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Physiological and biochemical changes in lettuce seeds during storage at different temperatures

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ABSTRACT

Dormancy in lettuce seeds is a process not yet fully understood. High storage temperatures can cause seed dormancy promoting physiological and enzymatic changes. The goal of this study was to investigate the influence of storage period and environments on the quality and dormancy of different lettuce cultivars. We also investigated the biochemical changes. A completely randomized experimental design was used in a 4x8x3 factorial arrangement to evaluate physiological quality on different storage periods (30, 60, 90 and 120 days) and environments (15, 25 and 35°C) of seeds from eight lettuce cultivars (Everglades, Babá de Verão, Elisa, Luiza, Grand Rapids, Hortência, Salinas 88 and Rubete). The biochemical activity was accessed by tetrazolium test and the activity of the endo-β-mannanase. There occurred physiological and biochemical changes on lettuce seeds under periods and high temperature storage environments. A reduction of seed quality occurred at high storage temperatures mainly when stored during 120 days. The storage period up to six months maintains the viability and vigor of lettuce seeds, when stored at 15°C. Germination is compromised when seeds are stored over 60 days on temperatures over 25°C. Everglades is a tolerant cultivar to germination conditions of 35°C and maintains its quality during storage at 15°C. Temperatures over 25°C induce thermodormancy on lettuce seeds during storage. The tetrazolium test indicates that the seeds were viable; nevertheless, there was no germination at high temperatures. Enzymatic changes occurred in seeds stored at 35°C due to dormancy. There was a decrease in the expression of endo-β-mannanase enzyme being influenced by the environment and storage period. The Everglades cultivar is thermotolerant.

Keywords: *Lactuca sativa*, thermotolerance, thermoinhibition, dormancy, enzymatic activity.

RESUMO

Alterações fisiológicas e bioquímicas em sementes de alface durante o armazenamento em diferentes temperaturas

Dormência em sementes de alface é um processo que ainda não está totalmente compreendido. As altas temperaturas de armazenamento podem causar dormência das sementes e promover mudanças fisiológicas e enzimáticas. O objetivo deste trabalho foi verificar a influência dos períodos e dos ambientes de armazenamento na qualidade e dormência de sementes de diferentes cultivares de alface e investigar as alterações bioquímicas. O delineamento experimental foi inteiramente casualizado, em esquema fatorial 4x8x3 para avaliar a qualidade fisiológica de oito cultivares de alface (Everglades, Babá de Verão, Elisa, Luiza, Grand Rapids, Hortência, Salinas 88 e Rubete) em diferentes períodos (30, 60, 90 e 120 dias) e ambientes de armazenamento (15, 25 e 35°C). A atividade bioquímica foi avaliada pelo teste de tetrazólio e pela a atividade da endo-β-mananase. Há alterações fisiológicas e bioquímicas nas sementes de alface em virtude do período e do ambiente de armazenamento em altas temperaturas. Há uma redução da qualidade das sementes a temperaturas elevadas de armazenamento, principalmente aos 120 dias. Verificou-se que o período de armazenamento até os seis meses mantém a viabilidade e vigor de sementes de alface, quando armazenadas a 15°C. As sementes armazenadas em ambientes com temperatura superior a 25°C não toleram o armazenamento a partir de 60 dias o que compromete a germinação e o vigor. A cultivar Everglades é tolerante à condição de germinação a 35°C e mantém sua qualidade ao longo do armazenamento em temperatura de 15°C. A temperatura acima de 25°C induz à termodormência das sementes nas cultivares de alface durante o armazenamento. O teste de tetrazólio indica que as sementes estavam viáveis, contudo não houve germinação em temperaturas elevadas. Ocorrem alterações enzimáticas nas sementes armazenadas em temperatura de 35°C devido à dormência. Houve decréscimo na expressão da enzima endo-β-mananase, sendo influenciada pelo ambiente e período de armazenamento. A cultivar Everglades é termotolerante.

Palavras-chave: *Lactuca sativa*, termotolerância, termoinibição, dormência, atividade enzimática.

