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Early flowering, genetic dissimilarity and clustering of lettuce cultivars with thermoinhibition tolerant seeds

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ABSTRACT

High temperatures interfere with lettuce cropping. Thermoinhibition of seed germination and early flowering cause important losses for farmers. The objectives of this work were to evaluate the tolerance to early flowering, to verify the genetic dissimilarity and to select lettuce cultivars tolerant to seed thermoinhibition. 18 cultivars were evaluated. The number of days until the first anthesis was verified for early flowering. Regarding seed thermoinhibition, the first and final germination counting and germination speed index were evaluated, using temperatures of 20, 25, 30 and 35ºC. The analyses were performed immediately after harvest and after six months under storage. The genetic dissimilarity was obtained using the Tocher Graph optimization and hierarchical UPGMA methods. Regression models were adjusted, and curves' clustering was performed by testing the identity of the models. Early flowering was observed in cultivars Floresta, Colorado, Grand Rapids, and Everglades. Cultivars showed genetic variability and are dissimilar regarding tolerance to thermoinhibition. By clustering the regression equations, it was possible to select thermoinhibition tolerant cultivars.

Keywords: *Lactuca sativa*, anthesis, genetic variability, multivariate statistics, temperature.

RESUMO

Florecimento precoce, dissimilaridade genética e agrupamento de cultivares de alface com sementes tolerantes à termoinibição

As altas temperaturas interferem no cultivo da alface. A termoinibição da germinação das sementes e florecimento precoce causam perdas importantes para os produtores. Os objetivos deste trabalho foram avaliar a tolerância ao florescimento precoce, verificar a dissimilaridade genética e selecionar cultivares de alface tolerantes à termoinibição de sementes. Foram avaliadas 18 cultivares. Para florecimento precoce verificou-se o número de dias para a primeira antese. Em relação à termoinibição de sementes avaliou-se a primeira e última contagem de germinação e o índice de velocidade de germinação, utilizando-se as temperaturas de 20, 25, 30 e 35ºC. As análises foram realizadas imediatamente após a colheita e após seis meses de armazenamento. O estudo da dissimilaridade genética foi realizado por meio do método de otimização de Tocher Gráfico e hierárquico UPGMA. Os modelos de regressão foram ajustados e o agrupamento das curvas foi realizado testando a identidade dos modelos. Florescimento precoce foi observado nas cultivares Floresta, Colorado, Grand Rapids e Everglades. As cultivares apresentaram variabilidade genética e são diferentes quanto à tolerância à termoinibição. Ao agrupar as equações de regressão foi possível selecionar cultivares tolerantes à termoinibição.

Palavras-chave: *Lactuca sativa*, antese, variabilidade genética, estatística multivariada, temperatura.

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Leafy vegetables occupy 174 thousand hectares of the planted area in Brazil and lettuce (*Lactuca sativa*) is the most produced and consumed leafy vegetable (Pessoa & Machado Junior, 2021). Seed production of these species has reached a high technological level in Brazil (Franco *et al*., 2018). However, temperature is one of the variables which most influence the production of vegetables and their seeds.

Lettuce plants are not easy to adapt to high temperature areas (Aquino *et al.,* 2014), neither their flowering nor their seed germination. At temperatures over 22^{o}C, lettuce plants are induced to early flowering (Azevedo *et al.,* 2014). Early flowering causes stem elongation, reduction of the number of leaves, influences the formation of commercial heads and stimulates the production of latex. This confers a bitter taste to lettuce leaves, makes them rigid and anticipates reproductive cycle, resulting in depreciation of lettuce and causing financial losses (Blind & Silva Filho, 2015).

On the other hand, when lettuce seeds are exposed to high temperatures during formation and/or storage, they show significant germination inhibition (Catão *et al.*, 2018). Such inhibition may be temporary (thermoinhibition) or permanent (thermodormancy), due to the hardening of the endosperm, which restricts radicular protrusion (Catão *et al.*, 2016). In addition, independently of germination temperature, some cultivars may show seeds with a dormancy denominated as primary, in periods near post-harvest, which is naturally surpassed after few months under storage (Catão *et al.*, 2016, 2018).

The existence of genetic variability for thermoinhibition tolerance in seeds of species from the genus *Lactuca* is already known. Previous research showed tolerance to germination at temperatures of 35ºC in seeds of cultivar Everglades (*L. sativa*) (Nascimento *et al.*, 2012; Catão *et al.,* 2014), and the accession of *Lactuca serriola* UC96US23 (Argyris *et al.,* 2011; Yoong *et al.*, 2016). In contrast, there are no commercial cultivars available holding such characteristic in the Brazilian market, forcing to depend on observation of previous seeding results, under temperature conditions within the region where one pretends to establish lettuce crops, turning difficult or even impossible to obtain quality seedlings.

