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Genetic dissimilarity for thermoinhibition in seeds of lettuce lines after defoliation

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ABSTRACT. Removal of lettuce basal leaves is a technique used by the seed industry; however, the effects on seed production and physiological potential are unknown. Genetic dissimilarity is fundamental in the identification of individuals in terms of traits of interest, although it is rarely considered in relation to seeds that are tolerant to thermoinhibition. The aim of this study was to ascertain the relationship between defoliation of lettuce plants and seed yield/physiological quality, as well as the genetic dissimilarity among genotypes with regard to seed thermoinhibition. We used 35 lines of biofortified lettuce, the cultivars Uberlândia 10000, Belíssima, UFU MC BIOFORT1, and Everglades (tolerant to thermoinhibition), and Grand Rapids and Verônica (susceptible to thermoinhibition). The seed yield and physiological quality of the genotypes with and without defoliation were evaluated, artificially aged, and germinated at four temperatures in a factorial arrangement. The genetic dissimilarity was estimated by Tocher graphing and the UPGMA clustering method, based on the Mahalanobis generalized distance (D^2_{ii}). Four UFU genotypes and Grand Rapids had high seed yields. With removal of basal leaves, there was higher seed yield per plant and there was an effect on their physiological quality. The genotypes exhibited genetic variability for thermoinhibition, with UFU-86#2#1#1 and Everglades showing similar performance.

Keywords: *Lactuca sativa* L.; defoliation; seed quality; thermodormancy; genetic variability.

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Introduction

Leafy vegetables represent 35% of the planted area of vegetable species grown in Brazil, and lettuce (*Lactuca sativa* L.) is the most produced and consumed crop, followed by arugula, collard greens, and watercress (ABCSEM, 2018). In relation to seed production of these species, a considerably high level of technology has been utilized in Brazil for some decades (Franco, Gomes, & Santos, 2018).

The success of vegetable seed companies is related to the seed production technology and the availability of cultivars, which generally come from plant breeding programs that seek to meet climate demands according to the specificity of each cultivar. All of these factors affect the ability to obtain high-quality seeds, considering genetic, physical, physiological, and seed health aspects (Nascimento & Melo, 2015).

Growing lettuce plants for seed production has similar requirements to growing lettuce for *in natura* consumption in relation to crop treatments; however, there are important differences regarding the choice of the production location, due to climate and plant spacing conditions (Sala & Nascimento, 2014), as well as the method of training the plants.

Regarding plant training, cultivars that do not form a head allow for normal bolting. However, in cultivars that form a head, manual opening of the head or gibberellic acid application are routine practices (Sala & Nascimento, 2014). Another common practice is the removal of the basal leaves of plants to mitigate problems from bacterial diseases. However, there is no information showing the effect of defoliation on the production and quality of lettuce seeds and the magnitude of that effect.

Climate conditions, especially temperature at the time of germination, also affect the quality of seed lots (Huo, Dahal, Kunusoth, McCallum, & Bradford, 2013; Wang et al., 2015). Most do not germinate at temperatures higher than 30°C, and temporary (thermoinhibition) or complete (thermodormancy) inhibition

of germination may occur due to the hardening of the endosperm, which restricts radicle emergence (Catão et al., 2018; Nascimento, Croda, & Lopes, 2012; Sung, Cantliffe, Nagata, & Nascimento, 2008). It is known, however, that there is genetic variability for this trait in species of the *Lactuca* genus, which has been observed in some studies on seeds of the cultivar Everglades (*L. sativa*) (Catão et al. 2014; Nascimento et al., 2012) and of the accession of *Lactuca serriola* US96UC23 (Agirys et al., 2011; Yoong et al., 2016), which showed tolerance to temperatures of 35°C during germination. However, there are no commercial cultivars with this trait available in the Brazilian market, and therefore there is a need to observe the temperature conditions of the region where the lettuce seedlings will be produced at the time of sowing.

There are few studies that relate the technique of lettuce plant defoliation with seed production, seed physiological quality, and high temperature tolerance during germination. Given this situation, understanding the effect of defoliation on these factors, as well as the genetic diversity in groups of genotypes for identification of those capable of germinating at higher temperatures, becomes highly relevant. This information can be important for the development of tropicalized lettuce breeding programs, to obtain cultivars that are more tolerant and of higher quality (Catão et al., 2016). Thus, the aim of this study was to ascertain the relationship between defoliation of lettuce plants and seed yield/physiological quality, as well as the genetic dissimilarity among genotypes regarding seed to thermoinhibition.

