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ARTICLE

Artificial neural networks discriminate lettuce seeds with different levels of thermoinhibition

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ABSTRACT: The thermoinhibition of lettuce seed germination causes important losses for producers, who do not have thermotolerant commercial cultivars. One of the obstacles has been the scarcity of optimizing techniques capable of efficiently discriminating thermotolerant and thermosensitive cultivars. The aim of this work was to evaluate the use of neural networks to discriminate different levels of thermoinhibition in lettuce seeds. Seeds of 18 cultivars were evaluated for thermoinhibition considering the characteristics of the first and last germination count and germination speed index, in seeds subjected to temperatures of 20, 25, 30 and 35 °C. The remaining seeds, which did not germinate, were subjected to the tetrazolium test. Analyses were performed immediately after seed harvesting and repeated after six months of storage. Discriminant analysis was performed and the Kohonen's Self-Organizing Map (SOM) was created using Artificial Neural Networks (ANNs). Neural networks discriminate lettuce cultivars and organizes them in terms of seed thermoinhibition tolerance through Kohonen's Self-Organizing Map. Discriminant analysis consistently identifies the Everglades and Luiza genotypes as tolerant to thermoinhibition.

Index terms: computational intelligence, harvest, *Lactuca sativa* L., seed dormancy, storage.

RESUMO: A termoinibição causa perdas importantes para os produtores, os quais não dispõem de cultivares comerciais com sementes termotolerantes. Há escassez de técnicas otimizadoras capazes de discriminar cultivares termotolerantes e termosensíveis com eficiência. Objetivou-se avaliar o uso de redes neurais para discriminar diferentes níveis de termoinibição em sementes de alface. Foram avaliadas sementes de 18 cultivares quanto à termoinibição considerando às características de primeira e última contagem de germinação e índice de velocidade de germinação, em sementes submetidas às temperaturas de 20, 25, 30 e 35 °C. As sementes remanescentes, que não germinaram, foram submetidas ao teste de tetrazólio. As análises foram realizadas imediatamente após a colheita das sementes e repetidas após seis meses de armazenamento. Uma análise discriminante e o Mapa Auto-Organizável de Kohonen (SOM) por Redes Neurais Artificiais (RNA's) foram realizados. As redes neurais discriminam as cultivares de alface e as organiza quanto a tolerância a termoinibição das sementes por meio do Mapa Auto-Organizável de Kohonen. A análise discriminante indentifica de maneira coerente o genótipo Everglades e Luiza como tolerantes a termoinibição.

Termos para indexação: inteligência computacional, colheita, *La*ctuca sativa L., dormência de sementes, armazenamento.

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INTRODUCTION

The COVID-19 pandemic brought several difficulties to Brazil with the closure of commercial food establishments. This caused a decrease in the consumption of leafy vegetables, resulting in a reduction in investments and in the planted area, mainly of lettuce (*Lactuca sativa* L.) (Anuário HF Brasil, 2020). Part of the planted area is expected to be recovered in the coming years, with increased consumption and prices.

Lettuce faces difficulties of adaptation in places with high temperatures, with regard to both flowering and seed germination (Catão et al., 2022a; Catão et al., 2022b). At temperatures greater than 22 °C, the plant is induced to flower early (Azevedo et al., 2014), resulting in the depreciation of the product, causing financial losses (Blind and Silva-Filho, 2015). On the other hand, seeds when exposed to high temperatures during imbibition and/or storage have their germination significantly inhibited (Catão et al., 2018), and this inhibition can be temporary (thermoinhibition) or complete (thermodormancy), due to the hardening of the endosperm, which ends up restricting root protrusion (Catão et al., 2016). Seed imbibition at high temperatures can denature enzymes (endo-β-mannanase), accelerate breathing and lead seeds to death due to deterioration (Catão et al., 2014; Nascimento et al., 2004).

It is also known that there is genetic variability for tolerance to thermoinhibition in seeds of species of the genus *Lactuca*. This has been observed in some studies that showed tolerance to germination at temperatures of 35 °C in seeds of the cultivar Everglades (*Lactuca sativa* L.) (Nascimento et al., 2012; Catão et al., 2014; Catão et al., 2022) and the *Lactuca serriola* accession UC96US23 (Argyris et al., 2011; Yoong et al., 2016).

In addition, regardless of germination temperature, some cultivars may have seeds with primary dormancy in periods close to harvest. This dormancy is established in the development and/or maturation stage of the seeds, while they are still physiologically linked to the mother plant. The mechanisms that lead to this type of dormancy are genetic, so seeds do not germinate shortly after harvest (Lopes and Nascimento, 2012). Thus, seeds need to be stored for their dormancy to be overcome (Catão et al., 2016; Catão et al., 2018).

In this context, the selection of lettuce plants that produce thermotolerant seeds is suggested. One of the major obstacles has been the difficulty in finding which statistical method is the most appropriate to evaluate multiple characteristics in seeds with different levels of thermoinhibition. One way to enable this type of analysis is through Computational Intelligence, especially artificial neural networks (ANNs), which mimic the functioning of biological neurons, acquiring knowledge and learning from errors (Cardoso et al., 2019).

ANNs can be used in classification and grouping, prediction of traits of interest, estimation of genetic diversity, fitting of models, study of adaptability and stability, and in genome-wide selection, among others (Oliveira et al., 2013; Hu et al., 2019; Cardoso et al., 2019). One type of ANNs is the Kohonen's Self-Organizing Maps (SOM), which simulate the functioning of the cerebral cortex, recognize patterns and create clusters (with neurons of the network), establishing strong connections with the closest neurons; likewise, close groups are more similar (Cruz and Nascimento, 2018). This occurs because the method of analysis has high accuracy, since even if there are experimental errors that do not meet the premises, as occurs in experiments using univariate statistics, there is the formation of groups of more representative similar and dissimilar materials in the ANNs, thus reducing subjectivity in the selection of genotypes.

Cardoso et al. (2021), for example, used ANNs to select colored-fiber cotton genotypes for adaptability and stability. However, there are no reports as to whether the technique can be effective for grouping lettuce cultivars with thermotolerant seeds. Commercial lettuce cultivars tolerant to thermoinhibition are not registered in the RNC/MAPA (Brasil, 2021). Thus, a demand is created in which tropicalized lettuce breeding programs start to accurately select genotypes that show tolerance to thermoinhibition. In view of the above, the objective of this study was to evaluate the efficacy of using artificial neural networks in the discrimination of lettuce cultivars with different levels of thermoinhibition in their seeds.