Predominantly, univariate methods have been used to select superior lettuce cultivars regarding tolerance to early flowering (Fiorini *et al.*, 2016) and tolerance to thermoinhibition (Catão *et al.*, 2014). The use of multivariate analysis to study genetic dissimilarity has been efficient for planning breeding programs and to define cropping strategies (Guedes *et al.*, 2013), with scarce information available on the topic of selection of tropical lettuce. The hierarchic method *Unweighted Pair Group Method Using Arithmetic Averages* (UPMGA) use the mean values of distances between pairs of genotypes to constitute groups, while the Tocher optimization method differs from the hierarchy method by the fact of the constituted groups being mutually exclusive, from a determinate clustering criterion (Cruz *et al.*, 2014).

Additionally, clustering of regression curves is a feasible alternative (Laurindo *et al.*, 2015), once when there are many genotypes the graphic representation becomes of hard visualization, due to the great number of regression curves (Azevedo *et al.*, 2012). Therefore, clustering of curves aids the graphic representation and superior genotypes' selection (Fiorini *et al.*, 2010). Diverse methods are available for curves' clustering using multivariate analysis (Azevedo *et al.,* 2012, 2015; Laurindo *et al.*, 2015). Although, the lack of statistical accuracy associated to significance levels make this strategy overlooked when compared to strategies based on test of identity of models, as in the method used by Vasconcelos *et al.* (2010). In view of these facts, the objective of this study was to evaluate the tolerance to early flowering, to verify genetic dissimilarity and to select, by means of clustering analysis of curves, lettuce cultivars with thermoinhibition tolerant seeds.

MATERIAL AND METHODS

The research was performed in an experimental area at the Center for Development and Transference of Technology from Federal University of Lavras (21°9'24''S, 44°55'34''W, 833 m altitude), in the municipality of Ijaci, Minas Gerais, Brazil. Initially, lettuce seedlings from different cultivars were produced: Everglades, Babá de Verão, Elisa, Lídia, Luiza, Regina 71, Regina 2000 (smooth leaves), Colorado, Floresta, Grand Rapids, Hortência, Marianne, Verônica (curly leaves), Salinas 88, Laurel, Raider Plus, Rubete, and Yuri (crisp head).

Seeds were seeded in expanded polystyrene trays with 128 cells containing commercial substrate. After 21 days seedlings were transplanted into flowerbeds in greenhouse conditions (maximum temperature: 18 to 36°C; minimum temperature: 15 to 27°C), with 0.4 x 0.6 m spacing and six plants per parcel. After transplanting, plants were daily evaluated to verify the data when the first flower opened, in order to determine the first anthesis. Cultivars Regina 71, tolerant to early flowering, and Grand Rapids, susceptible to early flowering, as determined by Fiorini *et al.* (2016), were used as controls.

Seeds from each cultivar were harvested individually, processed and then homogenized to constitute a unique lot. Then, part of the seeds was used to perform the experiments immediately after harvest, while other portion was stored in dry cool chamber (10ºC and 30% relative humidity of the air) and used in the experiments performed after six months storage.

Physiological quality of the seeds was determined through the following evaluations: **Germination and first count** - four samples with 50 seeds of each cultivar were sowed on two towel paper sheets dampened with water at a proportion of 2.5 times the weight of the dry substrate, in gerbox® plastic boxes. These boxes remained in BOD chamber at temperatures of 20, 25, 30 and 35ºC and 12 h photoperiod. The evaluation was constituted by two counts of normal plantlets with four and seven days in the BOD and the results were expressed as percentage of normal plantlets (Brasil, 2009). **Germination speed index** - was performed simultaneously to the germination test, computing during the same daily schedule the number of germinated seeds (defined as those with radicle protrusion). The index was determined according to Maguire (1962).

A randomized block design was used to evaluate early flowering, determining the number of days until the aperture of the first flower, with 18 treatments and three replicates. These data were submitted to analysis of variance and means were compared by the Scott-Knott test at 5% probability.

Genetic dissimilarity between cultivars was evaluated with multivariate analysis. The germination physiological responses at different temperatures immediately after the harvest and after six months under storage were evaluated. The measurements of dissimilarity were determined according to the multivariate analysis model;

determining a dissimilarity matrix, covariance matrix and the cultivar's measurements. Then, the canonical variables were determined as mentioned by Rao (1952). The generalized Mahalanobis distance (D^2ii') was used as a dissimilarity measure of distance, adopting the criterion by which the average of genetic divergence measures within each group must be lower than the distance between these groups. Thus, in order to facilitate the interpretation of dissimilarity measures between cultivars, the methods of clustering and optimization of Tocher (graphic), were used. A dendrogram was obtained by the UPGMA method and the cophenetic correlation coefficient (r) was calculated (Sokal & Rohlf, 1962), using the software GENES (Cruz, 2016).