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Lettuce (*Lactuca sativa*) is the most important leafy vegetable in the Brazilian diet (Nascimento *et al*., 2012). It is cultivated throughout the year in different regions and under different edaphic and climatic conditions. High temperatures are highly influential on germination of lettuce seeds and may lead to seed dormancy due to hardening of the endosperm, which ends up restricting radicle protrusion (Nascimento *et al*., 2012). Silva *et al*.

(2004) claim that, when seeds have germination limited by the endosperm, this tissue must be weakened to allow radicle protrusion, and this is the role of enzymes such as endo-β-mannanase. The endosperm cell wall in lettuce seeds consists mainly of galactomannans, and the endo-β-mannanase enzyme is part of the mechanism that degrades it (Nascimento *et al*., 2012). Increased activity of this enzyme in cultivars germinated at high temperatures is related to seed thermotolerance (Catão *et al*., 2014).

The ambient conditions for storage, such as air temperature and relative humidity, are preponderant factors for conservation of seeds until planting (Carvalho & Pinho, 2000). After planting, lettuce producers store packages containing remaining seeds in inappropriate locations, where ambient temperature is usually high, realizing later, at the following planting period, that seed quality was compromised. Thus dormancy in lettuce seeds is not well-understood process, and it is not known if it is acquired during storage at high temperatures.

High temperature, in addition to cause dormancy in lettuce seeds, also accelerates chemical reactions and intensifies metabolic processes. As a consequence of these metabolic events, seed respiration is greater, which leads to an increase in speed of deterioration.

The tetrazolium test is a quick biochemical test able to detect seed viability based on seed respiration, particularly in species which are slow to germinate and exhibit dormancy. The test is based on color change of living tissue in the presence of a tetrazolium solution. This color change reflects the activity of dehydrogenase enzymes involved in seed respiration. These enzymes, particularly malic acid dehydrogenase, catalyze the reaction of H^+ ions released by the reaction of living tissue with the salt (2,3,5-triphenyl tetrazolium chloride) to form a red-colored substance called trifenilformazan, which is stable and non-diffusible.

In the process of seed deterioration, one of the first degradation events is loss of membrane permeability. The membrane loses its selectivity and the enzymes and proteins become less efficient in catalytic activities (Smith & Berjak, 1995). Some of these enzymes and proteins may be used to measure seed quality (vigor), even when there is a fall in reserves during storage at high temperatures (Marcos Filho, 2005). Knowledge of the physiological and biochemical quality of the seeds may clarify if dormancy in lettuce seeds may be acquired during storage. The goal of this study was to verify the influence of storage period and environment conditions on the physiological and biochemical quality and dormancy of seeds of different lettuce cultivars.

MATERIAL AND METHODS

The production process of lettuce seeds was conducted in an experimental area in the municipality of Lavras, Minas Gerais State, Brazil (21°14′24″S, 45°00′34″W), where the soil is classified as an Oxisol with a clayey texture.

Initially, seedlings were produced from different lettuce cultivars: Everglades, Babá de Verão, Elisa, and Luiza (smooth leaf), Grand Rapids and Hortência (curly leaf), and Salinas 88 and Rubete (crisp head). They were sown in 128-cell expanded polystyrene trays containing a commercial substrate for vegetable seedlings. After 21 days, the seedlings were transplanted in seedbeds in a protected growth area. Each plot had 6 plants in one row, spaced 0.4 m apart in the row and 0.6 m between rows, summarizing 7.2 m^2 of area, following a randomized block design with three replications; looking for minimizing potential environmental effects during seed formation. During the seed production process the maximum and minimum mean temperatures of the air measured at 1.5 m from the soil were 45 and 31°C, respectively. Seeds of each plant from the same cultivar were harvested individually within each block and then mixed uniformly, composing a single seed lot.

Subsequently, seeds were treated with a carboxin fungicide before storage. The product was manually applied to the seeds in semi-impermeable plastic bags of a neutral chemical composition, and shaken until complete distribution. Before carrying out the tests, the seeds of the different cultivars were subjected to determination of moisture content and evaluation was performed according to the Rules for Seed Analysis (RAS) (Brasil, 2009) and the results were expressed in percentage.

