seeds were cleaned and dried until they reached around 6 to 7% of water content. Afterwards, the six samples of each cultivar were mixed homogeneously and packed, forming a lot of each cultivar. The seeds were stored in a cold chamber at 15 °C and relative humidity of 50%.

The analyses were carried out at the Central Seed Laboratory of the Federal University of Lavras at 40, 70, 100, 130, 160, 190 and 260 days after harvest (DAH), corresponded up to eight months after seeds harvest, using samples from 2,000 seeds of each lettuce cultivar, in each period. The tests described below were performed according to the Rules for Seed Analysis (RSA) for lettuce culture (Brasil, 2009).

For the germination test, sowing was performed on two

sheets of blotting paper, moistened 2.5 times the weight of the dry substrate with water in gerbox-type plastic boxes. The boxes with the seeds were kept in "biochemical oxygen demand" (BOD) chambers from the ELETROlab company, model EL202 / 4 LED, in alternating light and temperature regimes: 12 hours in the dark and 12 hours in light, at temperatures of 20 °C, 25 ºC, 30 °C and 35 ºC. For vigor assessment, the first germination count was performed on the fourth day after sowing. The first count was performed in conjunction with the germination test. On the seventh day, total germination was observed and seedlings were evaluated, according to Brasil (2009). Ten sub-samples of 50 seeds of each cultivar were analyzed. The results were expressed as a percentage of normal seedlings in the germination test. To evaluate the Germination Speed Index (GSI), daily germination counts were performed from the appearance of the first seedling and continuing until the seventh day. Using the data collected daily, the GSI was determined, using the formula proposed by Maguire (1962). After the end of each germination test, for the highest temperatures of 30 °C and 35 °C, the seeds that did not germinate were submitted to a Germination Test of Remaining Seeds (R) at 20 °C. The BOD temperature was adjusted to 20 °C and the seeds remained in this condition for seven days. A new evaluation was carried out on the seventh day, considering as germinated the normal seedlings of each repetition. At the end, the mean of the replicates was obtained, with data expressed as a percentage of germination, in relation to the total number of seeds that did not germinate.

A completely randomized design was used, with ten replicates for all tests. The data obtained in each test were submitted separately to the analysis of variance using the Sisvar software (Ferreira, 2011). The comparison of the means obtained in the germination tests, first count and germination speed index were made using the Scott-Knott test ($P < 0.05$) (Scott & Knott, 1974). Regression analyses were also carried out in order to adjust the germination response curves of the three cultivars according to the temperature and storage.

RESULTS

There were significant differences for all the characteristics evaluated, both in relation to the temperatures used in the analysis as well as the storage periods of the seeds.

Differences in germination results (Table 1) occurred more significantly for the tests performed at 30 °C and 35 °C. In all situations, the germination of the seeds of the three cultivars in lower environment temperatures, both at 20 °C and 25 °C, for the analyses carried out at 40 DAH and 70 DAH were equal to, or greater than 95%.

Table 1: Germination Test (%G) for Everglades, Luisa and Verônica cultivars between and within storage periods * for temperatures of 20 °C, 25 °C, 30 °C and 35 °C

	20 °C			25 °C			30 °C			35 °C		
DAC	**E**	**L**	**V**	**E**	**L**	**V**	**E**	**L**	**V**	**E**	**L**	**V**
40	98 Aa	98 Aa	95 Bb	99 Aa	99 Aa	98 Aa	15 Dd	26 Ca	5 Cc	1 Ea	0 Cb	0 Bb
70	99 Aa	99 Aa	98 Aa	99 Aa	98 Aa	99 Aa	37 Ca	33 Ca	10 Cb	1 Ea	1 Ca	0 Ba
100	98 Aa	99 Aa	98 Aa	97 Ba	99 Aa	97 Aa	50 Ba	63 Ba	34 Bb	4 Ea	0 Cb	0 Bb
130	98 Aa	98 Aa	99 Aa	99 Aa	98 Aa	98 Aa	98 Aa	91 Ab	91 Ab	21 Da	1 Cb	0 Bb
160	98 Aa	98 Aa	97 Aa	97 Ba	99 Aa	97 Aa	99 Aa	99 Aa	95 Ab	44 Ba	3 Cb	1 Bb
190	99 Aa	99 Aa	98 Aa	98 Aa	98 Aa	98 Aa	99 Aa	99 Ab	97 Aa	33 Ca	13 Bb	1 Bc
260	99 Aa	100 Aa	99 Aa	100 Aa	99 Aa	99 Aa	96 Aa	99 Aa	94 Ab	58 Aa	55 Aa	2 Ab

Means followed by the same lower-case letters in the lines and the same upper-case letters in the columns for each temperature belong to a same group by the Scott & Knott test ($P < 0.05$). Everglades (E), Luisa (L) and Verônica (V) * Days after harvest (DAH).