Completely random design with four replicates in a factorial scheme 18 x 4 (18 cultivars x 4 temperatures), was used to study seed thermoinhibition. To study the effect of temperature, quadratic regressions were adjusted for each cultivar. In order to obtain a better graphical representation and facilitate the interpretation of the results, we performed the clustering of curves with the aid of test for identity of models. In this test, when rejecting the hypothesis of equality of the regression models, the clustering of these models is implemented based in the estimation of the reduction mean-squared (QMRed) between pairs of regression models, which are tested by the F test at 5% probability, in order to establish clusters (Vasconcelos *et al*., 2010). This statistical analysis was performed with the aid of the software R.

RESULTS AND DISCUSSION

There was a significant effect for all characteristics evaluated (F test, $p \leq 0.05$). Differences were detected between cultivars regarding the number of days until the first anthesis by the Scott-Knott test at 5% probability, with four clusters formed (Figure 1).

The average number of days until the first anthesis (flowering beginning) varied between cultivars from 75 to 111 days. This represents amplitude of 36 days, a difference higher than the one observed between the control treatments Grand Rapids and Regina 71, which was of 30 days (Figure 1). Aquino *et al.* (2014) reported the average number of days for flowering in progenies of lettuce as being 70 and 91 days, representing amplitude of 13 days.

The higher averages for the number of days until anthesis were observed in cultivars Laurel, Salinas 88, Rubete, and Raider Plus. These cultivars exhibit later flowering than Regina 71 (tolerant control). Cultivar Regina 71 did not differ from cultivars Yuri, Luiza, Elisa, Regina 2000 and Lidia. According to Fiorini *et al.* (2016), cultivars considered as tolerant to early flowering usually achieve the first anthesis at more than 100 days. Sala & Costa (2012) reported that cultivar Regina with smooth leaves was the first to be developed for planting in hot regions. This was done in the 1980s decade and allowed the expansion of lettuce cropping into hot regions due to a slower bolting. The development of cultivars adapted to regions with high temperatures has been one of the main purposes in lettuce breeding programs in Brazil. New cultivars exhibiting higher tolerance to early flowering are frequently made available to the market in Brazil.

Cultivars Verônica, Marianne, Hortência, and Babá de Verão bloomed between 87 and 91 days. It is important to mention that cultivars Verônica, Marianne, and Hortência are commercial materials with curly leaves, classified as tolerant to early flowering. Differences in days until flowering of a specific cultivar may be observed according to the temperature (Fiorini *et al.*, 2016).

Cultivar Grand Rapids is regularly used as a sensitive control for early flowering (Fiorini *et al.*, 2016). At flowering period lower than 80 days, cultivars Floresta, Colorado and Everglades also show low tolerance to early flowering, not differing even from Grand Rapids.

Variations from canonical variables are shown in Figure 2A. Scores from the first two variables, concerning the cultivars within each germination temperature, explained 81.99% of the total variation. These results allowed to visualize graphically (bi-dimensionally) the evaluated cultivars, using the first and second canonical variables, expressed in parentheses in Figure 2A. The graphical dispersion allowed separating cultivars in clusters, a method which may be used as a strategy to select divergent materials for breeding.

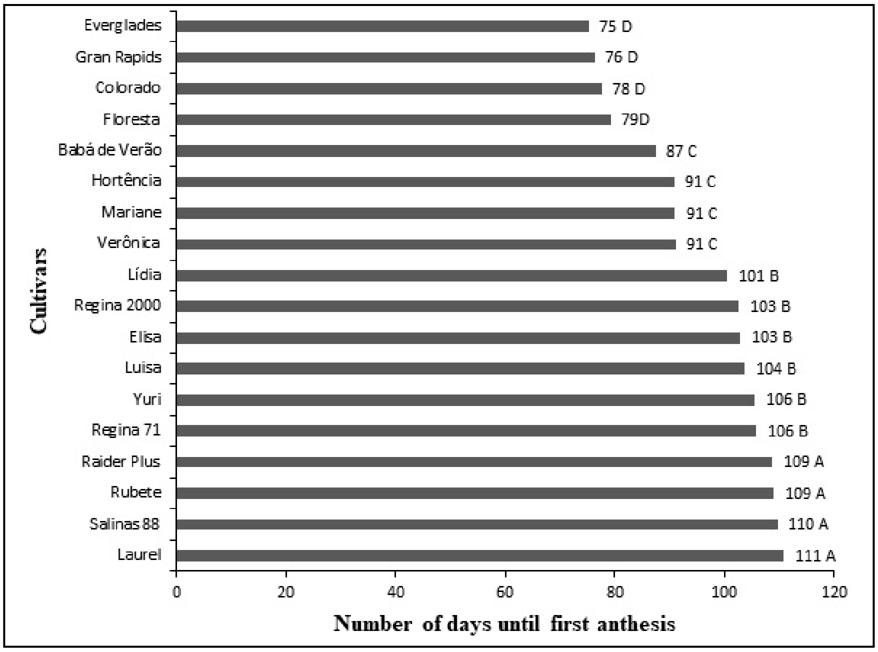

Figure 1. Average number of days until the first anthesis in lettuce cultivars. *Mean values followed by the same letter within the column are not statistically different among themselves by the Scott-Knott test at 5% probability. Ijaci, UFLA, 2021.