MATERIAL AND METHODS

The study was conducted at the Institute of Agrarian Sciences of the *Universidade Federal de Uberlândia* (UFU). Lettuce seeds of the following cultivars were produced: Butterhead lettuce (Everglades, Babá de Verão, Elisa, Lídia, Luiza, Regina 71, Regina 2000); Leaf lettuce (Colorado, Floresta, Grand Rapids, Hortência, Marianne, Verônica); and Iceberg lettuce (Salinas 88, Laurel, Raider Plus, Rubete, Yuri). The cultivar Everglades was used as a thermoinhibition-tolerant control (Catão et al., 2014) and the cultivar Grand Rapids was used as a thermoinhibition-sensitive control (Catão et al., 2018).

The seeds were sown in expanded polystyrene trays with 200 cells containing commercial substrate. After 21 days, the seedlings were transplanted to beds in an area under protected cultivation, using spacing of 0.4 x 0.6 meters with six plants per plot. The cultural practices and seed production were conducted as recommended for lettuce crop (Filgueira, 2013; Franco et al., 2018).

The seeds of each cultivar were harvested individually, processed and then homogenized to compose a single lot of seeds for each cultivar. Then, part of the seeds was analyzed shortly after harvest, while another part was stored in a cold and dry chamber (15 °C and 55% relative humidity), and the analyses were carried out after six months of storage.

The physiological quality of the seeds was determined by the following evaluations:

Germination and first germination count: four replications of 50 seeds of each cultivar were sown on two sheets of blotting paper, moistened with distilled water, in the proportion of 2.5 times the weight of the dry substrate, in transparent plastic boxes (Gerbox type). The boxes with the seeds were kept in four BOD-type chambers previously regulated at temperatures of 20 °C, 25 °C, 30 °C and 35 °C under a 12-hour photoperiod. The evaluation consisted of two counts of normal seedlings at four and seven days, and the results were expressed as percentage (Brasil, 2009).

Germination Speed Index: determined simultaneously with the germination test, by computing the number of germinated seeds daily and at the same time. Germinated seeds were those with radicle protrusion. The index was calculated according to the formula proposed by Maguire (1962).

Tetrazolium: performed on the remaining seeds (seeds that did not germinate) of the germination test, and the embryo was exposed after gently pressing the seed coat for its removal. Staining was performed in a solution of 2,3,5-triphenyl-tetrazolium chloride at a concentration of 1%, for three hours in the dark, at 30 °C. After this period and after checking the staining, the embryos were washed in running water and kept submerged in water until their evaluation, when they were analyzed individually to determine their viability. The interpretation was performed according to Brasil (2009), and the results were expressed in numbers of viable and dead seeds.

The experimental design used was completely randomized, with four replications. The statistical analysis of the data was in 18 x 4 factorial scheme (18 cultivars x 4 germination temperatures), and the evaluation times were analyzed independently.

Discriminant analysis was performed and the Kohonen's Self-Organizing Map (SOM) was created using artificial neural networks (ANNs). The SOM architecture is *feedforward* type with one input layer and one output layer, called topological map, divided into three steps (Cruz and Nascimento, 2018):

Step 1: to define the topological map and establish the random weights, three neurons in two dimensions (Figure 1A) (3 rows and 3 columns), 2000 epochs and neighborhood pattern with radius=2, the *dist* activation function (Euclidean distance), and hexagon-type topology were used for the formation of SOM. Subsequently, the synaptic weights and an input vector X_i were initialized.

Step 2: given the input values, the measure of the distance in competition was calculated, and the winning neuron was established as the one with the shortest distance; the input data of the neighboring neurons had their weights adjusted relative to the input, to determine their neighborhood by the learning rate (η), using the following expression:

$$i.w^{i+1}(winner) = W^{i} + (winner)^{+\eta(Xi-W^{1})}(winner)$$

$$ii. w^{i+1}(neighborhood) = W^{i} + (neighborhood)^{+f(x)\eta(Xi-W^{1})}(neighborhood)$$

η= Measure of learning rate; w = weight of neurons; xi= Input vector; f(x)= half the learning rate.

Step 3: Each input participated in the competition, ending an epoch, and stage 2 was resumed until there were no major changes between the input weights and the updated ones.

Discriminant analysis was performed by means of ANNs using a *Multilayer Perceptron* (MLP) neural network formed by two layers containing two to five neurons each, using the logarithmic activation function. The training algorithm chosen was Trainlm (Levenberg-Marquardt backpropagation). The training cycle was set at 5000 times with an error rate of 0.01%, with 80% of data relative to germination at different temperatures for training and 20% for validation of the neural network (Cruz and Nascimento, 2018) (Figure 1B). A 5 x 5 grid was established to represent a set of 25 neurons.

For the statistical analysis of the data, the F test and analysis of variance at 5% probability level were used. The means were grouped by the Scott-Knott test at 5% probability level. The analyses were performed in the GENE statistical program, integrated with R software and Matlab (Cruz, 2016).

RESULTS AND DISCUSSION

There was significant interaction between cultivars and germination temperatures shortly after harvest and with six months of storage (F test, 5% significance) for the variables analyzed. The seeds of most lettuce cultivars were sensitive to thermoinhibition or germination failure. Most of the seeds that did not germinate lost their viability at temperatures of 30 °C and 35 °C (Figure 3). Temperatures above 28 °C cause thermoinhibition of seeds (Yoong et al., 2016). It was possible to observe reduction of seed germination at temperatures of 30 °C and 35 °C, regardless of the evaluation time (shortly after harvest and with six months of storage) (Table 1). Imbibition at high temperatures can denature enzymes responsible for germination (endo-β-mannanase), besides accelerating respiration and causing death of seeds due to deterioration (Nascimento et al., 2004; Catão et al., 2014).

It is possible to observe that the cultivars Babá de Verão, Grand Rapids, Laurel, Lídia, Mariane, Raider Plus, Regina 71, Rubete and Salinas 88, shortly after harvest, show germination lower than the standard used for the commercialization of seeds of this species, which is 80% (Brasil, 2019) (Table 1). Probably, the seeds of these cultivars have primary dormancy, because the temperature of 20 °C is considered ideal for the germination of lettuce seeds (Brasil, 2009). It is worth pointing out that the seeds of these cultivars showed high viability, as can be verified in the tetrazolium test, but these results will be presented later and discussed throughout the text. The seeds of the cultivars Laurel, Marianne, Rubete and Salinas 88 also did not meet the commercialization standard at six months of storage (Table 1). It is suggested that these cultivars require more time to overcome dormancy.