Later, seeds from each cultivar were homogenized, placed in individual Kraft paper bags and stored in BOD chambers for a period of 30, 60, 90 and 120 days under temperatures of 15, 25 and 35°C. Physiological and biochemical quality were evaluated in each storage/temperature regimen.

For the germination test, seeds were put between two blotting paper sheets, moistened with water at the proportion of 2.5 times the weight of the dry substrate, in gerbox type plastic boxes. The boxes with the seeds were kept in BOD type chambers under an alternating light and temperature regimen, 12 hours in the dark and 12 hours in the presence of light at 20 and 35°C, respectively. Four subsamples of 50 seeds from each cultivar were analyzed.

The tetrazolium test was carried out on the remaining seeds (seeds that did not germinate) from the germination test, with the seed coats being removed and the embryos subjected to the test. Seeds were stained in a solution of 2,3,5-triphenyl tetrazolium chloride at a concentration of 1% during 3 hours in the dark at 30°C. After this period, having checked the staining, the embryos were washed in running water and kept submersed in water until their evaluation, when they were individually analyzed to verify their viability. Interpretation was made according to RAS (Brasil, 2009), and the results were expressed in percentage of viable seeds.

To evaluate the emergence, four replications of 50 seeds of each cultivar were used, distributed in multicell polystyrene trays containing the commercial substrate Multiplant for vegetables. The trays were kept in a greenhouse equipped with an intermittent mist system. Daily evaluations were made since the beginning of emergence, computing the number of emerged seedlings up to stabilization of stand (Queiroz *et al*., 2011).

For the enzyme endo-β-mannanase, the lettuce seeds from each treatment were placed to soak in plastic boxes on blotting paper moistened with distilled water at a quantity equivalent to 2.5 times the weight of the dry substrate. The seeds were kept in a germinator at 20°C during 24 hours and, after soaking, 150 seeds of each cultivar were macerated in a crucible with liquid nitrogen and polyvinylpyrrolidone (PVP). In each microtube, with 100 mg of powder from each sample, 300 μL of extraction buffer [0.1 M Hepes/0.5 M NaCl and ascorbic acid (5 mg of ascorbic acid per mL of buffer), pH 8.0] were added. The microtubes were then centrifuged during 30 minutes at 12,000 *g* and 2 μL of the supernatant were applied in gel containing 6 mL of LBG (Locust Bean Gum), 0.24 g of agarose, and 24 mL of buffer pH 5.0 (1 M citric acid/0.4 M of Na_2HPO_4 2 H_2O). The aliquots were applied in 2 mm holes made in the gel with the aid of an awl. The gel was incubated during 21 h in refrigerated conditions and revealed according to the methodology proposed by Silva *et al*. (2004). The activity of the enzyme endo-β-mannanase was calculated according to Downie *et al*. (1994).

In the test for evaluation of physiological quality of the lettuce seeds, we used a completely randomized experimental design in a 4x8x3 factorial arrangement, whose factors were four storage periods (30, 60, 90, and 120 days), eight lettuce cultivars, and three storage environments (15, 25, and 35°C). Then analysis of variance was performed at 5% probability by the F Test. For the comparison of means, the Scott-Knott test was used, at 5% probability.

RESULTS AND DISCUSSION

The moisture content of the seeds after drying ranged from 5 to 6%, values also reported by Barbosa *et al*. (2011) in different lettuce seed lots. Based on analysis of variance, significant differences were observed among the cultivars, storage periods, and storage environments, just as for interaction among the factors (p<0.05). The mean values of germination (%) at 20 and 35°C of the seeds of lettuce cultivars stored in different environments are shown in Table 1.

For germination at 20°C (Table 1), regardless of the storage period, the

Table 1. Germination (%) at 20 and 35°C of seeds from eight lettuce cultivars depending on ambient temperatures and storage periods. Lavras, UFLA, 2015.