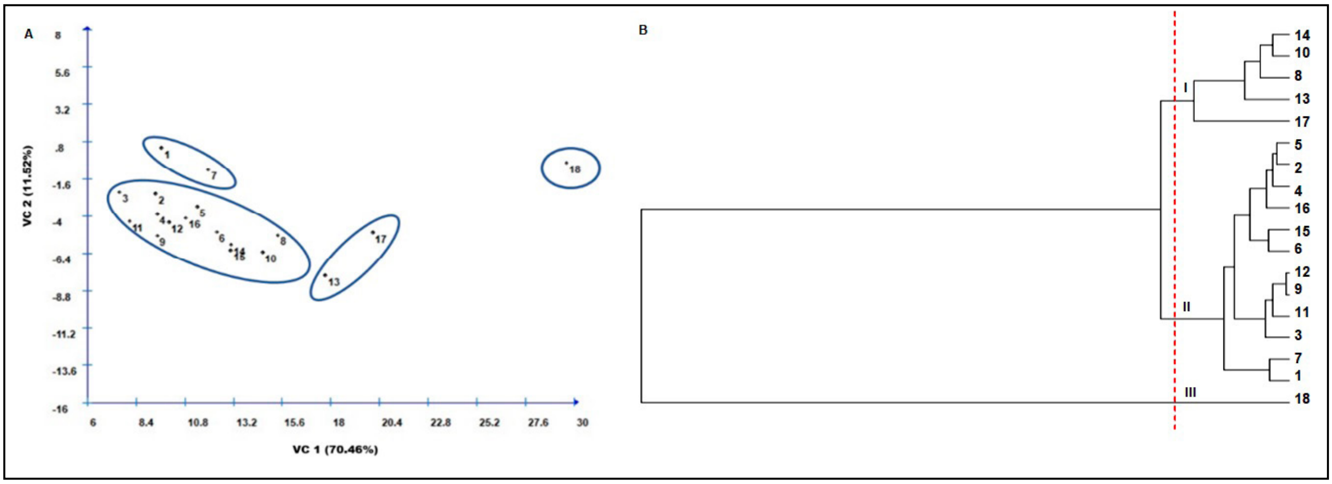

Figure 2. A: Dispersion of 18 lettuce cultivars regarding tolerance to thermoinhibition and considering the first two canonical variables. 1= Floresta; 2= Verônica; 3= Salinas 88; 4= Yuri; 5= Hortência; 6= Colorado; 7= Elisa; 8= Regina 2000; 9= Laurel; 10= Regina 71; 11= Rubete; 12= Marianne; 13= Babá de Verão; 14= Grand Rapids; 15= Raider Plus; 16= Lídia; 17= Luiza; 18= Everglades. B: Dendrogram developed from quantitative data with a generalized Mahalanobis distance ($D^2_{ii'}$) and the UPGMA clustering method in eighteen lettuce cultivars. Ijaci, UFLA, 2021.

It is possible to verify that cultivars are distributed in four different clusters, regarding tolerance to thermoinhibition. Cultivar Everglades was isolated from all others showing the high tolerance. Cultivar Luiza is the one closest to cultivar Everglades, showing higher tolerance when compared to the other cultivars. Certainly, such difference between canonical variables was due to the environmental effect, where the characters which contributed the most to the total genetic dissimilarity between cultivars, were precisely high germination temperatures (35ºC). Canonical variables, when used in genetic dissimilarity studies, have the purpose to identify similar genotypes in dispersion bi-dimentional or tri-dimentional graphics. In this kind of studies, the first canonical variables may comprehend as a minimum, 80% of the total variation of genotypes, where each variable is a linear combination of the original variables analyzed (Cruz *et al.*, 2014).

Cultivars were also separated in different clusters using dissimilarity measures (Figure 2B), which may be useful for tropical lettuce breeding (Araujo *et al.*, 2016). Using the generalized Mahalanobis distance, genetic dissimilarity between the eighteen cultivars varied from 1.10 (Grand Rapids) to 497.7 (Everglades). Thus, cultivar Everglades has a high dissimilarity when compared to the rest of cultivars, independently from germination temperature and the evaluation have been performed immediately after the harvest or after six months of seed storage. In contrast, cultivars Grand Rapids and Regina 71 showed to be sensitive to thermoinhibition. According to Marostega *et al.* (2017), the identification of genetic variability associated with seed germination allows to distinguish and better select the genotypes.