At 20 °C, a significant difference was observed only for the seeds of cultivar Verônica at 40 DAH whose germination was 95% (Table 1), a value lower than the germination of the other cultivars in this period, as well as the cultivar Verônica itself, considering the analyses that had been carried out from 70 DAH.

For the analyses carried out at 30 °C, there is a significant reduction in the germination of the three cultivars in the shortest storage periods until 100 DAH. The germination percentage of the seeds of the three cultivars were significantly lower in the first evaluation period at 40 DAH, with a progressive increase in germination for the analyses carried out at 70 DAH, 100 DAH and 130 DAH when they reached the values of 98% and 91% for Everglades

and both Luisa and Verônica, respectively. From the 160 DAH onwards, the cultivar Verônica, considered sensitive to thermoinhibition or denominated thermosensitive in this experiment, despite showing a germination equal to, or greater than 94%, differed significantly from cultivars Everglades and Luisa, considered tolerant to termoinibition, which showed no differences between each other at 160 and 260 DAH.

At 35 °C, a drastic reduction was observed in the germination of the seeds of all cultivars, until the analysis performed at 100 DAH. The values ranged between 0% and 1% for the three cultivars, with the exception of the Everglades, which showed a slight increase at 100 DAH when germination reached 4%. After this period, it was observed an increase in the germination value of the seeds of the cultivar Everglades, which were 21% at 130 DAH, 44% at 160 DAH, 33% at 190 DAH and 58% at 260 DAH, while seed germination of cultivars Verônica and Luisa remained at levels close to 1% until 160 DAH. For the analyses carried out at 190 DAH and 260 DAH, the cultivar Verônica continued with low values, 1% at 190 DAH and 2% at 260 DAH. On the other hand, the seeds of cultivar Luisa responded differently, with germination of 13% at 190 DAH and 55% at 260 DAH, not differing from cultivar Everglades in this last evaluation (Table 1).

In the first count test (%FC) performed at 20 °C, it is found that at 40 DAH, the Everglades cultivar presented a higher percentage of seedlings developed with a value of 70%, followed by the cultivar Luisa (23%) and finally, by the cultivar Verônica (3%) (Table 2).

Table 2: First Count Test (%FC) for Everglades, Luisa and Verônica cultivars between and within storage periods * for temperatures of 20 °C, 25 °C, 30 °C and 35 °C

	20 °C			**25 °C**			**30 °C**			**35 °C**		
DAC	**E**	**L**	**V**	**E**	**L**	**V**	**E**	**L**	**V**	**E**	**L**	**V**
40	70 Ba	23 Bb	3 Cc	93 Aa	77 Ba	48 Cb	12 Ca	11 Da	3 Db	1 Ca	0 Bb	0 Bb
70	52 Ba	16 Bb	13 Cb	96 Aa	57 Cb	72 Bb	34 Ba	8 Db	4 Db	0 Ca	0 Ba	0 Ba
100	96 Aa	96 Aa	89 Ab	10 Cb	32 Da	10 Db	33 Ba	23 Ca	14 Ca	0 Ca	0 Ba	0 Ba
130	89 Aa	95 Aa	95 Aa	81 Ba	89 Aa	72 Bb	90 Aa	65 Bb	57 Bb	2 Ca	0 Bb	0 Bb
160	97 Aa	96 Aa	77 Bb	97 Aa	99 Aa	93 Ab	93 Aa	95 Aa	83 Ab	2 Ca	0 Bb	0 Bb
190	95 Aa	99 Aa	93 Aa	98 Aa	98 Aa	97 Aa	94 Aa	85 Aa	50 Bb	18 Ba	6 Bb	0 Bb
260	96 Aa	97 Aa	95 Aa	99 Aa	99 Aa	99 Aa	92 Aa	94 Aa	87 Ab	46 Aa	31 Ab	2 Ac

Means followed by the same lower-case letters in the lines and the same upper-case letters in the columns for each temperature belong to a same group by the test of Scott & Knott test ($P < 0.05$). Everglades (E), Luisa (L) and Verônica (V) * Days after harvest (DAH).

In relation to the analyses performed at this same temperature at 70 DAH, the cultivar Everglades showed 52% FC, while the cultivars Luisa and Verônica did not differ one from each other, and presented 16% and 13% FC values, respectively. From the analyses performed at 100 DAH at 20 °C, there was a significant increase in the %FC values for all cultivars. However, there was no significant difference between cultivars Everglades and Luisa with average FC values of 96%. Cultivar Verônica differed for this characteristic only in the analyses carried out at 100 and 160 DAH, showing values of 89% and 77%, respectively. At 190 and 260 DAH, no difference was observed between cultivars and the %FC values were equal to or greater than 93%.