Material and methods

The study was conducted at the Vegetable Crop Experimental Station of the Universidade Federal de Uberlândia, Monte Carmelo, Minas Gerais State, Brazil (18°42′43.19″ S; 47°29′55.8″ W; 873 m asl). During the period of this study of lettuce seed production in the field, the rainfall was 1.474 mm and mean temperature was 22.6°C, according to the Instituto Nacional de Meteorologia (INMET).

The soil of the experimental area had the following characteristics: pH (H_2O) = 5.9; available P = 30.1 mg dm^{-3}; K = 0.22 $cmol_c$ dm^{-3}; Ca^{+2} = 2.8 $cmol_c$ dm^{-3}; Mg = 1.0 $cmol_c$ dm^{-3}; exchangeable H^+ Al = 3.40 $cmol_c$ dm^{-3}; organic matter = 4.2 dag kg^{-1}; SMP index = 3.40; aluminum = 0.0 $cmol_c$ dm^{-3}; CEC (pH 7.0) = 7.42 $cmol_c$ dm^{-3}; CEC (pH 7.0) base saturation = 54%; CEC effective saturation by aluminum = 0%; copper = 2.3 mg dm^{-3}; zinc = 6.6 mg dm^{-3}; and manganese = 6.6 mg dm^{-3}. The crop treatments were conducted as recommended for lettuce (Filgueira, 2013).

Seeds were evaluated from 35 lettuce lines arising from hybridization between the cultivars Belíssima × Uberlândia 10000, obtained after six successive self-pollinations carried out from 2013 to 2017. A pedigree breeding method was used in this study. These lines are part of the Biofortified Lettuce Breeding Program of UFU, with all the genealogy stored in the "BG α BIOFORT" software, registry no. BR512019002403-6 in the INPI (Maciel, Siquieroli, Gallis, Pereira, & Sales, 2019). In addition, the following check cultivars were used: the male parent (Uberlândia 10000), the female parent (Belíssima), the cultivars UFU MC BIOFORT1 and Everglades, considered tolerant to thermoinhibition (Catão et al., 2014; Nascimento et al., 2012), and the cultivars Grand Rapids and Verônica which were considered susceptible to thermoinhibition (Kano, Cardoso, Villas Bôas, & Higuti, 2011; Villela et al., 2010), for a total of 41 genotypes.

The seedlings were produced in a greenhouse using 200-cell expanded polystyrene trays containing coconut fiber-based commercial substrates. At 30 days after sowing, the seedlings were transplanted into final seedbeds having a width of 1.3 meters. Plots consisted of 16 plants at a spacing of 0.25 × 0.25 m in a randomized block design with four replications.

When the lettuce plants of each genotype were in the reproductive phase, two treatments were used to remove the basal leaves (with and without defoliation). In the plants without defoliation, no leaves were removed, whereas in the other treatments, the basal leaves were manually removed from the plants.

The inflorescences of the eight central plants in the plot having seeds at full physiological maturity were harvested. The seeds were processed, placed in multilayered paper bags, labeled, and weighed on a digital balance with 0.001 g precision. The moisture content of the seeds was determined by the laboratory oven method at 105 ± 3ºC for 24h, using two replicates for each genotype (Brasil, 2009), with results expressed as a percentage. Using the weight and moisture content (%) data, the seed yield of the plants from each plot was calculated, and the mean weight of seeds per plant (g) was obtained.

The seeds from each plant of the plot were mixed in a homogeneous manner, constituting one sample for each genotype per replication, resulting in four replications. They were then placed in multilayered paper bags and labeled to proceed with laboratory analyses.

The seeds were characterized based on their viability for each of the genotypes through the following tests. Germination (G%) was performed with four replicates of 50 seeds sown in a transparent acrylic box for germination (gerbox) plastic boxes containing two sheets of *germitest* (germination testing) paper previously moistened with distilled water in the proportion of 2.5 mL g^{-1} of paper (Brasil, 2009). The seeds were kept in a biochemical oxygen demand (BOD) chamber at a constant temperature of 20°C. The evaluation consisted of two counts of germination, the first at four days, evaluating root emergence, and the second at seven days, evaluating normal seedlings (Brasil, 2009). Results were expressed as the percentage of germination at the first count and the final count. Emergence (E%) was conducted in a greenhouse without controlled conditions; seeds were sown in 200-cell expanded polystyrene trays containing coconut fiber-based commercial substrate. Four replications with 20 cells were conducted for each lettuce genotype, with and without defoliation. The substrate was moistened daily up to approximately 60% of the water retention capacity. On the seventh day, the number of emerged seedlings was counted, and the results were expressed as a percentage. Germination speed index (GSI) and emergence speed index (ESI) were conducted simultaneously with the germination and emergence tests, and daily at the same time, the number of germinated seeds and emerged seedlings were calculated, respectively. Indices were determined according to the formula proposed by Maguire (1962). Electrical conductivity (EC) was measured with four replications of 50 seeds, which were weighed and placed in a disposable plastic cup (200 mL capacity) containing 50 mL of deionized water for 24h at 25°C. After the soaking period, readings were taken with a Digimed CD-21 conductivity meter, with a cell constant of 1, and data were expressed in μS cm^{-1} g^{-1} of seed. A completely randomized experimental design was used for the tests described in a 41 × 2 (genotypes × basal leaf removal) factorial arrangement.