Groups established in the UPGMA dendrogram (Figure 2B) showed cophenetic correlation coefficient of 91.7% (T test, $p<0.05$). Lopes *et al.* (2017) suggested values of cophenetic correlation above 80% to guarantee better adjustment between the original matrix distances and the graphic. However, lower values do not nullify the dendrogram, but show distortion, thus the dendrogram is still useful to evaluate the clusters formed.

Clusters were separated using a cutting value of 61.95%, established at points of abrupt change in the dendrogram branches (Cruz *et al.*, 2014). Cluster I was constituted of five cultivars, cluster II of 12 cultivars and cluster III constituted by cultivar Everglades only. Based on such information one can verify cultivar Everglades has genetic dissimilarity, significantly differing from the other cultivars.

Analyzing the results using the Tocher's optimization method, genetic dissimilarity was also verified. Results are shown in Figure 3 displaying a substantial genetic variability between cultivars. The method for optimization of Tocher (graphical) may be used to show minuscule genetic dissimilarities between two cultivars. Values near zero show higher similarity (yellow), while values near one, show higher genetic dissimilarity (black) (Figure 3A). Cultivar Everglades shows great dissimilarity in comparison to the other cultivars (Figure 3A). However, cultivar Luiza has more similarity with Everglades at temperatures of 30 and 35ºC (Figure 3B). Germination of cultivar Luiza was of 24% (30ºC) and 19% (35ºC) immediately after harvest and 75% (30ºC) and 63% (30ºC) after six months storage, respectively. A further analysis on this cultivar is recommendable to verify possible effects of primary dormancy.

Analysis of clustering methods allows the identification of auspicious cultivars as well of those with higher genetic similarity and dissimilarity. Contrasting cultivars may be used for hybridization, enabling to increase the number of desirable recombinants and to obtain superior genotypes to produce seeds tolerant to thermoinhibition.

