

Quality of pelleted and bare lettuce seeds at different temperatures

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Abstract

Two lettuce cultivars (Everglades and Virginia) were used to evaluate the performance of pelleted and bare seeds of lettuce under different germination temperatures. A completely randomized 2 x 2 x 5 factorial design was used. The factors consisted of two cultivars with pelleted and bare seeds, five intervals of germination temperatures (17 to 19°C, 21 to 23°C, 25 to 28°C, 30 to 32°C, 34 to 38°C), with four replications. The first germination count, T50, germination, germination speed index, emergence, emergence speed index and dry mass of plants were evaluated. Sowing germination test was carried out on two sheets of blotting-paper, moistened with water at a ratio of 2.5 times the paper weight in 'gerbox' plastic boxes. The boxes with seeds were kept in a thermogradient table, with alternating light procedure: 12 hours of darkness and 12 hours of light. The temperature was regulated to a gradient according to each treatment. On the 4th day, first germination count and germination test were performed. On the 7th day, germination was evaluated with normal seedlings as a parameter. The cultivar Everglades showed a higher germination, especially with pelleted seeds. The germination performance of the cultivars is influenced by the use of bare or pelleted seeds and by temperature. Better seed quality and early growth of lettuce are observed in temperatures between 17 to 23°C.

Keywords: Everglades, *Lactuca sativa* L., Thermodormancy, Thermoindhibition, Virginia.

Abbreviations: FGC_first germination count; T50_time for 50% of the seeds to germinate; GER_germination rate; GSI_germination speed index; ER_emergence rate; ESI_emergence speed index; PDM_plants dry mass.

Introduction

Lettuce (*Lactuca sativa* L.) is grown in all Brazilian regions and stands out among the most leafy vegetables consumed in Brazil due to its importance as a source of vitamins, minerals and fiber (Santi et al., 2010; Teodoro et al., 2016). The main obstacles in its cultivation are related to the sensitivity to temperature changes in the germination environment (Bertagnolli et al., 2003) and also to the size and shape of seeds, which can hinder the sowing.

The germination process involves several metabolic activities which occur during a scheduled sequence of chemical reactions; each of these reactions has its own temperature requirements (Marcos Filho, 2005). The temperature also affects the speed, the rate and the uniformity of germination, which results in poor quality and delay in seedlings production (Menezes et al., 2000) and, therefore, a direct damage to the producers (Nascimento and Cantiliffe, 2001). For most lettuce cultivars, the ideal temperature for seed germination is 20°C (Deng and Song, 2012). Temperatures above 25°C may negatively affect germination, germination speed, water absorption and may also affect the biochemical reactions, which are essential to the germination process (Schwember and Bradford, 2010).

Regarding size and shape, there are pelleted lettuce seeds in the market in order to solve these problems. Caldeira et al. (2016) reported that the commercial seeds are pelleted to increase the size and facilitate sowing. This process consists

of coating the seeds with a dry, inert, fine-grained and cementing (adhesive) material. However, the use of pelleted seeds can affect gas exchange and radicle issuance, inhibiting (Nascimento et al., 2009) or delaying germination when compared to bare seeds (Franzin et al., 2004; Pires et al., 2004). Another aspect that can affect seed's response to temperature during germination is the use of pelleted or bare seeds. For Bertagnolli et al. (2003) pelleted seeds have different hydrophilic and hydrophobic characteristics of the materials used in the coating. Thereby, when compared to bare seeds and exposed to different temperatures, they promote different results. Reports presented by Nascimento and Caliari (1989) show that pelleted seeds of lettuce are more sensitive to high temperatures. However, those results do not corroborate to the ones obtained by Salom et al. (2008) who found a higher germination rate of pelleted and bare seeds at 20 to 30°C and 15 to 25°C, respectively. The discrepancy between the results may be related to the thermotolerance of lettuce genotypes, considering that some cultivars are more tolerant to high temperatures, as verified by Catão et al. (2014). The authors observed that cultivar Everglades can be considered thermotolerant due to the germination rate at high temperature and with the increased activity of endo-β-mannanase enzyme. Given the above, it is necessary to determine the temperatures that promote maximum germination efficiency of pelleted and bare seeds.

Besides, it is also necessary to obtain more tolerant lettuce cultivars and with better physiological quality regarding the germination for tropical and subtropical regions. The aim of this study was to investigate the germination performance of lettuce seeds in two cultivars, under different coatings and temperatures.