After characterization of the initial quality, the seeds were tested for *accelerated aging*. Seeds of each genotype from plants with and without defoliation were distributed in a single, uniform layer on aluminum screens fixed within *gerbox-type* plastic boxes. Each *gerbox* contained 40 mL distilled water. Subsequently, lids were placed on the boxes, which were then identified and placed in a BOD chamber for 48h at 41°C (Frandoloso et al., 2017). At the end of the aging period, four replicates of 50 seeds of each genotype were placed to germinate according to the method described in the germination test. Here, the four BODs were used simultaneously, each previously regulated at germination temperatures of 20, 25, 30, and 35°C. The evaluation consisted of the determination of normal seedlings on the fourth and seventh day after setting up the test, following the method described by Brasil (2009). A completely randomized experimental design was used in a 41 × 4 × 2 formation (genotypes × temperatures × basal leaf removal).

The F test and analysis of variance at 5% probability were used for the statistical analysis of the data. In the event of significant effects, the mean values were compared using the Scott-Knott test at 5% probability using Sisvar 5.0 software (Ferreira, 2011). Normality tests (Lilliefors test) and homogeneity tests (Cochran test) were performed. The data for the analysis of physiological quality were transformed to $y = [(x + 1)^{\wedge}0.5]$.

Genetic divergence among the genotypes was evaluated by multivariate analysis, using the physiological responses of germination at different temperatures (20, 25, 30, and 35°C) at each level of defoliation. The Mahalanobis generalized distance (D^2ii') was used as a measure of dissimilarity, adopting the criterion by which the mean of the measures of genetic divergence in each group must be less than the mean distance between the groups. A cut-off line of 10% was used to separate the groups, which was established at points of abrupt change in the branches of the dendrogram (Cruz, Regazzi, & Carneiro, 2012). Thus, to facilitate interpretation of the measure of dissimilarity between genotypes, the Tocher optimization and the unweighted pair group method with arithmetic mean (UPGMA) clustering methods were used and processed using the GENES software (Cruz, 2016).

Results and discussion

In relation to seed production, there was a significant difference between genotypes and the defoliation level (whether basal leaves were removed or not); however, there was no interaction between these factors. The genotypes UFU-189#3#1#1, UFU-189#1#2#1, UFU-189#3#4#1, UFU-206#1#6#1, and Grand Rapids exhibited high seed production (g $plant^{-1}$). The mean seed production without leaf removal was 6.80 g $plant^{-1}$, and 5.40 g $plant^{-1}$ with leaf removal (Table 1). Therefore, defoliation can be an additional factor leading to a lower accumulation of dry matter in the seeds, with a consequent reduction in specific weight. The greater the intensity of defoliation, the lower is the weight of the seeds produced (Bastitella Filho et al., 2013). According to Medeiros and Nabinger (2001), defoliation reduces the photosynthetic area and the production of photoassimilates necessary for maintaining and filling seeds.

Table 1. Seed production (g plant^{-1}) and production systems with and without removal of the basal leaves of lettuce genotypes.