	Germination (20°C)											
	Storage periods (days)											
Cultivars	**30**			**60**			**90**			**120**		
	Storage temperatures (°C)											
	15	**25**	**35**	**15**	**25**	**35**	**15**	**25**	**35**	**15**	**25**	**35**
Luiza	94Ba	94Ba	81Cb	91Ba	89Ba	80Bb	91Ba	86Bb	75Bc	90Ba	82Ab	69Bc
Elisa	84Ca	82Ca	76Db	84Ca	75Db	68Cc	80Ca	76Ca	63Cb	81Ca	70Bb	59Cc
G. Rapids	96Aa	92Ba	70Eb	95Aa	73Db	53Ec	90Ba	51Eb	28Ec	88Ba	42Db	15Ec
B. Verão	96Aa	93Ba	70Eb	95Aa	83Cb	60Dc	89Ba	80Cb	60Cc	90Ba	51Cb	32Dc
Hortência	97Aa	94Ba	88Bb	94Aa	76Db	62Dc	91Ba	63Db	50Dc	88Ba	22Eb	11Fc
Rubete	98Aa	78Cb	60Fc	96Aa	64Eb	44Fc	94Aa	51Eb	21Fc	91Ba	19Eb	9Fc
Salinas 88	92Ba	89Ba	70Eb	88Ba	59Fb	44Fc	89Ba	51Eb	18Fc	85Ca	17Eb	12Fc
Everglades	99Aa	100Aa	99Aa	98Aa	99Aa	99Aa	96Aa	92Ab	90Ab	97Aa	83Ab	84Ab
CV (%)	3.89											
	Germination (35 °C)											
Luiza	94Ba	94Ba	81Cb	91Ba	89Ba	80Bb	91Ba	86Bb	75Bc	90Ba	82Ab	69Bc
Elisa	84Ca	82Ca	76Db	84Ca	75Db	68Cc	80Ca	76Ca	63Cb	81Ca	70Bb	59Cc
G. Rapids	96Aa	92Ba	70Eb	95Aa	73Db	53Ec	90Ba	51Eb	28Ec	88Ba	42Db	15Ec
B. Verão	96Aa	93Ba	70Eb	95Aa	83Cb	60Dc	89Ba	80Cb	60Cc	90Ba	51Cb	32Dc
Hortência	97Aa	94Ba	88Bb	94Aa	76Db	62Dc	91Ba	63Db	50Dc	88Ba	22Eb	11Fc
Rubete	98Aa	78Cb	60Fc	96Aa	64Eb	44Fc	94Aa	51Eb	21Fc	91Ba	19Eb	9Fc
Salinas 88	92Ba	89Ba	70Eb	88Ba	59Fb	44Fc	89Ba	51Eb	18Fc	85Ca	17Eb	12Fc
Everglades	99Aa	100Aa	99Aa	98Aa	99Aa	99Aa	96Aa	92Ab	90Ab	97Aa	83Ab	84Ab
CV (%)	16.86											

Mean values followed by the same uppercase letter in the column and lowercase letter in the row do not differ among themselves by the Scott-Knott Test at 5% probability.

Table 2. Viable (%) (V) and dead (%) (D) seeds remaining (not germinated), after 30, 60, 90 and 120 days of storage, by the tetrazolium test at 20°C of lettuce seeds stored at different ambient temperatures (A). Lavras, UFLA, 2015.

Cultivars	A (°C)	N	30		N	60		N	90		N	120	
			V	D		V	D		V	D		V	D
Luiza	15	12	25	75	18	22	78	18	39	61	20	30	70
Elisa	15	32	31	69	32	19	81	40	13	88	38	42	58
Grand Rapids	15	8	0	100	10	40	60	20	45	55	24	38	63
Babá Verão	15	8	38	63	10	30	70	22	41	59	20	35	65
Hortência	15	6	0	100	12	33	67	18	39	61	24	38	63
Rubete	15	4	25	75	8	13	88	12	33	67	18	39	61
Salinas 88	15	16	25	75	24	42	58	22	36	64	30	43	57
Everglades	15	2	0	100	4	25	75	8	25	75	6	33	67
Luiza	25	12	58	42	26	73	27	28	61	39	36	67	33
Elisa	25	36	69	31	50	64	36	48	65	35	60	60	40
Grand Rapids	25	16	81	19	54	83	17	98	73	27	116	68	32
Babá Verão	25	14	79	21	34	56	44	40	58	43	98	64	36
Hortência	25	12	75	25	48	58	42	74	61	39	156	62	38
Rubete	25	44	59	41	72	61	39	98	77	23	162	64	36
Salinas 88	25	22	64	36	82	70	30	98	66	34	166	61	39
Everglades	25	0	100	0	2	100	0	16	75	25	34	65	35
Luiza	35	38	61	39	40	63	38	50	76	24	62	66	34
Elisa	35	48	58	42	64	69	31	74	76	24	82	57	43
Grand Rapids	35	60	82	18	94	82	18	144	83	17	170	77	23
Babá Verão	35	60	88	12	80	76	24	80	61	39	136	63	38
Hortência	35	24	88	13	76	64	36	100	72	28	178	72	28
Rubete	35	80	63	38	112	65	35	158	87	13	182	69	31
Salinas 88	35	80	85	15	112	71	29	164	85	15	176	70	30
Everglades	35	2	0	100	2	100	0	20	85	15	32	63	38