The estimates for the coefficient

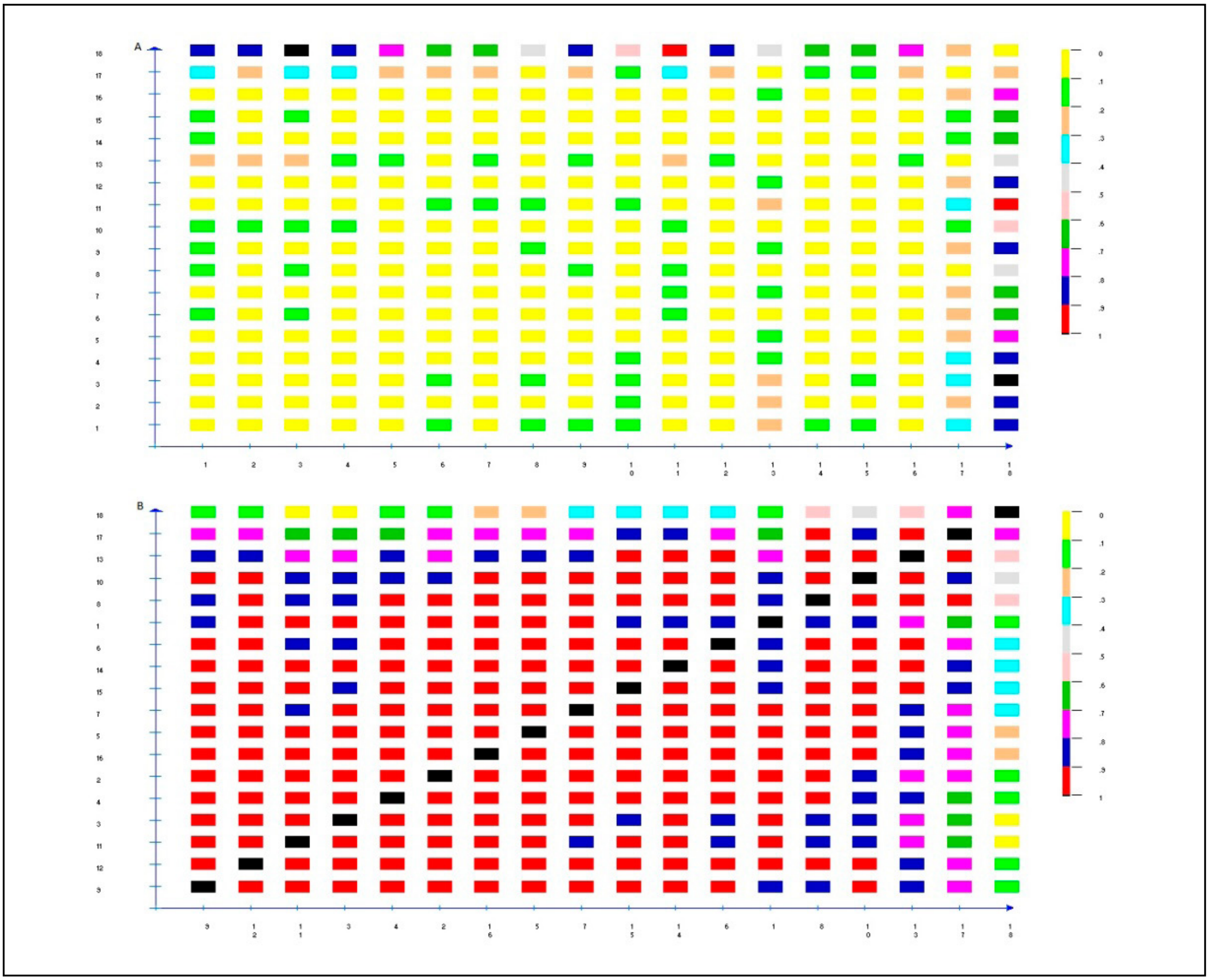

Figure 3. Clustering by the Tocher's optimization method (dissimilarity = A; similarity = B), based on eight physiological characteristics of germination from eighteen lettuce cultivars. 1= Floresta; 2= Verônica; 3= Salinas 88; 4= Yuri; 5= Hortência; 6= Colorado; 7= Elisa; 8= Regina 2000; 9= Laurel; 10= Regina 71; 11= Rubete; 12= Marianne; 13= Babá de Verão; 14= Grand Rapids; 15= Raider Plus; 16= Lídia; 17= Luiza; 18= Everglades. Ijaci, UFLA, 2021.

of second degree polynomial regression model and the coefficient of determination (R^2) for the clusters obtained by the test of model identity are shown in Table 1.

Using this technique allowed to reduce the number of regression curves that will be obtained for cultivar's germination. This fact promoted a better visualization and interpretation of data. Immediately after harvest four clusters were constituted. The third and fourth clusters contained cultivars Everglades and Luiza which showed the following coefficients: $\hat{a}$ = (68.350; 416.900) and c = (-0.060; 0.370), respectively. After six months storage nine cultivar clusters were constituted. Clusters seven and nine, also represented by Everglades and Luiza, showed the following coefficients: $\hat{a}$ (-32.375; -13.225) and c (-0.215; -0.145), respectively. Such results are in accordance with the germination percentages for these cultivars at high temperatures. Germination of seeds from cultivars in clusters one and two, immediately after the harvest, showed the lowest coefficients $\hat{a}$ and c, therefore those cultivars showed lower germination percentages under high temperatures. After six months of storage the lowest coefficients were verified in clusters six, five, one, two and three, respectively.

Differences between clusters from harvest and storage may probably be related with primary dormancy of seeds. Bessa *et al.* (2015) verified that crambe (*Crambe abyssinica*) seeds are able to surpass primary dormancy due to favorable conditions of storage environment. It is worth to remember that some cultivars did not germinate adequately immediately after harvest, therefore requiring a storage period in order to surpass primary dormancy. Using a multivariate clustering of regression curves, Azevedo *et al.* (2018) discriminated sweet-potato accesses with higher and lower tolerance to dehydration. Fiorini *et al.* (2010) and Azevedo *et al.* (2012) also selected