Genotypes	Production (g plant^{-1})
UFU-199#2#1#1	1.63 d
UFU-184#2#5#1	1.65 d
Belíssima	2.18 d
UFU-199#1#1#1	2.51 d
UFU-206#1#3#1	2.88 d
UFU-206#1#6#8	3.19 d
UFU-86#1#2#1	3.45 d
UFU-107#1#2#1	3.64 d
Everglades	3.67 d
UFU MC BIOFORT1	3.68 d
Uberlândia 10000	3.80 d
UFU-197#1#1	3.83 d
UFU-86#2#1#1	4.76 c
UFU-125#2#2#1	5.09 c
UFU-189#2#2#1	5.16 c
UFU-197#3#1#1	5.26 c
UFU-120#1#1#1	5.29 c
UFU-197#2#2#1	5.39 c
UFU-206#1#2#1	5.50 c
UFU-117#1#1#1	5.52 c
UFU-155#1#1#1	5.69 c
UFU-75#3#2#1	5.83 c
UFU-189#2#3#1	6.04 c
UFU-75#3#1#1	6.34 c
Verônica	6.43 c
UFU-206#1#1#1	6.57 c
UFU-7#1#2#1	6.76 c
UFU-206#3#2#1	7.08 b
UFU-117#1#3#1	7.21 b
UFU-206#1#4#1	7.83 b
UFU-125#1#1#1	7.99 b
UFU-040#5#5#1	8.35 b
UFU-189#2#1#1	8.36 b
UFU-75#1#1#1	8.43 b
UFU-206#1#5#1	8.63 b
UFU-189#3#2#1	8.98 b
UFU-189#3#1#1	9.99 a
UFU-189#1#2#1	10.65 a
UFU-189#3#4#1	11.06 a
UFU-206#1#6#1	11.32 a
Grand Rapids	12.25 a
Production systems	
With defoliation	5.40 b
Without defoliation	6.80 a
CV (%)	34.21

*Mean values followed by the same letter do not differ from each other according to the Scott-Knott test at 5% probability.

Considering 70,000 plants ha^{-1}, seed yield is 476 kg ha^{-1} without defoliation and 378 kg ha^{-1} with defoliation. The yield of lettuce seeds without plant defoliation was higher than the mean yield (368 kg ha^{-1}) reported by Souza et al. (2019). According to Viggiano (1990), seed yield can range from 372 to 1,179 kg ha^{-1}, depending on the cultivar, production location, and climate conditions. It should also be noted that the seeds were produced in the spring/summer, the period in which lettuce normally has a lower seed yield, as reported by Souza et al. (2019).

In lettuce plants without defoliation, more assimilates may have been synthesized from photosynthesis. This flow of assimilates is governed by metabolic processes and transport, which occurs in a source-sink system and results in the distribution of dry matter in different plant organs (Santos et al., 2020). Thus, plants without defoliation have a greater chance of redirecting their photoassimilates for the formation of reproductive and seed production structures.

The mean moisture content of the seeds of the different lettuce genotypes after drying was 5.6%, which was also reported by Carvalho et al. (2017). The initial characterization of the physiological quality of the seeds of the lettuce genotypes is shown in Table 2. In the evaluation, there was a significant

interaction between the genotypes and removal of the basal leaves of the lettuce plants. The physiological quality of the seeds of the genotypes differed, and Uberlândia 10000, Belíssima, and Everglades exhibited high germination percentages under conditions of defoliation, and lower results without defoliation. According to Heidari (2015a), plant defoliation can increase germination of the seeds because the plants remain in the vegetative stage for a longer time, increasing the photosynthetic period to compensate for the absence of leaves.

Table 2. Initial characterization of the physiological quality of seeds from lettuce produced with removal (WD) and without removal (WoD) of the basal leaves.