For the first count tests performed at 25 °C, a more vigorous response of the seeds was observed in the tests performed at 40 DAH and 70 DAH. It is also observed that the greatest vigor of the thermotolerant cultivar Everglades was maintained.

The results obtained for the analyses carried out from 160 DAH are similar to those obtained at 20 °C, with the vigor of the seeds of all cultivars being equaled from 190 and 260 DAH, in this case with values equal to or greater to 97%.

When the environmental temperature of the analysis was raised to 30 °C, as in the case of the germination test (Table 1), a significant drop in the vigor of the seeds was observed in all cultivars in the shortest storage periods until 100 DAH. A low vigor is observed, which was significantly lower in the first analysis period (40 DAH) where the values of 12%, 11% and 3% were observed for cultivar Everglades, Luisa and Verônica, respectively. At this temperature, the vigor has recovered from 100 DAH for the thermotolerant cultivar Everglades, whose FC value

was 90%, differing significantly from the cultivars Luisa and Verônica (Table 2). From 160 DAH, the FC% value of cultivar Luisa is equal to that of Everglades, differing from Verônica, reaching the FC values of 92%, 94% and 87% for seeds of cultivars Everglades, Luisa and Verônica, respectively, at 260 DAH.

It is observed that the Everglades cultivar, which is tolerant to thermoinhibition, presented a vigor relatively superior to the other cultivars until 130 DAH and also superior to Verônica, which is thermosensitive, until the last analysis carried out at 260 DAH (Table 2).

When considering the analyses carried out at 35 °C, a significantly low vigor is observed for all cultivars, which do not differ until the evaluations carried out at 160 DAH and show values of FC% equal to 2% at most (Table 2). At 190 DAH, Everglades cultivar had an increase in the FC% value of its seeds, reaching 18% and differing from the cultivars Luisa and Verônica, which presented values of 6% and 0% respectively. For the analyses carried out at 260 DAH, there was an increase in the vigor of the seeds of the cultivar Everglades, whose FC reached 46%, differing from the cultivar Luisa, which also had an increase in vigor, but reaching the value of 31%, only. The cultivar Verônica, on the other hand, did not show an increase in vigor, with the FC value remaining at 2%.

Regarding the germination speed index (GSI), the Everglades cultivar showed the highest GSI, followed by the cultivar Luisa and then by the cultivar Verônica at a temperature of 20 °C until 70 DAH, being equal from 130 DAH on (Table 3).

Table 3: Germination Speed Index (GSI) for Everglades, Luisa and Veronica cultivars between and within storage periods * for temperatures of 20 °C, 25 °C, 30 °C and 35 °C

	20 °C			25 °C			30 °C			35 °C		
DAC	**E**	**L**	**V**	**E**	**L**	**V**	**E**	**L**	**V**	**E**	**L**	**V**
40	11,6 Ca	9,7 Cb	8,1 Dc	14,2 Ca	13,6 Ca	11,0 Cb	2,2 Db	4,3 Ea	0,8 Ec	0,1 Da	0,0 Cb	0,0 Bb
70	11,2 Ca	9,8 Cb	9,1 Cb	13,9 Ca	12,2 Db	11,8 Cb	5,5 Ca	3,5 Eb	1,2 Ec	0,1 Da	0,1 Ca	0,0 Bb
100	14,4 Aa	14,9 Aa	12,1 Bb	9,9 Da	10,7 Ea	9,5 Db	5,9 Ca	6,4 Da	3,6 Db	0,3 Da	0,0 Cb	0,0 Bb
130	14,0 Aa	12,9 Ba	13,3 Aa	13,4 Ca	13,1 Ca	12,1 Cb	14,2 Ba	11,8 Cb	11,3 Cb	2,2 Ca	0,1 Cb	0,0 Bb
160	12,3 Ca	12,3 Ba	11,7 Bb	15,1 Ba	14,2 Ba	13,6 Bb	13,8 Ba	13,1 Ba	12,3 Ba	4,1 Ba	0,3 Cb	0,1 Bb
190	13,1 Ba	13,1 Ba	12,3 Ba	16,1 Aa	16,3 Aa	14,8 Ab	14,3 Aa	13,4 Ba	10,9 Cb	3,8 Ba	1,5 Bb	0,0 Bc
260	13,2 Ba	12,6 Ba	12,4 Ba	16,3 Aa	15,4 Aa	15,2 Aa	15,7 Aa	15,4 Aa	14,6 Ab	7,6 Aa	6,5 Aa	0,4 Ab

Means followed by the same lower-case letters in the lines and the same upper-case letters in the columns for each temperature belong to a same group by the test of Scott & Knott test ($P < 0.05$). Everglades (E), Luisa (L) and Verônica (V) * Days after harvest (DAH).