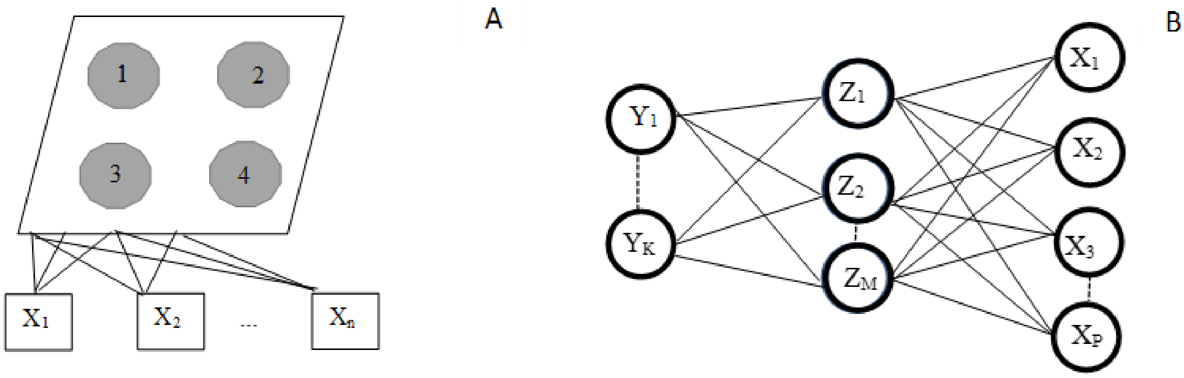

Figure 1. (A) Architecture and topology of a two-dimensional SOM neural network (adapted from Cruz and Nascimento, 2018); (B) Scheme of the only hidden layer of the neural network (Adapted from Nascimento et al., 2013).

Table 1. Average percentages of first germination count (FGC), germination (G) and germination speed index (GSI) of lettuce seeds shortly after harvest and with six months of storage at different temperatures.

Cultivars	Shortly after harvest											
	Temperature											
	20 °C			25 °C			30 °C			35 °C		
	FGC	G	GSI	FGC	G	GSI	FGC	G	GSI	FGC	G	GSI
B. Verão	44Ba	47Ba	9.0Ba	29Bb	31Ba	11.7Ba	27Bb	29Ba	10.8Ba	7Bc	7Bb	3.0Bb
Colorado	96Aa	98Aa	29.4Aa	93Aa	95Aa	32.3Aa	50Ab	77Aa	14.1Bb	1Bc	2Bb	0.1Bc
Elisa	90Aa	92Aa	23.6Aa	78Aa	85Aa	22.9Aa	22Bb	27Bb	5.5Bb	6Bc	16Bb	2.0Bb
Everglades	94Aa	96Aa	32.7Aa	92Aa	94Aa	40.9Aa	91Aa	93Aa	33.9Aa	82Aa	84Aa	23.3Aa
Floresta	90Aa	94Aa	23.9Aa	90Aa	93Aa	30.9Aa	26Bb	28Bb	7.8Bb	10Bb	11Bb	2.9Bb
G. Rapids	27Ba	37Ba	9.1Ba	20Ba	24Ba	7.3Ba	12Bb	18Ba	5.2Ba	1Bb	1Bb	0.1Ba
Hortência	95Aa	97Aa	26.2Aa	86Aa	92Aa	29.8Aa	58Ab	74Ab	17.4Aa	1Bc	1Bc	0.1Bb
Laurel	29Ba	33Ba	8.4Ba	48Ba	52Ba	15.3Ba	26Ba	39Ba	5.5Ba	0Bb	1Bb	0.1Bb
Lídia	63Aa	65Aa	15.8Ba	44Bb	47Ba	18.6Ba	13Bb	18Bb	3.6Bb	2Bc	3Bc	0.4Bb
Luiza	87Aa	90Aa	35.0Aa	40Bb	49Bb	11.9Bb	21Bb	24Bb	8.8Bb	10Bc	19Bb	3.6Bb
Marianne	61Aa	67Aa	29.1Aa	37Ba	40Bb	17.2Ba	25Bb	30Bb	5.5Bb	6Bc	7Bc	2.1Bb
R. Plus	66Aa	68Aa	28.3Aa	46Ba	40Bb	14.9Bb	32Ba	39Bb	7.9Bb	3Bb	5Bc	0.6Bc
Reg. 2000	90Aa	91Aa	30.3Aa	68Ab	81Aa	22.5Aa	52Ab	58Ab	20.1Aa	20Bc	4Bc	6.4Bb
Regina 71	25Ba	31Ba	13.9Ba	17Bb	29Ba	4.2Bb	8Bb	15Bb	1.8Bb	3Bb	3Bc	0.7Bb
Rubete	43Ba	44Ba	17.6Ba	26Ba	28Ba	8.4Ba	1Bb	1Bb	0.1Bb	0Bb	1Bb	0.1Bb
Salinas 88	63Aa	67Aa	20.2Aa	57Aa	62Aa	18.4Ba	30Bb	44Ba	8.8Bb	0Bc	1Bb	0.1Bc
Verônica	83Aa	87Aa	33.4Aa	78Aa	83Aa	24.1Aa	64Aa	76Aa	22.4Aa	1Bb	1Bb	0.1Bb
Yuri	82Aa	86Aa	27.6Aa	77Aa	80Aa	30.9Aa	71Aa	78Aa	20.1Aa	1Bb	2Bb	0.2Bb
CV (%)	15.4	11.4	25.7	15.4	11.4	25.7	15.4	11.4	25.7	15.4	11.4	25.7