'N= not germinated seeds from a total of 200 seeds

ambient temperature of 15°C conserved the seed quality of all the cultivars. The lettuce seeds are not affected by external factors when cold stored and this contributes to reduce the speed of deterioration processes (Nascimento *et al*., 2012). Regarding seeds stored at 25°C, we observed that 60 days of storage compromised the seed quality, except for the cultivars Everglades, Luiza, and Babá de Verão, which exhibited the greatest germination percentages, with values over 80%, a value considered as the minimum for commercialization of lettuce seeds (Brasil, 1986). Nevertheless, at 120 days of storage at 25°C we observed a decrease in germination of the cultivar Babá de Verão, and viability was reduced to 51%. Thus, only the cultivars Everglades and Luiza met the minimum standards for commercialization (Table 1) when stored at this temperature during 120 days. Increasing the storage temperature from 25 to 35°C, there was interference in the viability of the seeds of all the lettuce cultivars, except for the cultivar Everglades (Table 1). According to Martins & Lago (2008), high storage temperature has great influence on seed conservation, affecting the biochemical reactions that regulate seed metabolism.

As may be observed, high storage temperature greatly influences germination already at 30 days; only the cultivars Everglades, Luiza, and Hortência maintained quality under these conditions (Table 1). However, after 60 days of storage, cv. Hortência was not able to tolerate the ambient temperature of 35°C, and cv. Luiza was not able to tolerate it after 90 storage days. Only the Everglades cultivar meets the minimum standards of commercialization after 90 days of storage, with 84% germination at 20°C. Nascimento *et al*. (2012) reported that few lettuce genotypes met the minimum standards of commercialization when germinating at a high temperature. According to Nascimento *et al*. (2012), these standards were 98%, 93%, and 82% for the cultivars Vitória de Verão, Camila, and Vitória Verdinha, respectively.

The storage environment at high temperature contributed to reducing seed germination of most cultivars, especially of Hortência, Rubete, and Salinas 88, which exhibited the lowest germination (Table 1). It may be noted that the germination of these cultivars was inhibited (thermoinhibition) when seeds were stored in a milder environment (15°C) but their viability was maintained. Testing germination at 35°C, cvs. Grand Rapids, Hortência, Babá de Verão, Rubete, and Salinas 88 exhibited a germination percentage equal to or near zero throughout the entire storage period, even when stored in a mild environment (15°C) (Table 1). Thermosensitive cultivars have a greater quantity of mannose and galactose in the cell wall; this causes rigidification of the endosperm and impedes root protrusion (Nascimento *et al*., 2001).

Germination temperature of 35°C assists the identification of thermotolerant cultivars, such as 'Everglades' (Catão *et al*., 2014). It may be observed that the seeds of this cultivar presented over 30% of germination, even when stored at 35°C, while the germination percentage of the other cultivars was zero or near zero.

However, cv. Everglades maintained high germination throughout storage in all environments (Table 1). The Everglades seed germination decreased from 74% to 68% germination over the whole storage period, indicating its superiority in tolerating adverse conditions, as was also observed in other studies (Nascimento, 2003; Kozarewa *et al*., 2006). These results indicate Everglades as a potential genotype for breeding programs.