At a temperature of 25 °C, the GSI of cultivars Everglades and Luisa only differed in the analysis carried out at 70 DAH, when seeds of cultivar Luisa showed lower GSI than those of cultivar Everglades. The cultivar Verônica had the lowest IVG in all evaluated periods, apart from the last evaluation period, 260 DAH.

At 30 °C, the results for cultivars Luisa and Everglades differed for the periods of 40, 70 and 130 DAH, in which cultivar Luisa showed a lower GSI. For the cultivar Verônica, only in 160 DAH the seeds reached the same germination speed, but did not maintain this increase in the GSI, with a performance below of those observed in cultivars Luisa and Everglades in the other evaluated periods.

At 35 °C, GSI had a dramatically dropin germination speed. Seeds of cultivars Everglades and Luisa reached the same speed in the last period, at 260 DAH, but there was a reduction of around 50% in germination speed, when compared with the other temperatures evaluated. 'Verônica' seeds did not show germination speed, only in the last period when the means differed from zero, but lower than the GSI of cultivars Luisa and Everglades.

Figure 1A shows the percentages of seeds that germinated at a temperature of 30 °C, in the germination test (%G), and those that germinated at a recommended temperature of 20 °C, in the evaluation of the remaining seeds (%R). As previously mentioned, the shorter storage periods, 40, 70 and 100 DAH had a low germination in %G for all cultivars under temperatures higher than 30 ºC. The germination of the three cultivars showed values below than 70% in these storage periods. However, after the germination test at 30 ºC, the seeds of each cultivar at 40, 70 and 100 DAH were able to germinate at 20 ºC, in the remaining germination test, reaching the value of 100% of germination of normal seedlings.

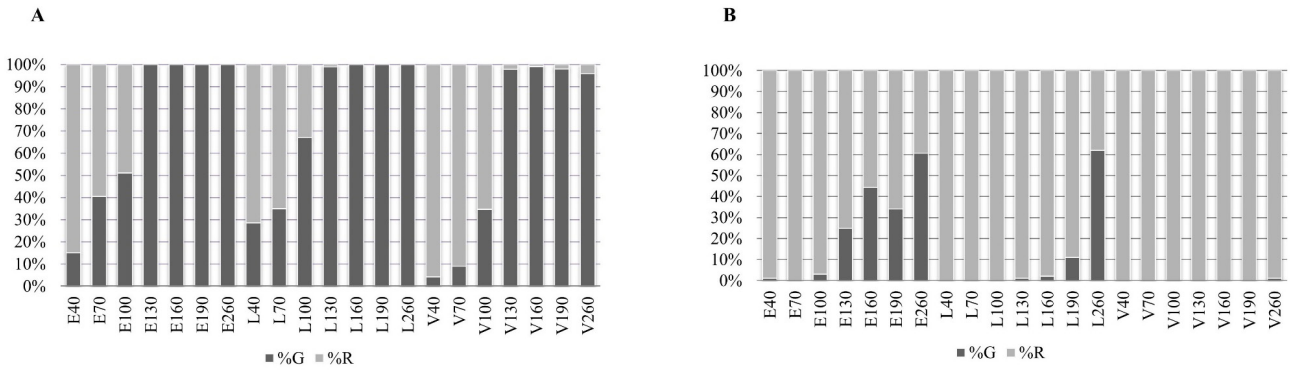

Figure 1: Germination test (%G): A- at 30 °C and test of remaining seeds (%R) at 20 °C of the cultivars Everglades (E), Luisa (L) and Verônica (V) as a function of the days after harvest (40, 70, 100,130, 160, 190 and 260). B - at 35 °C and test of remaining seeds (%R) at 20 °C of cultivars Everglades (E), Luisa (L) and Verônica (V) as a function of the days after harvest (40, 70, 100, 130, 160, 190 and 260).

When the seeds were submitted to the germination test at 35 °C, germination of less than 60% occurred for all cultivars and in all storage periods (Figure 1B). For the percentage of normal seedlings for each cultivar, Everglades and Luisa approached this percentage in the last evaluated period, at 260 DAH. When the temperature was lowered to 20 °C, the seeds were viable as the remaining test shows, and was observed the maintenance of seed quality due to the lack of dead and or abnormal seeds, as shown in Figure 1B.

The responses of each cultivar were analyzed by regression analyses (Figures 2 A, B and C) for four storage periods as a function of the different temperatures. Only four intermediate storage periods, representing the seven analyzed periods were used for a better visualization of the graph.