Results and discussion

All the variables, except T50, were significantly influenced ($p<0,01$) by the cultivars (Table 1). These results are similar to the ones checked by Catão et al. (2014), who also showed the effect of lettuce cultivars in the germination and emergence variables. This fact is probably related to different characteristics regarding genetic background.

For coating, temperatures, and the interaction cultivar x coating, significant differences were observed in all the characteristics under evaluation. Nascimento et al. (2012) also observed the effect of temperature on physiological quality of lettuce seeds. These findings are probably related to the hydrophilic and hydrophobic characteristics of the materials used in the coating (Bertagnolli et al., 2003), to lettuce sensitivity to temperature gradients and to the intrinsic characteristics of each cultivar.

In the interaction cultivar x temperature, there was a significant effect on the emergence, emergence speed index and dry mass of plants. The interaction coating x temperatures significantly influenced ($p<0,01$) all the variables, except the first germination counting and emergence. For the interaction genotype x coating x temperatures, significant differences for germination, germination speed index, emergence speed index and seedlings dry mass were observed. The cultivars differ from each other since each genotype has different morphophysiological and genetic characteristics. Thus, it was not the objective of this study to unfold the interactions between cultivars x coating and cultivars x temperatures.

Overall, it was observed that cultivar Everglades showed better means in the tests related to germination in blotting paper (Table 1). On the other hand, assessing the tests related to emergence on commercial substrate, it was noted that cultivar Virginia showed a better performance. These results differ from those obtained by Catão et al. (2014), who concluded that Everglades showed higher germination and emergence rates in both substrates.

Pelleted seeds showed better results in all traits for coating (Table 1). These results corroborate with those obtained by Bertagnolli et al. (2003), who also verified a reduction in physiological quality of bare seeds of lettuce subjected to thermal stress, when compared to the pelleted seeds. The authors reported that these findings are probably due to the characteristics of the pellet material which helped to maintain the performance of the seeds as the temperature is not the most suitable.

In general, it was found that the two smallest temperature gradients (17 to 19°C; 21 to 23°C) provided better physiological performance in lettuce seeds (Table 1). Temperatures above 25°C reduced the physiological potential, as evidenced by the germination test, first count of germination, emergence speed index and dry mass of plants. According to Catão et al. (2014), temperatures above 30°C and especially at 35°C lead to thermoinhibition or thermodormancy in lettuce seeds. These factors associated with the negative potential make the germination unfeasible. Additionally, high temperatures cause the weakening of the seeds endosperm, which prevents the embryo growth and restricts root protrusion (Sung et al., 2008).

In general, the higher means of T50 were at 34-38°C for the coated and bare seeds. However, for coated seeds, it was observed that there were no statistical differences between the temperature gradients (Table 2). In temperature ranges of 30-32°C and 34-38°C, only the bare seeds had higher means of T50. Therefore, it is evident that the coating protects the seeds that are subjected to high temperatures, promoting a more uniform germination under adverse temperature conditions. Similar results were found by Bufalo et al. (2012), who studied the germination of lettuce seeds under different temperatures and stratification periods.

Cultivar Everglades showed higher germination rates in all temperature gradients for pelleted seeds (Table 3). With coating, Everglades germination was impaired only in higher temperatures; for cultivar Virginia, there was a reduction in germination at temperatures of 25 to 28°C. However, when assessing the bare seeds, both cultivars showed unsatisfactory germination, with average values below the minimum standard of 80% that is required for the marketing of lettuce seeds in Brazil, established by Normative Instruction No. 45 (BRASIL, 2013).

By analyzing the interaction between cultivars x temperature within coating (Table 4) it was observed that cultivar Everglades showed better germination rates when pelleted seeds were used. However, there were no differences in gradients of 25 to 28°C and 30 to 32°C for cultivar Virginia. These results corroborate with the study that characterizes the cultivar Everglades as thermotolerant (Nascimento et al., 2004; Kozarewa et al., 2006; Catão et al., 2014). Bertagnolli et al. (2003) which also verified a better performance of pelleted lettuce seeds regarding the tolerance to heat stress.

The higher germination rate in pelleted seeds of cultivar Everglades is probably related to the ability that the coating has to rapidly absorb and store water. Therefore, water is readily available to the seed, which accelerates the germination as reported by Manohar and Heydecker (1964). In addition, water is also efficient in conducting heat energy. Thus, lower temperatures can retard the germination of pelleted seeds (Salum et al., 2008).