Cultivars	Six months of storage											
	Temperature											
	20 °C			25 °C			30 °C			35 °C		
	FGC	G	GSI	FGC	G	GSI	FGC	G	GSI	FGC	G	GSI
B. Verão	100Aa	100Aa	46.4Aa	96Aa	97Aa	43.9Aa	94Aa	99Aa	38.3Aa	38Bb	40Bb	14.2Bb
Colorado	100Aa	100Aa	43.2Aa	93Aa	99Aa	45.0Aa	87Aa	91Aa	40.2Aa	7Cb	8Cb	1.7Cb
Elisa	89Aa	90Aa	42.7Aa	88Aa	93Aa	41.8Aa	43Db	43Db	17.0Cb	7Cc	9Cc	2.3Cc
Everglades	94Aa	95Aa	45.5Aa	97Aa	97Aa	47.5Aa	88Aa	92Aa	43.6Aa	72Ab	84Aa	28.7Ab
Floresta	73Ba	87Aa	28.5Ba	71Ba	81Ba	22.7Ca	14Eb	18Eb	3.7Db	4Cc	5Cc	1.0Cb
G. Rapids	80Ba	82Aa	31.6Ba	68Ba	72Bb	19.8Cb	56Cb	63Cb	20.0Cb	31Bc	31Bc	13.1Bb
Hortência	91Aa	92Aa	30.1Bb	86Aa	86Aa	40.1Aa	54Cb	62Cb	14.9Cc	10Cc	13Cc	3.6Cd
Laurel	74Ba	76Ba	33.1Ba	71Ba	72Ba	30.3Ba	67Ba	67Ba	31.9Ba	10C	10Cb	3.3Cb
Lídia	89Aa	91Aa	34.5Ba	82Aa	92Aa	32.0Ba	58Cb	60Cb	22.1Cb	6Cbc	8Cc	1.6Cc
Luiza	74Ba	80Aa	34.5Ba	74Ba	75Ba	33.8Ba	69Ba	75Ba	25.9Ca	61Aa	63Ab	28.4Aa
Marianne	72Ba	77Ba	34.4Ba	70Ba	73Ba	28.3Ba	65Ba	72Ba	26.3Ca	5Cb	8Cb	1.8Cb
R. Plus	91Aa	91Aa	42.9Aa	89Aa	91Aa	43.7Aa	89Aa	89Aa	39.8Aa	17Cb	18Cb	7.6Cb
Reg. 2000	93Aa	95Aa	33.5Ba	80Ab	88Aa	24.5Cb	69Bb	82Ba	20.7Cb	31Bc	32Bb	13.3Bb
Regina 71	89Aa	92Aa	32.2Ba	80Ba	87Aa	25.8Ca	61Cb	67Cb	19.9Cb	27Bc	33Bc	12.0Bb
Rubete	64Ba	66Ba	24.2Ba	60Ba	63Ba	25.0Ca	49Da	52Db	22.7Ca	6Cb	9Cc	3.1Cb
Salinas 88	72Ba	73Ba	34.1Ba	61Ba	65Ba	29.0Ba	44Db	48Db	18.1Cb	1Cc	2Cc	0.5Cc
Verônica	87Aa	88Aa	38.2Aa	69Bb	73Ba	23.9Cb	42Dc	49Db	11.8Dc	5Cd	6Cc	2.0Cc
Yuri	83Aa	84Aa	39.5Aa	71Ba	76Ba	33.6Ba	71Ba	75Ba	25.7Cb	2Cb	2Cb	0.7Cc
CV (%)	15.8	13.2	27.9	15.8	13.2	27.9	15.8	13.2	27.9	15.8	13.2	27.9

*Means followed by the same uppercase letter in the column and lowercase letter in the row of the analyzed variable do not differ from each other, by the Scott-Knott test, at 5% significance level.

Primary dormancy is common in most lettuce cultivars, which prevents or impairs germination immediately after seed harvesting. This dormancy can be overcome naturally after the fourth month of storage (Kano et al., 2011). Primary dormancy contributed to the low germination percentage of seeds of these cultivars at 20 °C and at higher temperatures. Thus, primary dormancy can influence the evaluation of thermoinhibition when germination is performed immediately after seed harvesting. Another aspect to be considered is related to the vigor of the seeds, as those with low vigor may lead to erroneous evaluations. For a more reliable evaluation of thermoinhibition, the primary dormancy must be overcome, and lettuce seeds should have high physiological quality. Additionally, thermoinhibited seeds, soaked and exposed to high temperatures can be induced to deteriorate since they undergo changes in their metabolism and because they are exposed to the action of pathogens.

In some cultivars, the reduction in seed germination with increasing temperature was clear (Table 1), as also reported by Catão et al. (2014) and Nascimento et al. (2012). The critical period for the induction of thermoinhibition is within the first 8–12 h of imbibition under high temperature (Argyris et al., 2008). At temperatures of 30 °C and 35 °C there was thermoinhibition of seed germination. It was observed that the imbibition occurred, but the germination of the seeds of the vast majority of the cultivars was extremely low, causing a reduction in physiological potential (Table 1).

It is worth noting that the germination test was evaluated seven days after its installation, following the guidelines prescribed in the Rules for Seed Testing (Brasil, 2009). Almeida et al. (2019) suggested that, at high temperatures, seeds should be evaluated 11 days after setting up the germination test. However, when analyzing the data of these authors, no significant increases in germination that justify prolonging the test were found. The largest increase was observed in the cultivar Grand Rapids (9%). In addition, Catão et al. (2014) and Catão et al. (2018) considered the cultivar Grand Rapids as sensitive to thermoinhibition, based on the reduction of the physiological potential of its seeds and the low activity of the enzyme endo-β-mannanase at high temperatures. Thermoinhibition is not a condition that can be overcome over time, but rather with the reduction in the temperature to which the seeds are exposed to germinate.

There was a significant reduction in seed germination and vigor at 35 °C (Table 1). This temperature caused a reduction in germination speed, regardless of the time at which the test was performed. Catão et al. (2014) and Catão et al. (2016) also reported reduction in the germination speed of lettuce seeds under high temperatures. These authors observed that many lettuce cultivars had their germination process interrupted due to the denaturation of the enzyme endo-β-mannanase and this led to drastic reductions in germination speed at 35 °C.

Several studies have reported that thermoinhibition is caused by a close link between endosperm weakening, endo-β-mannanase enzyme activity, ethylene production, heat-tolerant proteins (heat shock proteins-HSP) and germination of lettuce seeds at high temperatures (Nascimento and Cantliffe, 2002; Nascimento et al., 2004; Schwember and Bradford, 2010; Catão et al., 2014).

The highest germination percentage at 35 °C was observed in seeds of the cultivar Everglades (84%), shortly after harvest and with six months of storage. Everglades is shown to be a cultivar with potential for use in tropicalized lettuce breeding programs. Almeida et al. (2019), Catão et al. (2018), Zuffo et al. (2017), Catão et al. (2016) and Catão et al. (2014) analyzed the germination of the cultivar Everglades at 35 °C and reported that this cultivar, despite being tolerant to thermoinhibition, shows reduction in germination, but with less intensity compared to other cultivars.

Gonai et al. (2004) stated that the maximum temperature for lettuce seed germination depends on antagonistic interactions between the hormones gibberellin and abscisic acid. Studies conducted with lettuce showed that the content of abscisic acid is maintained at very high levels when the seeds are soaked at high temperatures but decreases rapidly as the imbibition occurs at optimal temperatures for germination (Argyris et al., 2008). Gibberellin is necessary for the germination of lettuce seeds at high temperatures, even when abscisic acid biosynthesis is inhibited (Argyris et al., 2011).