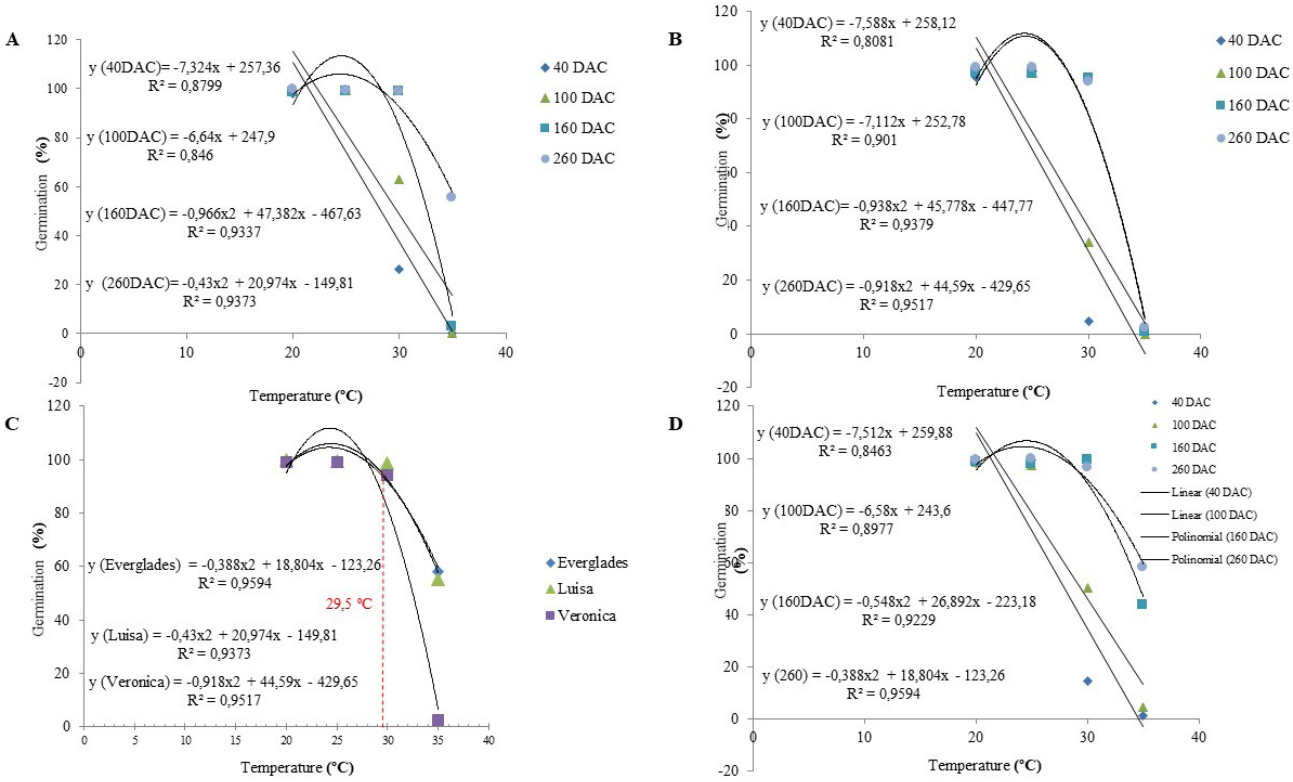

Figure 2: Germination of lettuce seeds in four storage periods (40, 100, 160, 260), as a function of the germination temperature: A- cultivar Everglade; B- cultivar Luisa; C- cultivar Verônica (the vertical red line marks the point that initiates the thermoinhibition). D - Germination of the seeds of three lettuce cultivars as a function of the analysis temperature at 260 days after harvest.

In relation to Everglades cultivar (Figure 2A), it is observed that in the shortest storage periods, that is, 40 DAH and 100 DAH, there was a linear response of germination that decreased as the temperature increased, reaching levels close to 0% germination at 35°C.

On the other hand, for the analyses carried out at 160 DAH and 260 DAH, the responses showed a quadratic behavior, whose equations of the curves demonstrate that from 20.6 °C to 27.8 °C, the seeds reached 100% germination. After, germination is reduced as the temperature is risen. This decrease is more evident in the analysis carried out at 160 DAH, when germination reached 52.4%, than at 260 DAH, when germination reached 59.6%. Similar to Everglades, the germination response showed by cultivar Luisa (Figure 2B) for the different storage periods was similar to 40 DAH and 100 DAH with linear equations that demonstrated the decrease in germination as temperature rose, also reaching values close to 0% at 35 °C.

Regarding the analyses carried out at 160 DAH and 260 DAH, quadratic responses were also observed, with germination reaching 100% at 20.7 °C and remaining at this level up to 28.2 °C. However, from this temperature, there is a difference in relation to the Everglades cultivar as at 160 DAH, the reduction in germination is greater, reaching 17.9%. For the analysis at 260 DAH, the equation shows a smaller reduction in germination as a function of temperature, reaching 57.5%.

Considering the cultivar Verônica (Figure 2C), it was observed that germination at 40 DAH and 100 DAH showed a linear response, similar to the cultivars Everglades and Luisa, reaching the values of 4.1% and 5.4% respectively, when the temperature was 35 °C.