Throughout this discussion, it

Table 3. Viable (%) (V) and dead (%) (D) seeds remaining (not germinated), after 30, 60, 90 and 120 days of storage, by the tetrazolium test at 35°C of lettuce seeds stored at different ambient temperatures (A). Lavras, UFLA, 2015.

Cultivars	A (ºC)	N	30		N	60		N	90		N	120	
			V	M		V	M		V	M		V	M
Luiza	15	156	57	43	156	79	21	164	76	24	162	55	45
Elisa	15	154	62	38	162	70	30	164	78	22	164	58	42
Grand Rapids	15	194	59	41	198	71	29	200	58	43	200	56	44
Babá Verão	15	186	60	40	196	69	31	196	63	37	198	57	43
Hortência	15	200	64	36	196	62	38	200	60	40	200	64	37
Rubete	15	200	68	33	200	64	37	200	67	34	200	62	38
Salinas 88	15	200	59	42	200	67	34	200	68	32	200	61	39
Everglades	15	52	60	40	56	63	38	54	59	41	64	55	45
Luiza	25	168	62	38	176	72	28	194	64	36	198	61	39
Elisa	25	166	69	31	178	61	39	190	66	34	198	62	38
Grand Rapids	25	198	61	39	198	67	33	200	64	36	200	66	35
Babá Verão	25	194	63	37	198	64	36	200	65	36	200	64	36
Hortência	25	200	68	32	200	59	41	200	68	32	200	66	34
Rubete	25	200	71	30	200	60	41	200	62	38	200	63	38
Salinas 88	25	200	59	42	200	66	35	200	70	30	200	67	34
Everglades	25	66	59	41	74	68	32	90	62	38	120	54	46
Luiza	35	178	61	39	186	61	39	198	77	23	200	86	15
Elisa	35	182	63	37	184	64	36	200	80	21	200	89	11
Grand Rapids	35	200	61	40	200	60	41	200	84	17	200	91	9
Babá Verão	35	198	71	29	200	66	35	200	81	19	200	93	8
Hortência	35	200	66	35	200	63	37	200	81	20	200	94	7
Rubete	35	200	56	44	200	55	45	200	84	17	200	93	8
Salinas 88	35	200	57	43	200	67	34	200	81	19	200	93	8
Everglades	35	120	62	38	122	57	43	130	65	35	134	72	28

'N= not germinated seeds from a total of 200 seeds was mentioned several times that the temperature of germination and of the storage environment, as well as longer periods of storage compromised the quality of lettuce seeds, thus reducing their viability. According to Harrington (1972), every 5.5 degrees of decrease in temperature doubles the potential storage time. This statement is warranted upon analyzing the tetrazolium test in seeds remaining from the germination test at 20 and 35°C (Tables 2 and 3, respectively).

Testing germination at 20°C, it becomes evident that the room temperatures of 25 and 35°C led to greater dormancy of the seeds, as they did not germinate, which may be verified by the quantity of viable seeds (Table 2). Increasing the storage period, the high ambient temperature led to a greater number of not germinated seeds, and most of these seeds were viable. For germination at 35°C, the tetrazolium test showed that although the seeds did not germinate, they were still viable (Table 3).

The cultivars Hortência, Rubete, and Salinas 88 did not germinate after 30 days of storage, even the seeds maintained at 15°C; however, around 60% of the seeds were still viable, as observed in Table 3. This may have occurred due to the dormancy of the lettuce seeds imposed by thermal stress. Results of studies showed that the tetrazolium and germination tests may be considered as complementary and, in combination, they allow evaluation of the physiological quality of the seeds by means of their viability (Dias & Alves, 2008). It is important to use the two tests, tetrazolium and germination, to know the quantity of viable and dormant seeds, information essential for quality control.

In relation to the emergence test (Table 4), it is noteworthy that seeds stored during 30 days at a temperature of 25 and 35°C have less vigor than those stored at 15°C. Analyzing each cultivar, it may be seen that the ambient temperature also reduced the vigor of lettuce seeds. Only cv. Everglades tolerated longer thermal stress, showing reduction in emergence after 90 days of storage at 25 and 35°C. Comparing the cultivars, we observed that Everglades has greater vigor at high temperatures, regardless of the storage period (Table 4).