The cultivar Luiza did not differ statistically from Everglades (tolerant to thermoinhibition) based on the germination test at 35 °C when performed six months after storage (Table 1). It is worth noting that the germination of the seeds of the cultivar Luiza was higher when compared to those of the other cultivars under high temperatures.

Catão et al. (2014) considered that the cultivar Luiza has moderate tolerance to thermoinhibition and recommended a more detailed study. Regardless of the evaluation time (shortly after harvest and with six months of storage), it is possible to observe that the germination of the seeds of the other cultivars is maintained at 20 °C and 25 °C. At temperatures of 30 °C and 35 °C, there is a decrease in germination and germination speed index (Table 1). Thus, the use of high temperatures is essential to select the dissimilar materials that best adapt to thermal stress conditions (Catão et al., 2018; Catão et al., 2022a).

The remaining seeds of the germination test were soaked, regardless of the evaluation time, which consequently facilitated the softening of their tissues to conduct the tetrazolium test. Shortly after harvest (Figure 2) and with six months of storage (Figure 3) at temperatures of 20 °C and 25 °C, it was possible to observe a greater number of viable remaining seeds. This is indicative of primary dormancy in the seeds of the cultivars Babá de Verão, Grand Rapids, Laurel, Lídia, Marianne, Raider Plus, Regina 71, Rubete and Salinas 88. As can be observed in Figure 2, the remaining (non-germinated) seeds of these cultivars showed high viability at temperature of 20 °C, so it can be inferred that the seeds were dormant. The tetrazolium test is a fast biochemical test, capable of detecting the viability of seeds based on respiration, particularly in species whose germination is slow or have dormancy (Dias and Alves, 2008).

However, when the seeds were exposed to high germination temperatures (30 °C and 35 °C) there was a considerable increase of dead seeds shortly after harvest (Figure 2) and with six months of storage (Figure 3). As mentioned, the seeds were soaked, but root protrusion did not occur, as also observed by Catão et al. (2014) when working with thermoinhibition in lettuce seeds. Schwember and Bradford (2010), Argyris et al. (2011) and Deng and Song (2012) found that the endosperm surrounding the embryo constitutes a physical barrier to radicle emergence and is necessary for the imposition of thermoinhibition on lettuce seeds. The rate and extent of weakening of the micropylar endosperm involving the tip of the radicle may be involved in determining the occurrence of thermoinhibition. The rise

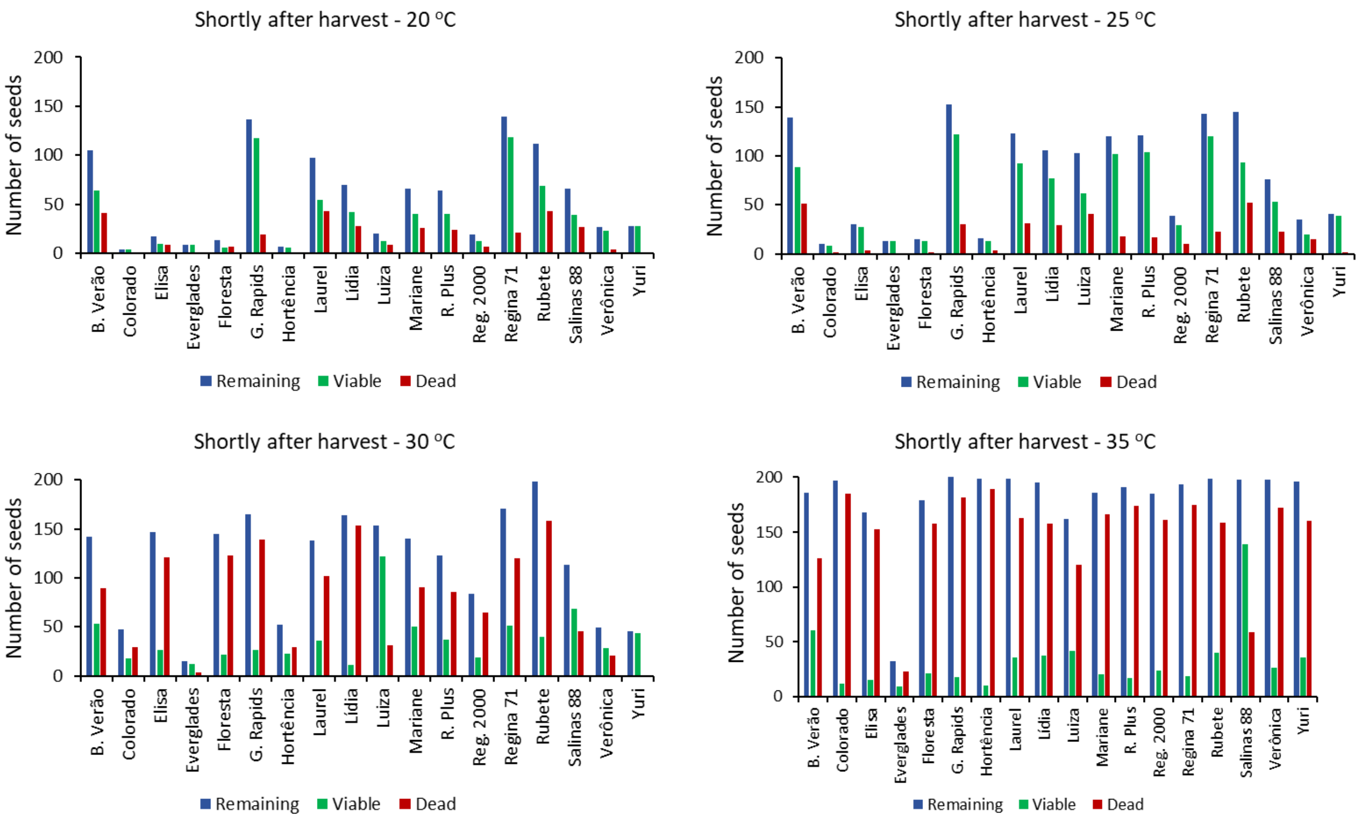

Figure 2. Number of remaining seeds (viable and dead) of the germination test of lettuce cultivars subjected to different temperatures shortly after harvest. *Ungerminated seeds of the germination test, in a total of 200 seeds.