As for the 160-DAH and 260-DAH storage periods, the responses to the rise in temperature in the germination test were also quadratic, similar to Everglades and Luisa cultivars. Nevertheless, in this case, with a reduction in germination at higher levels, therefore differentiating the cultivar Verônica from the cultivar Everglades at 160 DAH, when cultivar Verônica showed a germination of only 6.4% at 35 °C and differentiating itself, both from the cultivar Everglades as for cultivar Luisa at 260 DAH, when the temperature of 35 °C reduced germination to 5.4%.

Thus, it was found that in this storage period, the germination of the three cultivars showed a quadratic response as a function of temperature (Figure 2D). It is observed that up to the temperature of 27.9 °C, all cultivars maintained a germination of 100%.

DISCUSSION

The seeds germination rates equal or higher than 95% under lower environment temperatures at 40 DAH and 70 DAH is an indication that they did not have primary dormancy, which is common in newly-harvested lettuce seeds and can last up to a few months after harvest, when it is naturally overcome (Kano *et al.*, 2011). However, some cultivars such Everglades may not have dormancy, even after some months of seeds harvest (Catão *et al.*, 2018).

Although 20 °C is considered the ideal temperature for germination of lettuce seeds, even adopted in the germination patterns for this species (Brasil, 2009), it appears that at 25 °C, the germination of seeds from all three cultivars, in all assessment periods, was 97% or over. Thus, this temperature can be considered relatively mild, once the germination rate was around the same observed at 20 ºC for these cultivars, not affecting the germination of their seeds (Bufalo *et al.*, 2012; Villela *et al.*, 2010).

Under higher temperatures, such 30 ºC, there was observed a significant reduction in the seed's germination from the three cultivars, and the increase of germination tax after 70 DAH, reaching more than 91% after 130 DAH, and equal or more than 94% at 160 DAH for cultivar Verônica (sensitive to thermoinhibition). This probably suggests that seed storage may contribute to a higher tolerance to germination at high temperatures. Tolerance to thermoinhibition is related to factors such as softening of the endosperm, an increment in the ethylene production, activity of the endo-β-mannanase enzyme, an increase in the gibberellin and a reduction in abscisic acid (Nascimento *et al.*, 2012).

In the present study, cultivar Verônica was sensitive to thermoinhibition at temperatures of 35 °C, as also reported by Villela *et al.* (2010) and Kano *et al.* (2011). The higher tolerance to germination of cultivar Everglades at high temperatures is also confirmed, in addition to a possible tolerance at 260 DAH observed in cultivar Luisa (Catão *et al.*, 2014; Nascimento & Pereira, 2007). The tolerance to the thermoinhibition observed on cultivar Everglades, as well as a possible tolerance of cultivar Luisa only manifested after a certain period of storage, confirming what

had already been demonstrated at 30 °C for all cultivars. At 35 °C, the response of the three cultivars was different. Cultivar Verônica did not show any significant germination values in its seeds, whereas cultivar Luisa showed an increase that started at 190 DAH and starting on 130 DAHS for cultivar Everglades (Table 1).

The results of first count test % (FC) (Table 2) under 20 °C and 25 °C indicate the superiority of cultivar Everglades at 40 DAH and further storage periods, with increase of FC for all cultivars along the time. There was an increment in the seed vigor of all cultivars after a given storage period and the thermotolerant cultivar, Everglades, had greater vigor already in the shorter storage periods of 40 and 70 DAH in relation to the others cultivars. However, under temperature above of 30 °C, the vigor of the seeds had a significant drop, reaching only 2% of FC for the cultivar Verônica under 35 ºC at 260 DAH.

In this sense, it is observed that the vigor of lettuce seeds tends to increase after 70 days of seed storage, when the analyses are performed at the ideal temperature for germination, which is 20 °C. As the germination temperature is raised, the storage period necessary for the seeds to reach greater vigor increases; however, this storage period tends to be shorter for the thermotolerant genotype Everglades. At more extreme temperatures, such as 35 °C, the time necessary for seeds to show vigor is longer, and only observed for the thermotolerant genotypes such as Everglades and possibly thermotolerant such as cultivar Luisa, but with less vigor than observed at lower temperatures. In this condition of high temperatures, on the other hand, the Verônica thermosensitive genotype has no vigor (Table 2). Our results are according with the ones obtained by Catão *et al.* (2018), in which the authors demonstrated that the Everglades cultivar showed greater vigor when compared to the other cultivars, regardless of the storage period.

The mechanisms involved in germination at high temperatures are also related to the vigor of lettuce seeds, both in thermosensitive and in thermotolerant cultivars (Soares *et al.*, 2017). As already mentioned, the shorter storage periods, less than 100 DAH, had a lower germination and vigor for all cultivars under temperatures higher than 30 ºC. However, the seeds of each cultivar at 40, 70 and 100 DAH were able to germinate when the temperature was reduced from 30 ºC to 20 °C, reaching the value of 100% of germination of normal seedlings.