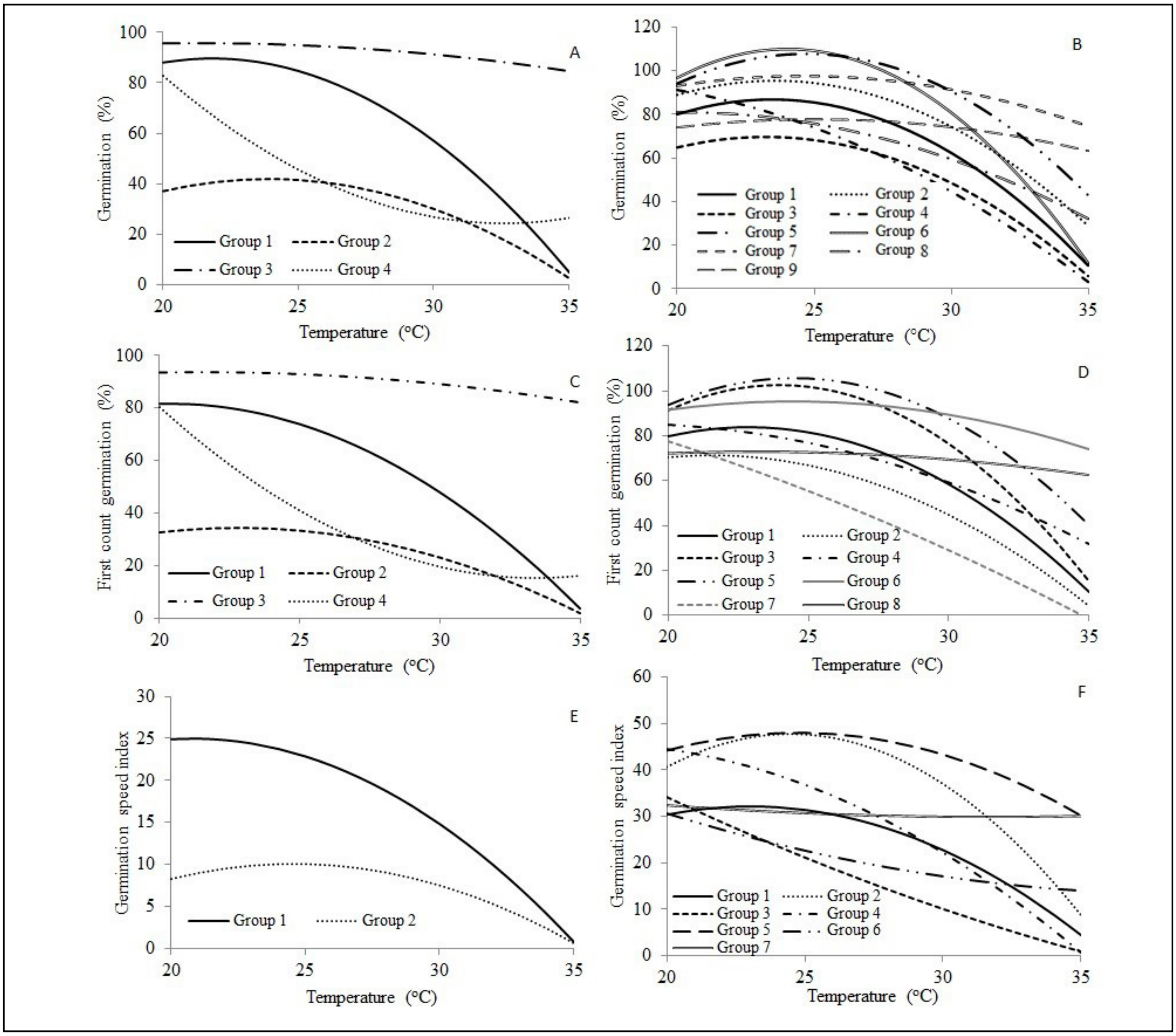

Figure 4. Predictions for germination, first count germination and germination speed index of lettuce seeds immediately after harvest (A, C, E) and after six months storage (B, D, F) at different temperatures obtained by the second degree polynomial model in clusters formed by the test of identity of models. Ijaci, UFLA, 2021.

tomato accessions showing resistance against *Phytophthora infestans*. Thus, multivariate clustering based on the model's coefficients clusters accesses according to the similarity of the curve shape, and consequently, with similarity on their performance along the evaluation period (Silveira *et al.*, 2011).

Germination of cultivars Everglades and Luiza (clusters 3 and 4) immediately after harvest and after six months of storage (clusters 7 and 9), can be observed in Figures 4A and 4B, respectively. It is possible to verify that Everglades has a tendency superior to Luiza regarding tolerance to high temperatures. However, it is important to emphasize that cultivar Luiza has superior germination when compared to the other cultivars at high temperatures.

Catão *et al.* (2014) considered that cultivar Luiza has moderate tolerance to thermoinhibition. Thus, these authors recommend that this cultivar may be introduced in genetic breeding programs in order to accomplish a more comprehensive research. Amongst all other clusters, independently from the evaluation period, seed germination remained up to 25°C. Above this temperature germination declined as the temperature raised (Figures 4A and 4B).

Estimates for the coefficient of second degree polynomial regression model and coefficient of determination (R^2) for clusters, obtained by the identity of models test between lettuce cultivars and temperatures, in the first count germination test, are shown in Table 1 and Figure 4C and 4D. Four clusters of cultivars were constituted after harvest, corroborating with data from the germination test. However, after six months storage, seeds constituted eight clusters, differing form germination for the same period. It has to be emphasized that first count germination test is

Table 1. Estimates for the coefficient of second degree polynomial regression model and coefficient of determination (R^2), for clusters obtained by the test of model identity from germination test, first count and germination speed index. Ijaci, UFLA, 2021.