At 120 days at 35°C, susceptible cultivars (Grand Rapids, Babá de Verão, Hortência, Rubete, and Salinas 88) exhibited a low percentage of emergence. The emergence test, upon being performed under greenhouse conditions, simulated what occur to rural producers upon storing their seeds at high temperatures. The results of this study showed that the storage temperature reduced the quality and led to dormancy (thermodormancy) of lettuce seeds.

Similar results were observed by other authors in the performance of tomato seedlings (Ferreira *et al.*, 2013). These authors affirm that emergence and speed of emergence are affected by temperatures above 33°C since these temperatures affect the speed of reorganization of the cellular membranes, thus altering seed metabolism and thereby reducing the speed of seedling emergence. Seed lots also behaved in a different manner in regard to the effects of high temperatures, when they have had a different history. Moreover, the speed of deterioration of the seeds is different, and even the component parts of a single seed deteriorate at different speeds (Marcos Filho, 2005), and this interferes in the physiological potential and in the capacity to resist adverse conditions imposed at the time of germination and emergence.

In addition to the physiological alterations caused by high temperatures in the seeds, there are alterations in the protein and/or enzymatic patterns. Lettuce seeds have germination limited by the presence of the endosperm, and there is thus the need for softening of this tissue for root protrusion to occur. This role is performed by various enzymes, such as endo-β-mannanase, which is present in the endosperm in different

Table 4. Mean seedling emergence (%) from lettuce seeds stored at different temperatures and periods. Lavras, UFLA, 2015.

Cultivars	Storage periods (days)											
	30			60			90			120		
	Storage temperatures (°C)											
	15	25	35	15	25	35	15	25	35	15	25	35
Luiza	93Aa	83Cb	66Cc	94Aa	83Bb	52Bc	93Ba	69Cb	36Cc	84Ba	53Cb	26Bc
Elisa	93Aa	82Cb	55Dc	93Aa	83Bb	50Bc	91Ba	75Bb	40Bc	83Ba	72Bb	28Bc
G. Rapids	91Ba	78Db	65Cc	89Ba	75Cb	47Bc	87Ba	68Cb	30Dc	80Ba	54Cb	8Cc
B. Verão	94Aa	87Bb	73Bc	90Ba	86Bb	48Bc	87Ba	68Cb	36Cc	78Ca	50Db	8Cc
Hortência	90Ba	83Cb	58Dc	88Ba	84Bb	40Cc	83Ca	69Cb	30Dc	82Ba	53Cb	4Cc
Rubete	95Aa	74Db	65Cc	92Aa	73Cb	41Cc	89Ca	67Cb	31Dc	82Ba	54Cb	7Cc
Salinas 88	91Ba	81Cb	54Dc	87Ba	75Cb	41Cc	87Ca	63Db	32Dc	76Ca	48Db	6Cc
Everglades	96Aa	95Aa	93Aa	97Aa	93Aa	95Aa	96Aa	83Ab	85Ab	94Aa	81Ab	79Ab
CV(%)	3.99											

Mean values followed by the same uppercase letter in the column and lowercase letter in the row do not differ among themselves by the Scott-Knott Test at 5% probability.