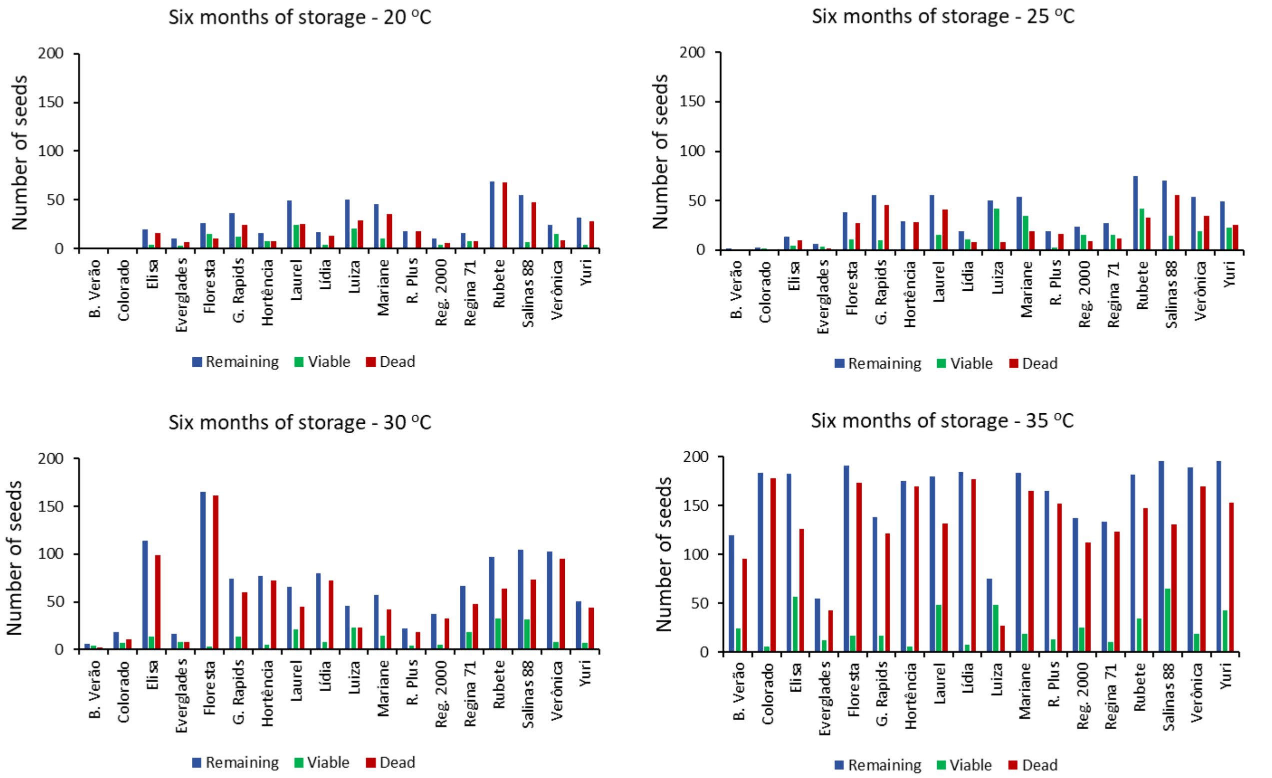

Figure 3. Number of remaining seeds (viable and dead) of the germination test of lettuce cultivars subjected to different temperatures with six months of storage. *Ungerminated seeds of the germination test, in a total of 200 seeds.

in temperature, besides causing thermoinhibition in lettuce seeds, also accelerates chemical reactions and intensifies metabolic processes. As a result of these metabolic events, seed respiration is higher, which increases the rate of deterioration (Marcos-Filho, 2015).

The Everglades cultivar has the lowest number of remaining seeds at 35 °C, regardless of the evaluation time (Figures 2 and 3). By the tetrazolium test it is possible to observe that few remaining seeds were dead and not thermoinhibited. Although this cultivar is considered thermotolerant, high temperatures reduce its germination (Catão et al., 2014). For Nascimento et al. (2012), the germination of seeds of most lettuce cultivars is erratic or completely inhibited at temperatures above 30 °C. This occurs because of the weakening of their endosperm, which prevents the growth of the embryo and restricts root protrusion (Sung et al., 2008).

The lighter colors (yellow) in Figure 4 represent lettuce cultivars with higher tolerance to seed thermoinhibition. Thus, at the temperature of 35 °C (Figure 4), there was greater formation of dark colors, indicating greater similarity of cultivars with thermoinhibition (Figure 4). The light colors (yellow) have shorter distance between neurons, which means that the characteristics are more important for the distinction of dissimilarity groups regarding thermoinhibition and do not make strong connections with neighboring neurons. On the other hand, dark colors (brown and black) represent greater dissimilarity distances (Cardoso et al., 2021).

The lighter colors (yellow) are represented by the cultivars Everglades and Luiza (Figure 5), as could also be verified in the physiological data (Table 1), which confirm the thermotolerance of these cultivars. The cultivars Everglades and Luiza are dissimilar to the other cultivars (Figure 5), that is, they do not have strong connections with the neighboring clusters (neurons) represented by thermoinhibition-sensitive cultivars. According to Braga et al. (2011) and Cruz and Nascimento (2018), Kohonen's Self-Organizing Map (SOM) using ANNs is able to detect and organize the similarities of input patterns through competitive learning, simulating the cerebral cortex with connections between the strongest neurons due to their proximity.