Genotypes	FGC (%)		G (%)		GSI		E (%)		ESI		EC	
	WD	WoD	WD	WoD	WD	WoD	WD	WoD	WD	WoD	WD	WoD
UFU-117#1#3#1	35 dB	70 aA	57 cA	72 aA	5.25 bB	9.41 aA	27 dB	81 aA	1.04 dB	2.87 bA	6.85 aA	20.52 dB
UFU-206#1#6#8	69 bA	52 aA	72 bA	53 bA	8.18 aA	7.56 aA	55 bA	68 aA	2.61 bA	0.61 eB	6.11 aA	10.26 bB
UFU-86#1#2#1	67 bA	44 bA	62 bA	51 bA	7.35 aA	5.88 bA	33 dA	38 cA	1.95 bA	0.82 eB	6.36 aA	8.17 bB
UFU-75#1#1#1	48 cA	64 aA	60 bA	71 aA	6.70 aA	8.23 aA	63 bA	76 aA	2.04 bA	2.16 cA	7.04 aA	6.50 aA
UFU-189#3#1#1	57 cA	75 aA	65 bA	66 aA	7.87 aA	8.85 aA	53 bA	63 aA	1.77 cA	1.10 dB	8.28 aA	6.52 aA
UFU-197#3#1#1	74 bA	51 aA	67 bA	59 aA	9.11 aA	6.76 aB	46 cA	50 bA	2.13 bB	2.84 bA	4.15 aA	7.37 aA
UFU-125#1#1#1	35 dA	52 aA	46 cA	58 aA	4.91 cA	6.82 aA	21 dB	61 aA	0.80 dB	2.05 cA	16.45 cB	9.42 bA
UFU-7#1#2#1	53 cA	59 aA	65 bA	77 aA	6.34 bB	9.00 aA	44 cB	61 aA	1.40 cB	1.98 cA	6.00 aA	9.31 bA
UFU-155#1#1#1	39 dA	40 bA	68 bA	48 bA	5.99 bA	5.71 bA	25 dA	21 dA	1.62 cA	0.75 eB	9.87 aA	15.42 cB
UFU-206#3#2#1	22 dA	22 bA	37 cA	36 bA	3.51 cA	4.70 bA	14 dB	49 bA	0.62 dB	1.31 dA	12.64 bB	5.66 aA
UFU-189#2#3#1	50 cA	59 aA	72 bA	66 aA	6.97 aA	7.18 aA	38 cB	63 aA	1.87 bA	0.72 eB	19.05 cA	16.24 cA
UFU-184#2#5#1	61 bA	41 bA	47 cA	49 bA	7.45 aA	5.37 bA	39 cB	15 dA	0.82 dA	0.48 eA	5.53 aA	15.33 cB
UFU-107#1#2#1	70 bA	36 bB	73 bA	46 bB	9.13 aA	4.80 bB	49 cA	64 aA	2.45 bA	1.19 dB	7.99 aA	10.56 bA
UFU-86#2#1#1	64 bA	51 aA	75 bA	64 aA	8.14 aA	7.08 aA	58 bA	38 cB	1.50 cA	1.35 dA	7.19 aA	6.66 aA
UFU-75#3#2#1	58 cA	51 aA	62 bA	60 aA	7.39 aA	7.43 aA	31 dB	60 aA	1.02 dB	2.22 cA	6.69 aA	8.27 bA
UFU-120#1#1#1	46 cA	55 aA	64 bA	61 aA	7.00 aA	7.18 aA	28 dB	55 bA	0.52 dB	2.09 cA	6.70 aA	9.68 bA
UFU-189#1#2#1	67 bA	61 aA	58 cA	68 aA	8.10 aA	7.45 aA	46 cA	48 bA	1.93 bA	1.12 dB	6.91 aA	8.83 bA
UFU-206#1#1#1	58 cA	52 aA	57 cA	60 aA	7.23 aA	6.79 aA	47 cA	26 dB	1.48 cA	0.91 eB	7.35 aA	8.41 bA
UFU-75#3#1#1	58 cA	54 aA	72 bA	64 aA	6.90 aA	6.82 aA	34 dB	58 bA	1.00 dB	1.97 cA	8.17 aA	8.65 bA
UFU-197#1#1	44 cA	45 bA	47 bA	48 bA	5.73 bA	5.37 bA	41 cA	36 cA	0.79 dB	1.79 cA	10.27 bA	12.85 cA
UFU-189#2#2#1	52cA	44 bA	43 cA	58 aA	6.04 bA	6.13 aA	24 dB	55 bA	1.23 dA	0.85 eA	5.01 aA	4.42 aA
UFU-197#2#2#1	27 dB	59 aA	33 cB	71 aA	3.81 cB	7.81 aA	25 dA	33 cA	0.66 dA	1.82 cB	7.03 aA	9.14 bA
UFU-189#2#1#1	73 bA	64 aA	52 cA	62 aA	7.56 aA	7.92 aA	53 bA	51 bA	2.01 bA	0.60 eB	5.33 aA	5.24 aA
UFU-199#2#1#1	28 dA	36 bA	35 cA	39 bA	4.52 cA	4.70 bA	20 dA	23 dA	1.43 cA	0.86 eB	10.45 bA	8.45 bA
UFU-206#1#6#1	80 bA	54 aB	71 bA	58 aA	9.15 aA	6.42 bB	40 cB	74 aA	1.75 cA	1.58 dA	6.89 aA	6.06 aA
UFU-206#1#3#1	51 cA	65 aA	60 bA	65 aA	6.45 bA	7.86 aA	15 dB	44 cA	1.15 dA	0.69 eA	6.43 aA	8.43 bA
UFU-189#3#4#1	56 cA	46 bA	61 bA	35 bB	7.69 aA	6.18 bA	51 bA	49 bA	1.83 cA	1.71 cA	5.92 aA	7.83 bA
UFU-206#1#4#1	72 bA	40 bB	65 bA	46 bA	8.31 aA	5.33 bB	24 dA	38 cA	0.74 dA	0.42 eA	4.00 aA	7.50 aA
UFU-125#2#2#1	77 bA	52 aB	78 bA	53 bB	9.43 aA	6.84 aA	59 bA	60 aA	2.05 bA	1.75 cA	12.25 bB	5.84 aA
UFU-206#1#2#1	58 cA	41 bA	56 cA	43 bA	6.88 aA	5.74 bA	30 dA	33 cA	1.58 cA	0.85 eB	10.33 bB	4.99 aA
UFU-117#1#1#1	67 bA	65 aA	71 bA	69 aA	8.40 aA	7.90 aA	46 cB	64 aA	1.79 cB	2.48 bA	7.55 aA	17.08 cB
UFU-189#3#2#1	50 cA	47 bA	70 bA	57 aA	7.26 aA	6.06 bA	20 dB	61 aA	0.77 dA	0.93 eA	6.15 aA	10.39 bB
UFU-199#1#1#1	41 dA	69 aB	65 bA	75 aA	7.38 aA	8.09 aA	39 cB	66 aA	1.14 dA	1.56 dA	4.84 aA	5.24 aA
UFU-206#1#5#1	34 dA	49 bA	44 cA	59 aA	4.38 cA	6.38 bA	23 dA	31 cA	1.65 cA	0.66 eB	11.51 bA	8.74 bA
UFU-040#5#5#1	41 dA	42 bA	52 cA	51 bA	5.29 bA	5.86 bA	40 cA	44 cA	1.40 cA	1.48 dA	13.00 bB	7.32 aA
UFU MC BIOFORT1	35 dA	45 bA	30 cA	49 bA	4.08 cA	5.67 bA	30 dA	40 cA	1.54 cA	1.06 dA	7.58 aA	10.91 bA
Grand Rapids	50 cA	38 bA	56 cA	40 bA	7.00 aA	5.80 bA	40 cA	38 cA	1.00 dB	1.65 cA	9.95 bA	12.98 cA
Uberlândia 10000	99 aA	46 bB	99 aA	49 bB	5.99 bA	5.86 bA	98 aA	40 cB	2.00 bA	1.31 dA	3.83 aA	7.06 aA
Belíssima	100 aA	61 aB	100 aA	61 aB	6.23 bA	7.44 aA	98 aA	45 cB	4.41 aA	1.28 dB	2.57 aA	8.60 bB
Everglades	99 aA	58 aB	99 aA	66 aB	6.03 bA	8.25 aA	100 aA	68 aB	4.46 aA	2.00 cB	3.75 aA	21.98 dB
Verônica	48 cA	59 aA	73 bA	64 aA	7.00 aA	7.36 aA	62 bA	19 dB	1.10 dA	4.41 aA	10.05 bA	11.08 bA
CV (%)	19.53		17.56		11.28		14.46		8.33		14.30	