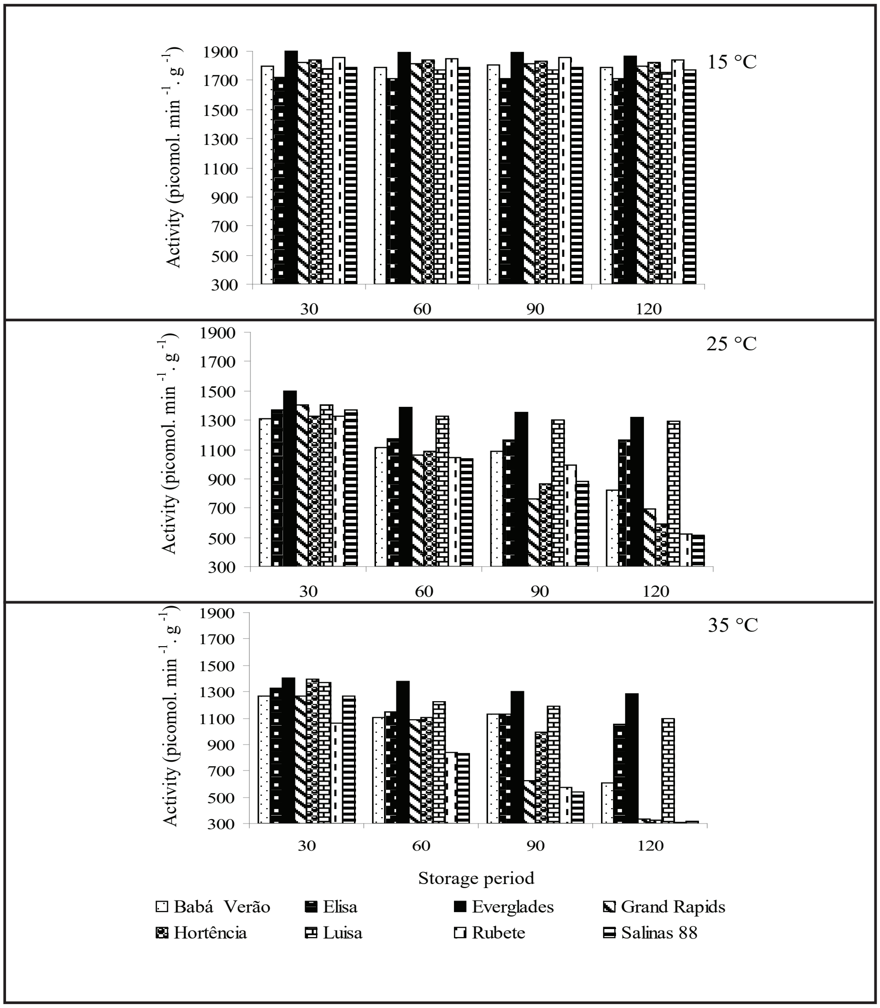

Figure 1. Activity of the enzyme endo-β-mannanase (picomol/min/g) in lettuce seeds depending on ambient temperatures (15; 25 and 35°C) and storage periods (30, 60, 90 and 120 days). Lavras, UFLA, 2015.

isoforms (Silva *et al*., 2004). At the beginning of lettuce seeds germination, soaking results in hydration of the matrices, such as the cells of the cell wall (Nonogaki *et al*., 2010), composed of mannose, glucose, galactose, and arabinose, which are degraded by endo-β-mannanase (Mo & Bewley, 2003).

It was possible to observe a reduction in the activity of the enzyme endo-β-mannanase throughout the storage period, especially when the lettuce seeds pre-soaked at 20°C were stored at 25 and 35°C (Figure 1), manifesting the natural process of deterioration that occurs in seeds, according to the results of germination and vigor. Other researchers observed that storage interferes in the enzyme endo-β-mananase activity of lettuce seeds (Diniz *et al*., 2009). The same behavior was observed for tomato seeds (Albuquerque *et al*., 2010). When the seeds were stored at 15°C, there was maintenance in the activity of this enzyme, regardless of the storage period (Figure 1).

The seeds of the cultivar Everglades showed enzymatic activity of 1900 pmol/min/g when stored at 15°C, but there was reduced activity (1400 pmol/min/g) when the seeds were stored at 35°C. It is noteworthy that Everglades at 120 days of storage and 35°C also exhibited greater enzymes activity than the other cultivars. It becomes evident at high temperature that this cultivar can express its germination potential, confirming the results observed in Table 1. The result indicates the importance of the enzyme endo-β-mannanase for viability of lettuce seeds, and it is one more indication that Everglades is a thermotolerant cultivar (Catão *et al*., 2014). It is also fitting to note that this behavior may vary according to the species, the genotype, and the cultivar, especially when there are differences in the storage temperature.

Based on all the physiological and biochemical results discussed above, it is evident that storage temperature is fundamental in conserving the quality of lettuce seeds due to its action on viability, vigor, and the biochemical reactions that determine this entire process. There are physiological and biochemical changes in lettuce seed as a result of the storage period and the storage environment. These changes are responsible for maintaining seed quality. The cultivars respond differently to the conditions to which they were exposed. The cultivar Everglades is thermotolerant.

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