This result shows that the seeds submitted to high temperatures underwent a process of thermoinhibition, which was evident for the three cultivars in the first three evaluation periods. This is confirmed as, for these periods, the remaining seeds germinated normally again when the ideal temperature of germination was restored. It is also observed that there was no evolution in thermodormism, and that the stress to which the seeds were submitted did not promote harmful changes in the embryonic tissues since the germination originated normal seedlings (Figure 1A).

When the germination test was conducted under 35 °C at 260 DAH, there was not observed results in the thermo-dormancy of the seeds in the cultivars Everglades, Luisa and Verônica, showing that there was a temporary thermoinhibition caused by high environmental temperature during germination in all seven evaluated periods, and not a thermo-dormancy, maintaining the ability of germination at 20 ºC and the seed quality.

Regarding the seed germination response of the Everglades, Luisa and Verônica cultivars as a function of the temperatures in the analysis and the storage period, regression analysis was also used to adjust equations that would provide a better understanding and a more adequate response for each case. Our results indicate that the germination is reduced as the temperature is risen and are similar to those found for the Everglades cultivar in other studies (Catão *et al.*, 2014; Nascimento *et al.*, 2004; Nascimento & Pereira, 2007).

It is observed that the curves obtained for the germination of the seeds of the cultivar Everglades demonstrated that the storage of the seeds for longer periods contributes to activate the mechanisms involved in the thermoinhibition control, such as the induction of GA synthesis and ABA repression. (Chaves *et al.*, 2011; Gonai *et al.*, 2004).

Once the time after seeds harvest is increased, there is a smaller reduction in germination as a function of temperature. These results suggest a need for a longer storage period, indicating that cultivar Luisa is able to develop the mechanisms involved in the control of tolerance to thermoinhibition in relation to the Everglades. In addition, these results corroborate those obtained by Villela *et al.* (2010) and Catão *et al.* (2014).

The behavior of regression curves for cultivar Verônica was similar to those observed for Everglades and Luisa. These results corroborate those reported by some authors in order to confirm the tolerance to the thermoinhibition of the cultivar Everglades and the susceptibility of the cultivar Verônica (Catão *et al.* (2014) and Kano *et al.* (2011)).

Based on the graphs that showed the germination curves

of each cultivar for each storage period as a function of the temperature, it can be inferred that at 260 DAH, it is possible to identify, with a greater clarity, genotypes that are tolerant to thermoinhibition. Thus, it was decided to obtain the seed germination curves of each cultivar after this storage period, as a function of the temperature, seeking to characterize the most appropriate temperature to identify genotypes tolerant to thermoinhibition.

From the temperature of 27.9 °C on, there was a differentiation in the results of germination, in such a way that the equation of the curve of the cultivar Verônica, thermosensitive, showed a significantly more drastic reduction in the germination, differentiating it from the tolerant cultivars Luisa and Everglades. Under temperature values above 29.5 °C, the germination percentage of cultivars tends to decrease due to thermoinhibition. According to Sung *et al.* (1998), once the maximum tolerated temperature limit is exceeded, there is a drastic reduction in germination after an increase of 2 to 3 °C, becoming sensitive to thermoinhibition. Thus, it is considered that from a temperature of 30.5 °C at 260 days, thermo-tolerant and thermosensitive cultivars can be differentiated. As a result, this can be taken as a temperature to be used in germination tests, in breeding programs, for the selection of genotypes tolerant to thermoinhibition.

CONCLUSIONS

The mechanisms related to overcoming thermoinhibition in lettuce seeds are activated after a period of seed storage, which varies according to the genotype. The cultivars Everglades and Luisa were tolerant to thermoinhibition, while the cultivar Verônica was sensitive to it. The temperature at which the thermoinhibition process in lettuce seeds begins is 29.5 °C while the most suitable temperature for differentiating tolerant and sensitive thermoinhibition lettuce genotypes is 30.5 °C. The identification of lettuce genotype tolerant to thermoinhibition was effective in seeds from 260 days after harvest.

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REFERENCES

Almeida FA, Silva-Mann R, Santos HO, Pereira RW & Blank AF (2019) Germination temperatures affect the physiological quality of seeds of lettuce cultivars. Bioscience Journal, 35:1143-1152.

Argyris J, Dahal P, Hayashi, E, Still D & Bradford K (2008) Genetic Variation for Lettuce Seed Thermoinhibition Is Associated with Temperature-Sensitive Expression of Abscisic Acid, Gibberellin, and Ethylene Biosynthesis, Metabolism, and Response Genes. Plant Physiology, 148:926-947.