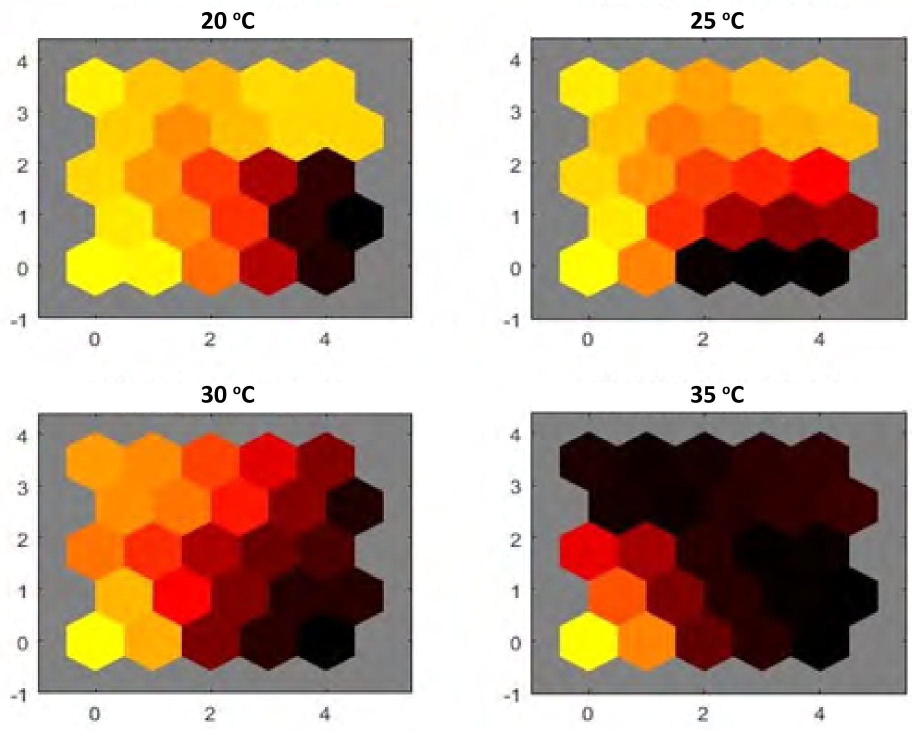

Figure 4. Weight of input variables in network neurons in which lighter colors represent a greater effect of a variable on a given network of neurons to define the cluster. Input 1 = 20 °C; Input 2 = 25 °C; Input 3 = 30 °C; Input 4 = 35 °C.

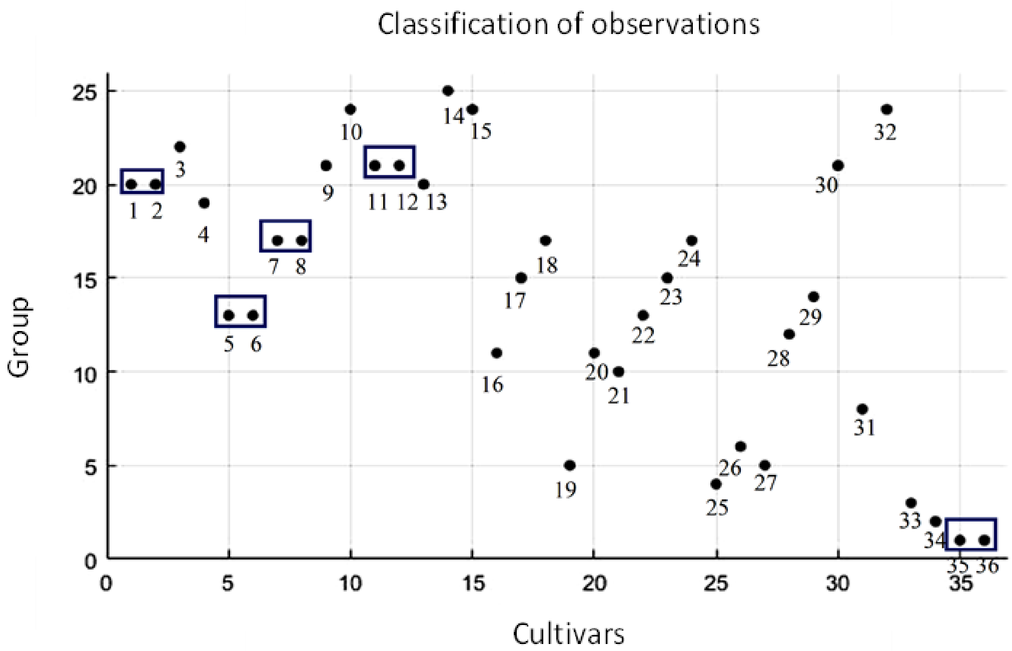

Figure 5. Classification of lettuce cultivars in each cluster by SOM in 25 groups of neurons: 1-2 Floresta; 3-4 Verônica; 5-6 Salinas 88; 7-8 Yuri; 9-10 Hortência; 11-12 Colorado; 13-14 Elisa; 15-16 Regina 2000; 17-18 Laurel; 19/20 Regina 71; 21-22 Rubete; 23-24 Marianne; 25-26 Babá de Verão; 27-28 Grand Rapids; 29-30 Raider Plus; 31-32 Lídia; 33-34 Luiza and 35-36 Everglades. *Odd numbers represent the cultivar shortly after harvest and even numbers represent the cultivar with six months of storage. ☐ **Cultivars classified in the same cluster shortly after harvest and with six months of storage.

Figure 4 shows that the high germination temperatures influenced the activation of neurons, defining the clusters with respect to the dissimilarity, where dark colors (brown and black) represent thermoinhibition-sensitive cultivars, while light colors (yellow) represent cultivars with thermotolerant seeds. With the increase in temperature, it is possible to observe the decrease of clusters, that is, most cultivars are thermosensitive and were grouped by their similarity. The temperatures 20 °C and 25 °C have great similarity because they have almost the same color pattern, that is, germination behaves similarly. Thus, at 20 °C and 25 °C the great majority of cultivars have seeds that can germinate, and it was not possible to observe dissimilar groups regarding thermoinhibition. In general, these temperatures allow a higher germination of lettuce seeds; however, some cultivars did not germinate at 25 °C, already showing sensitivity to this temperature (black colors) (Figure 4).

Everglades, represented by the neuron of row 5 column 1, was the only cultivar activated at 35 °C, shortly after harvest and with six months of storage (Figures 5 and 6), followed by the cultivar Luiza in row 5 column 2, both showing tolerance to thermoinhibition, which suggests that the seeds of these cultivars possibly have genes for tolerance to high temperatures.

The method of data analysis using neural networks allows the results to have high accuracy, because even if there are experimental errors that do not meet the premises, as occurs in experiments using univariate statistics, groups of more representative similar and dissimilar materials are formed in the ANNs (Cardoso et al., 2021).

Another relevant point is that ANNs are not only based on means and variances (Cruz and Nascimento 2018), but also increase the number of observations and reduce the apparent error rate, quantifying the weights between neurons (Cardoso et al., 2019).

The largest clusters were not responsive to temperatures of 30 °C and 35 °C, that is, 97% of the cultivars had their germination inhibited, being determinant only for the clusters of Everglades, Luiza and Babá de Verão, represented by the lighter colors (Figure 4), corroborating Catão et al. (2014), who observed tolerance of Everglades and Luiza to thermoinhibition at 35 °C.

The cultivars were grouped by the SOM in pairs in Figure 5, and the pair of cultivars corresponded to germination shortly after harvest and with six months of storage. It is possible to observe that there was an increase in seed germination after storage, so most cultivars (73%) showed primary dormancy from maternal inheritance. Thus, the cultivars were not located in the same clusters at the evaluation times (shortly after harvest and with six months of storage) (Figure 5).