*Mean values followed by the same uppercase letter in the row and lowercase letter in the column do not differ from each other according to the Scott-Knott test at 5% probability. FGC, first germination count; G, germination; GSI, germination speed index; E, emergence; ESI, emergence speed index; EC, electrical conductivity.

Most of the genotypes did not differ statistically in relation to defoliation in the first germination count and germination analyses. Only the genotypes UFU-117#1#3#1, UFU-107#1#2#1, UFU-197#2#2#1, UFU-206#1#6#1, UFU-206#1#4#1, UFU-125#2#2#1, UFU-199#1#1#1, Uberlândia 10000, Belíssima, and Everglades were statistically different in relation to defoliation when evaluating the first germination count. The genotypes UFU-107#1#2#1, UFU-197#2#2#1, UFU-189#3#4#1, UFU-125#2#2#1, Uberlândia 10000, Belíssima, and Everglades were statistically different in relation to defoliation in the evaluation of germination.

Stresses from defoliation can change the source-sink relationship of plants and affect seed physiological quality (Pereira et al., 2012), although there is little information regarding the effects of defoliation on seed germination traits (Heidari, 2015a). In other crops, such as maize, defoliation resulted in higher seed germination than that of a control group with no defoliation (Heidari, 2015b). It is also noteworthy that many genotypes did not achieve a minimum germination percentage of 80% (Brasil, 2019). However, lettuce seeds have primary dormancy, which can be naturally overcome through storage (Kano et al., 2011).