By analyzing the remaining seeds from the germination test (Table 5), it was observed by the tetrazolium test that the pelleted seeds of cultivar Virginia (in all temperature gradients) and the bare seeds (in the smallest and the largest temperature gradient) showed dormancy. Bertagnolli et al. (2003) and Bufalo et al. (2012), analyzing the performance of bare and pelleted seeds of lettuce subjected to heat stress, also found that at 25°C or above 25°C, there was a reduction in germination rate and speed, and at 35°C there was a cease of seeds germination. This last fact has not been reported in the present study, even for cultivar Virginia which showed a lower tolerance to heat stress. For Valdes and Bradford (1987), the thermodormancy is aggravated in pelleted seeds. This fact was observed only in pelleted seeds of Virginia (Table 5), probably due to the characteristics of this cultivar. The germination speed was reduced when temperatures between 34 and 38 ° C were used in pelleted seeds of Everglades and in Virginias bare seeds (Table 3). On the other hand, there was a reduction in vigor in pelleted seeds in the gradients of 30 to 32°C and 34 to 38°C for bare seeds of Everglades and pelleted seeds of Virginia. The pelleted seeds of Everglades presented a greater germination speed index in all temperature gradients (Table 4).

For emergence speed index, the pelleted seeds of Everglades were not affected by temperature gradients. For cultivar Virginia, there was a reduction in the emergence speed index

Table 1. Mean values for the first germination counting (FGC), time for 50% of the seeds to germinate (T50), germination rate (GER), germination speed index (GSI), emergence rate (ER), emergence speed index (ESI) and plants dry mass (PDM) obtained in the assay with bare and pelleted seed of lettuce cultivars exposed to different temperatures. Lavras, Minas Gerais, Brazil, 2014.

Source of variation	FGC	T50	GR	GSI	ER	ESI	PDM
	%	unid.	%	-	%	-	mg ⁻¹
Cultivar¹							
Everglades	60 a	1.55 a	77 a	30.22 a	63 b	20.24 b	6.24 b
Virginia	53 b	1.67 a	70 b	26.32 b	72 a	22.13 a	7.61 a
Coating¹							
Pelleted	60 a	1.32 b	85 a	32.62 a	87 a	25.83 a	8.17 a
Bare	51 b	1.90 a	62 b	23.92 b	47 b	16.64 b	5.67 b
Temperatures²							
17 – 19°C	65 a	1.31 b	85 a	33.76 a	70 a	23.49 a	8.48 a
21 – 23°C	61 a	1.31 b	83 a	32.72 a	71 a	23.19 a	8.08 a
25 – 28°C	57 b	1.31 b	78 b	31.09 a	71 a	21.01 b	6.27 b
30 – 32°C	53 b	1.68 b	70 c	26.00 b	67 a	19.90 b	6.36 b
34 – 38°C	46 c	2.43 a	49 d	17.79 c	57 b	18.60 b	5.41 c
Mean	56.00	1.61	73.06	28.27	67.31	21.24	6.92
CV (%)	13.82	21.93	11.32	13.93	9.68	13.62	15.80

¹Means followed by the same letter do not differ by F test. ² Means followed by the same letter in the column do not differ statistically by Scott and Knott test at 5% probability. CV - coefficient of variation.

Table 2. Mean values of the time for 50% of the seeds to germinate regarding the interaction between coating vs. temperature obtained in the test with bare and pelleted seeds of lettuce cultivars exposed to different temperatures. Lavras, Minas Gerais, Brazil, in 2014.

Temperature	Coating	
	Pelleted	Bare
17 – 19 °C	1.0 Aa	1.0 Ba
21 – 23 °C	1.0 Aa	1.0 Ba
25 – 28 °C	1.0 Aa	1.0 Ba
30 – 32 °C	1.0 Ab	2.0 Aa
34 – 38 °C	2.0 Ab	3.0 Aa

Means followed by the same capital letter in the column and. lowercase in line. They belong to the same group. by Scott test Knott (1974). at 5% of probability.

Table 3. Mean values of germination (GER), germination speed index (GSI), emergence speed index (IVE) e seedling dry mass of lettuce regarding the interaction between cultivars vs coating within the temperatures. Lavras, MG, Brasil, 2014.