Brasil (2009) Regras para análise de sementes. Brasília, Ministério da Agricultura, Pecuária e Abastecimento - Secretaria de Defesa Agropecuária. 399p.

Bufalo J, Amaro ACE, Araújo HS, Corsato JM, Ono EO, Ferreira G & Rodrigues JD (2012) Stratification periods in seeds germination of lettuce (*Lactuca sativa* L.) under different conditions of light and temperature. Semina: Ciências Agrárias, 33:931-940.

Catão HCR, Gomes LAA, Guimarães RM, Fonseca PHF, Caixeta F & Galvão AG (2018) Physiological and biochemical changes in lettuce seeds during storage at different temperatures. Horticultura Brasileira, 36:118-125.

Catão HCRM, Gomes LAA, Guimarães RM, Fonseca PHF, Caixeta F & Marodin JC (2016) Physiological and isozyme alterations in lettuce seeds under different conditions and storage periods. Journal of Seed Science, 38:305-313.

Catão HCRM, Gomes LAA, Santos HOD, Guimarães RM, Fonseca PHF & Caixeta F (2014) Aspectos fisiológicos e bioquímicos da germinação de sementes de alface em diferentes temperaturas. Pesquisa Agropecuária Brasileira, 49:316-322.

Chaves IDS, Alvarenga AAD, Dousseau S, Soares GCM, Souza EDS & Artur MAS (2011) Germination of Miconia ligustroides (Melastomataceae) diaspores submitted to different treatments for dormancy overcoming. Brazilian Journal of Botany, 34:335-341.

Deng Z & Song S (2012) Sodium nitroprusside, ferricyanide, nitrite and nitrate decrease the thermo-dormancy of lettuce seed germination in a nitric oxide-dependent manner in light. South African Journal of Botany, 78:139-146.

Ferreira DF (2011) Sisvar: a computer statistical analysis system. Ciência e agrotecnologia, 35:1039-1042.

Gonai, T, Kawahara S, Tougou M, Satoh S, Hashiba T, Hirai N, Kawaide H, Kamiya Y & Yoshioka T (2004) Abscisic acid in the thermoinhibition of lettuce seed germination and enhancement of its catabolism by gibberellin. Journal f Experimental Botany, 55:111-118.

Huo H, Dahal P, Kunosoth K, McCalum C & Bradford K (2013) Expression of 9-cis-EPOXYCAROTENOID DIOXYGENASE4 is essential for thermoinhibition of lettuce seed germination but not for seed development or stress tolerance. Plant Cell, 25:884-900.

Kano C, Cardoso AII, Bôas RLV & Higuti ARO (2011) Germinação de sementes de alface obtidas de plantas cultivadas com diferentes doses de fósforo. Semina: Ciências Agrárias, 32:591-597.

Maguire JD (1962) Speed of germination aid in selection and evaluation for seedling and vigour. Crop Science, 2:176-177.

Soares PAM, Souza DAMS, Bernardino DLMP, Azevedo QR, Lacerda JPN & Silva FG (2017) Germination and vigor of lettuce seeds under different temperatures. Brazilian Journal of Agriculture, 92:271-280.

Nascimento WM, Cantliffe DJ & Huber DJ (2004) Ethylene evolution and endo-beta-mannanase activity during lettuce seed germination at high temperature. Scientia Agricola, 61:156-163.

Nascimento WM, Croda MD & Lopes ACA (2012) Produção de sementes, qualidade fisiológica e identificação de genótipos de alface termotolerantes. Revista Brasileira de Sementes, 34:510-517.

Nascimento WM & Pereira RS (2007) Testes para avaliação do potencial fisiológico de sementes de alface e sua relação com a germinação sob temperaturas adversas. Revista Brasileira de Sementes, 29:175-179.

Sala FC & Costa CPD (2012) Retrospectiva e tendência da alfacicultura brasileira. Horticultura brasileira, 30:187-194.

Scott AJ & Knott M (1974) A cluster analysis method for grouping means in the analysis of variance. Biometrics, 30:507-512.

Sung Y, Cantliffe DJ & Nagata RT (1998) Seed developmental temperature regulation of thermotolerance in lettuce. Journal of the American Society for Horticultural Science, 123:700-705.

Villela RP, Souza RJD, Guimarães RM, Nascimento WM, Gomes LAA, Carvalho BO & Bueno ACR (2010) Produção e desempenho de sementes de cultivares de alface em duas épocas de plantio. Revista Brasileira de Sementes, 32:158-169.

Yoong FY, O'Brien LK, Truco MJ, Huo H, Sideman R, Hayes R, Michelmore RW & Bradford KJ (2016) Genetic variation for thermotolerance in lettuce seed germination is associated with temperature-sensitive regulation of ethylene response factor1 (ERF1). Plant physiology, 170:472-488.