The germination test is performed under optimal conditions of water availability, aeration, and temperature (Brasil, 2009). Therefore, the use of tests that evaluate seed vigor is necessary for identification of possible differences among the seed lots (Marcos Filho, 2015). When the emergence, electrical conductivity, germination speed index, and emergence speed index tests were performed, differences could be detected in seed vigor.

The genotypes Uberlândia 10000, Belíssima, and Everglades had higher percentages of seed emergence and plant defoliation (Table 2). The seeds of the genotypes had different vigor levels according to the emergence test, with or without defoliation. This test is considered the best indicator for hypotheses regarding seed vigor because it utilizes conditions that simulate those to which the seeds will be subjected at the time of sowing (Pereira, Torres, & Linhares, 2015). Differences were also observed in seed vigor in the germination and emergence speed indices, and in general, seeds exhibited lower vigor in association with defoliation. Rodolfo, Souza, Coelho, and Belizario (2017) also reported that the vigor of wheat seeds declined in association with plant defoliation. Thus, defoliation can affect seed quality; intense defoliation can reduce total photoassimilates, leading to a reduction in seed vigor (Medeiros & Nabinger, 2001).

Differences in seed vigor were also observed in the electrical conductivity test. The plants without defoliation produced seeds with lower membrane integrity, as shown by the electrical conductivity test. Castilho et al. (2019) highlighted that the genotype itself can alter the results of the electrical conductivity test.

Regarding thermoinhibition, the seeds of all lettuce genotypes showed a reduction in germination caused by high temperatures, regardless of defoliation status (Table 3). Germination declined in all genotypes evaluated at 35°C. At that temperature, imbibition occurred, but the germination of the seeds of most of the genotypes was extremely low. The highest percentages of germination at that temperature were in the genotypes UFU-125#1#1#1, UFU-7#1#2#1, UFU-86#2#1#1, UFU-206#1#6#1, and Everglades.

Catão et al. (2018) reported that high temperatures are associated with thermoinhibition and, during the evaluation of the germination test, soaked seeds with no root emergence were observed. The critical period for induction of thermoinhibition is within the first 8–12 h of imbibition at high temperature (Argyris, Dahal, Hayashi, Still, & Bradford, 2008). The cultivars susceptible to thermoinhibition have a larger amount of mannose and galactose in the cell wall, which results in rigidity of the endosperm and impedes root emergence (Nascimento et al., 2012). The endosperm surrounding the embryo constitutes a physical barrier to radicle emergence and is required for the imposition of thermoinhibition in lettuce seeds. The rate and extent of weakening of the micropylar endosperm enclosing the radicle tip may be involved in determining whether thermoinhibition occurs (Schwember & Bradford, 2010).

Several studies have reported the causes of this sensitivity and the existence of a close link between endosperm weakening, endo-β-mannanase enzyme activity, ethylene production, heat-tolerant proteins such as heat shock proteins (HSP), and germination of lettuce seeds under high temperatures (Catão et al., 2014; Schwember & Bradford, 2010; Nascimento, Cantliffe, & Huber, 2004; Nascimento & Cantliffe, 2002).

The highest percentage of germination at 35°C was found in the seeds of the cultivar Everglades, 51% with defoliation, and 53% without. Everglades exhibit potential as a genotype for use in tropicalized lettuce breeding programs. Similar results were observed by Zuffo et al. (2017), Catão et al. (2018), and Almeida, Silva-Mann, Santos, Pereira, and Blank (2019), upon analyzing the germination of seeds of this cultivar at high temperatures.

Most of the genotypes had high vigor seeds at 20°C after the accelerated aging test (Table 3). After aging, many genotypes germinated superior to the initial quality evaluation. High temperature and relative humidity conditions can increase the permeability of the seed coat, resulting in an increase in germination (Ataíde, Flores, & Lima e Borges, 2012).

Table 3. Germination (%) of lettuce seeds from different genotypes at different temperatures (°C) after accelerated aging, in accordance with the cropping system with and without defoliation.