Coating	Temperature	GER (%)		GSI		ESI		SDM (mg ⁻¹)	
		Ever.	Virg.	Ever.	Virg.	Ever.	Virg.	Ever.	Virg.
Pelleted	17 – 19°C	100 Aa	90 Aa	41.8 Aa	32.6 Ab	33.6 Aa	26.8 Ab	11.0 Ab	13.1 Aa
	21 – 23°C	100 Aa	89 Ab	40.9 Aa	31.5 Ab	31.1 Aa	21.6 Bb	9.2 Bb	12.5 Aa
	25 – 28°C	100 Aa	73 Bb	41.7 Aa	26.8 Ab	29.1 Aa	17.6 Cb	3.4 Cb	9.3 Ba
	30 – 32°C	98 Aa	63 Cb	39.0 Aa	19.6 Bb	28.7 Aa	16.9 Cb	6.6 Ba	7.4 Ca
	34 – 38°C	76 Ba	59 Cb	33.5 Ba	18.3 Bb	28.9 Aa	23.5 Bb	4.3 Ca	4.7 Da
Bare	17 – 19°C	75 Aa	76 Aa	27.0 Ab	33.5 Aa	15.0 Ab	28.4 Aa	4.8 Aa	4.9 Aa
	21 – 23°C	70 Aa	75 Aa	26.9 Aa	31.4 Aa	13.2 Ab	26.6 Aa	5.5 Aa	5.1 Aa
	25 – 28°C	68 Aa	70 Aa	25.5 Aa	30.3 Aa	14.0 Ab	23.1 Ba	6.3 Aa	6.1 Aa
	30 – 32°C	54 Bb	68 Aa	16.3 Bb	28.8 Aa	11.8 Ab	22.1 Ba	4.7 Ab	6.5 Aa
	34 – 38°C	26 Ca	34 Ba	9.2 Ca	10.0 Ba	7.6 Bb	14.2 Ca	6.4 Aa	6.5 Aa

Means followed by the same capital letter in the column and. lowercase in line. They belong to the same group. by Scott test Knott (1974). at 5% of probability. Ever - Everglades, Virg - Virginia.

Table 4. Mean values of germination (GER), germination speed index (GSI), emergence speed index (ESI) e seedling dry mass of lettuce regarding the interaction between cultivars vs temperature within coating. Lavras, MG, Brazil, 2014.

Temperature	Coating	GER (%)		GSI		ESI		SDM (mg ⁻¹)	
		Ever.	Virg.	Ever.	Virg.	Ever.	Virg.	Ever.	Virg.
17 – 19°C	Pelleted	100 A	90 A	41.8 A	32.6 A	33.6 A	26.8 A	11.0 A	13.1 A
	Bare	75 B	76 B	27.0 B	33.5 A	5.0 B	28.4 A	4.8 B	4.9 B
21 – 23°C	Pelleted	100 A	89 A	40.9 A	31.5 A	31.1 A	21.6 B	9.2 A	12.5 A
	Bare	70 B	75 B	26.9 B	31.4 A	13.2 B	26.6 A	5.5 B	5.1 B
25 – 28°C	Pelleted	100 A	73 A	41.7 A	26.8 A	29.1 A	17.6 B	3.4 B	9.3 A
	Bare	68 B	70 A	25.5 B	30.3 A	14.0 B	23.1 A	6.3 A	6.1 B
30 – 32°C	Pelleted	98 A	63 A	39.0 A	19.6 B	28.7 A	16.9 B	6.6 A	7.4 A
	Bare	54 B	68 A	16.3 B	28.8 A	11.8 B	22.1 A	4.7 B	6.5 A
34 – 38°C	Pelleted	76 A	59 A	33.5 A	18.3 A	28.9 A	23.5 A	4.3 B	4.7 A
	Bare	26 B	34 B	9.2 B	10.0 B	7.6 B	14.2 B	6.4 A	6.1 A

Means followed by the same capital letter in the column and. lowercase in line. They belong to the same group. by Scott test Knott (1974). at 5% of probability. Ever - Everglades, Virg - Virginia.

Table 5. Number of remaining seeds (N) and viability (%) of lettuce seeds subjected to the tetrazolium test performed 7 days after sowing, obtained at the test of pelleted and bare seeds of cultivars exposed to different temperatures. Lavras, Minas Gerais, Brazil, 2014.