Only the cultivars Floresta, Salinas 88, Yuri, Colorado and Everglades were located in the same cluster. This indicates that the cultivars behaved similarly shortly after harvest and with six months of storage (Figure 5). Therefore, according to the data obtained in Table 1, one can infer that the cultivars Floresta, Colorado and Everglades did not have primary dormancy, under the experimental conditions, and that the cultivars Salinas 88 and Yuri had low germination at both evaluation times. This result differs from the observations made by Kano et al. (2011), who suggested the storage of seeds for a minimum period of four months to overcome primary dormancy.

The suggestion that these cultivars (Floresta, Colorado and Everglades) did not show primary dormancy is because the ANNs have high simulation capacity, expanding the input data by estimating new values, validating them and adjusting the weights for each variable in the connections between neurons, organizing the groups by similarity through competitive learning (Cruz and Nascimento, 2018). Thus, ANNs make it possible to detect variations more accurately, when compared to the traditional statistical method, since the simulations eliminate possible experimental errors and have low statistical error, allowing a better distinction of cultivars.

Figure 6 shows groups of individuals with high dissimilarity for germination at different temperatures. It is also possible to observe in the Topological Map of the SOM network the number of cultivars located within each neuron. This indicates high dissimilarity among lettuce cultivars regarding the tolerance to thermoinhibition of seeds. The most distant neurons did not show competitiveness and their dissimilarity was amplified with the presence of neurons with zero individuals, forming a barrier of distancing, since the clusters in the SOM are more similar due

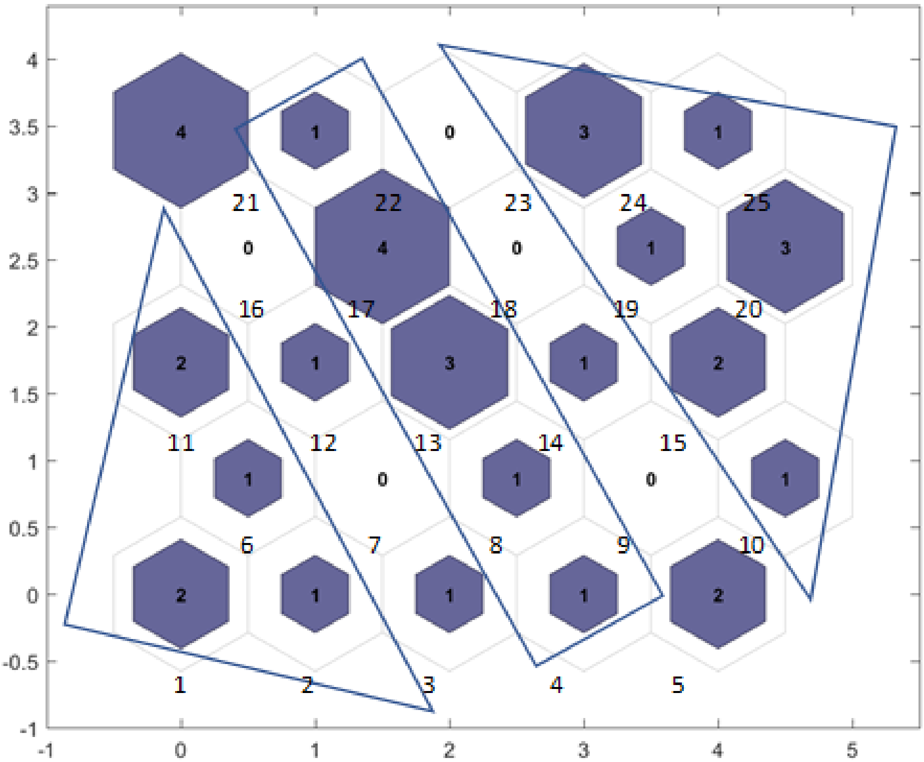

Figure 6. Topological map in Kohonen's Self-Organizing network for 25 classes of neurons by means of artificial neural network and classification of the number of lettuce cultivars shortly after harvest and with six months of storage with respect to thermoinhibition within each hexagon (neuron).

to their physical proximity. Everglades, Luiza and Babá de Verão are the closest cultivars, forming a cluster of more similar individuals in relation to seed thermoinhibition, as can also be observed in Figure 5. Two more groups were formed (central and an upper one), distinguishing cultivars into three groups, with several subgroups for germination under high temperatures (Figure 5).

In the first cluster it is possible to observe the Everglades cultivar at the two evaluation times. The cultivars Luiza and Babá de Verão were classified in the same cluster as Everglades after six months of storage (Figure 5). This suggests that storage was determinant for seeds to express their germination potential, that is, the seeds of these cultivars possibly have genes for tolerance to high temperatures, for instance through physiological responses induced by thermal stress. Argyris et al. (2011) identified that the *L. serriola* accession UC96US23 (wild parent closest to cultivated lettuces) has a quantitative trait locus (QTL) for germination at high temperature (Htg6.1). Clemente et al. (2015) also observed in seeds with thermoinhibition that the expression of the LsNCED4 gene occurs exclusively at high temperatures.

Oliveira et al. (2021) found that thermoinhibition tolerance in lettuce seeds is a characteristic controlled by one or a few genes, with additive effect and high heritability, allowing the use of contrasting cultivars for the selection of genotypes tolerant to the effects of high temperature. Moreover, Oliveira et al. (2021) stated that there is a maternal effect for the characteristic of tolerance to germination at high temperatures. In view of the above, Everglades and Luiza show potential for hybridization and selection of progenies in tropicalized lettuce breeding programs. The validation of the use of neural networks can also become an alternative to assist in the selection of lettuce genotypes for the tolerance to thermoinhibition of seeds at high temperatures.

The cultivars Floresta and Elisa with thermoinhibited seeds were grouped on the upper right diagonal, regardless of whether the evaluation of germination at high temperatures was carried out after harvest or with six months of storage (Figures 5 and 6). In addition, on the upper right diagonal are also the cultivars Verônica, Rubete, Laurel, Marianne, Hortência, Regina 2000 and Lídia (Figures 5 and 6). However, these cultivars had germination influenced by both primary dormancy and high temperatures shortly after harvest and with six months of storage.

CONCLUSIONS

Neural networks discriminate lettuce cultivars and organize them with respect to seed thermoinhibition tolerance through Kohonen's Self-Organizing Map. Discriminant analysis consistently identified the Everglades and Luiza genotypes as tolerant to thermoinhibition.

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