Cluster	Cultivars	Coefficients a	Coefficients b	Coefficients c	R^2
Germination					
Immediately after harvest					
1	Colorado, Hortência, Yuri, Verônica, Regina 2000, Floresta, Elisa, Salinas 88, Raider Plus	-164.331	22.418	-0.503	1.000
2	Babá de verão, Grand Rapids, Laurel, Lídia, Marianne, Regina 71, Rubete	-111.975	12.951	-0.276	0.998
3	Everglades	68.350	2.560	-0.060	0.950
4	Luiza	416.900	-24.110	0.370	0.864
Six months storage					
1	Laurel, Marianne, Yuri, Hortência, Lídia	-227.880	26.832	-0.572	0.964
2	Regina 2000, Regina 71, Raider Plus	-187.550	24.037	-0.510	0.980
3	Rubete, Salinas 88	-179.888	21.493	-0.463	0.999
4	Elisa, Verônica, Floresta	-38.958	7.442	-0.242	0.966
5	Babá de Verão	-267.675	30.395	-0.615	0.940
6	Colorado	-360.575	39.155	-0.815	0.959
7	Everglades	-32.375	10.575	-0.215	0.816
8	Grand Rapids	-8.400	8.860	-0.220	0.980
9	Luiza	-13.225	7.265	-0.145	0.871
First count germination					
Immediately after harvest					
1	Colorado, Elisa, Floresta, Hortência, Regina 2000, Salinas 88, Yuri, Verônica. Raider Plus	-70.742	14.972	-0.367	0.997
2	Babá de Verão, Grand Rapids, Laurel, Lídia, Marianne, Regina 71, Rubete	-80.700	10.101	-0.221	0.989
3	Everglades	63.425	2.805	-0.065	0.970
4	Luiza	417.950	-24.080	0.360	0.889
Six months storage					
1	Elisa, Hortência, Laurel, Lídia, Marianne, Regina 71, Yuri	-227.880	26.832	-0.572	0.944
2	Rubete, Salinas 88, Verônica	-187.550	24.037	-0.510	0.960
3	Colorado, Raider Plus	-179.888	21.493	-0.463	0.999
4	Grand Rapids, Regina 2000	-38.958	7.442	-0.242	0.996
5	Babá de Verão	-267.675	30.395	-0.615	0.970
6	Everglades	-360.575	39.155	-0.815	0.989
7	Floresta	-32.375	10.575	-0.215	0.802
8	Luiza	-8.400	8.860	-0.220	0.985
Germination speed index					
Immediately after harvest					
1	Colorado, Elisa, Everglades, Floresta, Hortência, Lídia, Luiza, Marianne, Raider Plus, Regina 2000, Salinas 88, Verônica, Yuri	-26.888	4.995	-0.120	0.810
2	Babá de Verão, Grand Rapids, Laurel, Regina 71, Rubete	-42.609	4.298	-0.088	0.882
Six months storage					
1	Hortência, Laurel, Lídia, Marianne, Regina 2000, Regina 71, Rubete, Salinas 88, Yuri	-70.122	8.871	-0.193	0.994
2	Babá de Verão, Colorado, Raider Plus	-163.988	17.289	-0.353	0.920
3	Floresta, Verônica	105.447	-4.332	0.038	0.969
4	Elisa	5.618	4.723	-0.139	0.906
5	Everglades	-55.721	8.377	-0.169	0.940
6	Grand Rapids	87.170	-3.803	0.049	0.934
7	Luiza	47.903	-1.141	0.018	0.899

considered a vigor predictor once seeds with higher physiological potential are able to show higher tolerance to stresses.

One of the oldest concepts of seed vigor is associated with germination speed (Maguire, 1962). Although seed lots show similar germination percentages, differences in germination speed are frequently observed, therefore, seeds with higher germination speed are more vigorous. Only two clusters were constituted immediately after harvest for the germination speed index (Table 1 and Figures 4E and 4F). The second cluster contains only cultivars Grand Rapids, Laurel and Regina 71 which shows low tolerance to thermoinhibition. These cultivars have slow germination speed and therefore they are more susceptible to injuries caused by high germination temperatures. Seeds from cultivars Floresta, Verônica, Elisa and Grand Rapids evaluated after six months storage showed a reduction of the germination speed index from 25ºC onwards. Cultivars from other clusters also showed a reduction of germination speed when the temperature increased to 35°C (Figures 4E and 4F). Catão *et al.* (2014) also verified lower indices of germination speed at high temperatures, in agreement with the results observed in the present work.

As observed, lettuce cultivar Everglades is tolerant to thermoinhibition, however, it blooms early after only 75 days of sowing. As previously stated, cultivar Regina 71 is tolerant to early flowering (Fiorini *et al*., 2016), however as verified it is totally dissimilar from Everglades with its germination inhibited at high temperatures. Therefore, it is not possible to associate cultivars with tolerance to early flowering as being tolerant to thermoinhibition and vice-versa. Based on the results shown and discussed, it is evident that temperature is an aggravating factor for plant development and the quality of lettuce seeds.

Therefore, cultivars Floresta, Colorado, Grand Rapids and Everglades bloom prematurely. Cultivars show dissimilarity regarding their tolerance to thermoinhibition. It is possible to separate sensitive cultivars and select those tolerant to thermoinhibition. Everglades is tolerant to thermoinhibition.

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