Temperature	Everglades					
	N ¹	Pelleted		N ¹	Bare	
		Viable (%)	Dead (%)		Viable (%)	Dead (%)
17 – 19°C	0	0	0	20	0	100
21 – 23°C	0	0	0	24	0	100
25 – 28°C	0	0	0	26	4	96
30 – 32°C	2	0	100	37	0	100
34 – 38°C	19	0	100	59	36	54
Surface	Virginia					
	N ¹	Pelleted		N ¹	Bare	
		Viable (%)	Dead (%)		Viable (%)	Dead (%)
17 – 19°C	8	88	12	19	32	68
21 – 23°C	9	44	56	20	0	100
25 – 28°C	22	18	82	24	0	100
30 – 32°C	30	10	90	26	0	100
34 – 38°C	33	6	94	53	30	70

¹ seeds do not germinate in the germination test in a total of 80 seeds.

from the temperature gradient above 21 to 23°C (Table 3). The pelleted seeds of Everglades showed higher emergence and germination speed index in all temperature gradients (Table 4). These results are similar to those observed by Catão et al. (2014), who verified that the germination and the emergence speed index reduced when the temperature increased from 20°C to 35°C. Regarding the dry mass (Table 3), the pelleted seeds of Everglades showed higher value in the lowest temperature gradient. For the seeds of Virginia, the dry mass was affected at the gradient of 25 to 28°C. On the other hand, the bare seeds of both cultivars did not differ statistically among the temperature gradients. By comparing the interaction between cultivars vs temperature within coating (Table 4), the pelleted seeds of Everglades showed higher means in all the temperature gradients. This fact is probably related to the seedlings that emerge first, as evidenced by the emergence speed index (Table 4). The seedlings certainly grow more and present more dry mass due to the photosynthesis in the early growth stages.

Materials and methods

Experiment establishment and management

The experiment was performed in a completely randomized 2 x 2 x 5 factorial design. The factors consisted of two lettuce cultivars (Everglades and Virginia), with pelleted and bare seed and five gradients of germination temperatures (17 to 19°C, 21 to 23°C, 25 to 28°C, 30 to 32°C, 34 to 38°C), with four replications. Cultivar Everglades can be considered thermotolerant. The tests were conducted with a sample of 20 seeds per replication, in a total of 80 seeds per treatment.

Determined variables

The physiological quality of seeds was evaluated by the following determinations:

Germination test

Sowing was carried out on two sheets of blotting-paper, moistened with water at a ratio of 2.5 times the paper weight in *gerbox* plastic boxes. The boxes with seeds were kept in a thermogradiant table, with alternating light procedure: 12 hours of darkness and 12 hours of light. The temperature was regulated for a gradient according to each treatment. On the 4th day, first germination count and germination test were

performed. On the 7th day, germination was evaluated, with normal seedlings as parameter, according to the Rules for Seed Analysis (2009). The results were expressed as percentage of normal seedlings at the germination test. The time required for 50% of the seedlings to germinate (T50) was calculated (Côme, 1970).

Tetrazolium test

It was performed with the remaining seeds from the germination test (seeds that did not germinate), removing the seed coats and embryos submitted to the tetrazolium test. A solution of 2,3,5-triphenyl tetrazolium chloride was used in a concentration of 1% during 3 hours in the dark at 30°C. The percentage of viable seeds was determined according to the Rules for Seed Analysis (2009).

Emergence test

The seeds were sown in *gerbox* plastic boxes containing commercial substrate Tropstrato HA Hortaliças®, with water retention capacity adjusted to 60%. The boxes with seeds were kept in thermogradiant table under the same conditions of the germination test. Daily evaluations were performed from the beginning of the emergence, computing the number of emerged seedlings until stabilization.

Germination and emergence speed

They were performed simultaneously with germination and emergence tests. It was computed, daily and at the same time, the number of seedlings which presented two fully open leaflets. The indices calculation was performed with formula proposed by Maguire (1962).

Seedlings dry mass

On the 7th day after test sowing, the seedlings were washed and 5 uniform seedlings (shoot and root) were collected from each *gerbox*. Later, they were placed in paper bags model 'Kraft' to be dried at 60°C for 72 hours. The dry mass was composed by the average weight of seedlings expressed as mg⁻¹.

Statistical analysis

Analysis of variance was performed by the adoption of a statistical model and a similar analysis procedure provided by Ramalho et al. (2012). The means were grouped by the Scott-Knott test (1974). Statistical analysis was performed with the aid of SISVAR® statistical package (Ferreira 2011).

Conclusions

Cultivar Everglades showed higher germination, especially when pelleted seeds were used. The germination performance of cultivars is influenced by temperature and by the use of bare or pelleted seeds. From 17 to 23°C, it is observed a higher quality seed and an early growth of lettuce.

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