Genotypes	With defoliation Temperature (°C)				Without defoliation Temperature (°C)			
	20	25	30	35	20	25	30	35
UFU-117#1#3#1	62 cA	56 cA	26 dB	12 cC	71 aA	62 bA	58 bA	11 bB
UFU-206#1#6#8	67 cA	52 cB	75 aA	3 cC	52 cA	44 cA	7 eB	1 bB
UFU-86#1#2#1	55 cA	49 cA	23 dB	2 cC	58 bA	44 cB	8 eC	0 bC
UFU-75#1#1#1	65 cA	54 cA	11 eB	1 cB	82 aA	70 bA	5 eB	4 bB
UFU-189#3#1#1	75 bA	50 cB	65 aA	0 cC	60 bA	23 eB	1 eC	2 bC
UFU-197#3#1#1	61 cA	68 bA	11 eB	4 cB	52 cA	49 cA	1 eB	1 bC
UFU-125#1#1#1	72 bA	73 bA	20 dB	19 bB	76 aA	63 bA	71 aA	6 bB
UFU-7#1#2#1	74 bA	75 bA	17 dB	27 bB	78 aA	56 cB	39 cC	12 bA
UFU-155#1#1#1	20 fA	24 eA	2 eB	0 cB	28 eA	14 fB	2 eC	0 bC
UFU-206#3#2#1	62 cA	54 cA	1 eB	1 cB	68 bA	31 dB	3 eC	2 bC
UFU-189#2#3#1	61 cA	23 eB	21 dB	1 cC	75 aA	12 fB	2 eB	1 bB
UFU-184#2#5#1	8 fA	14 eA	2 eA	1 cA	21 eA	8 fB	8 eB	3 bB
UFU-107#1#2#1	68 cA	63 bA	8 eB	6 cB	79 aA	32 dB	42 cB	3 bC
UFU-86#2#1#1	50 dA	58 cA	55 bA	25 bB	63 bA	57 cA	51 cA	22 bB
UFU-75#3#2#1	54 cA	55 cA	45 cA	0 cB	43 dA	50 cA	45 cA	1 bB
UFU-120#1#1#1	67 cA	19 eB	2 eC	1 cC	65 bA	21 eB	16 dC	0 bC
UFU-189#1#2#1	54 cA	28 dB	0 eC	1 cC	67 bA	22 eB	1 eC	1 bC
UFU-206#1#1#1	64 cA	36 dB	4 eC	1 cC	74 aA	36 dB	2 eC	0 bC
UFU-75#3#1#1	71 bA	44 cB	19 dC	3 cD	66 bA	48 cB	7 eC	3 bC
UFU-197#1#1	68 cA	46 cB	27 dC	1 cD	39 dA	24 eB	11 eC	0 bC
UFU-189#2#2#1	71 bA	49 cB	0 eC	0 cC	72 aA	54 cB	22 dC	2 bD
UFU-197#2#2#1	32 eA	23 eA	5 eB	0 cB	37 dA	41 dA	25 dB	0 bC
UFU-189#2#1#1	63 cA	12 eB	2 eB	1 cB	49 cA	31 dB	2 eC	1 bC
UFU-199#2#1#1	31 eA	27 dA	3 eB	0 cB	39 dA	21 eB	4 eC	0 bC
UFU-206#1#6#1	81 aA	69 bB	56 bC	24 bD	70 bA	67 bA	25 dB	8 bC
UFU-206#1#3#1	49 dA	36 dB	5 eC	3 cC	42 dA	28 dB	7 eC	0 bC
UFU-189#3#4#1	76 bA	55 cB	1 eC	0 cC	72 aA	72 bA	6 eB	2 bB
UFU-206#1#4#1	51 dA	51 cA	3 eB	1 cB	49 cA	34 dB	1 eC	0 bC
UFU-125#2#2#1	49 dA	49 cA	39 cA	4 cB	56 cA	33 dB	28 dB	8 bC
UFU-206#1#2#1	80 aA	73 bA	5 eB	0 cB	68 bA	30 dB	7 eC	0 bC
UFU-117#1#1#1	70 bA	69 bA	38 cB	4 cC	67 bA	68 bA	15 dB	11 bB
UFU-189#3#2#1	66 cA	56 cA	3 eB	6 cB	79 aA	9 fB	1 eB	6 bB
UFU-199#1#1#1	60 cA	66 bA	14 dB	2 cB	78 aA	43 cB	6 eC	2 bC
UFU-206#1#5#1	62 cA	59 cA	68 aA	5 cB	40 dA	39 dA	2 eB	1 bB
UFU-040#5#5#1	66 cA	46 cB	7 eC	0 cC	57 bA	58 cA	6 eB	0 bB
UFU MC BIOFORT1	42 dA	33 dA	9 eB	0 cB	40 dA	36 dA	21 dB	2 bC
Grand Rapids	36 eA	30 dA	5 eB	0 cB	25 eA	19 eA	4 eB	0 bB
Uberlândia 10000	33 eA	17 eB	7 eC	2 cC	52 cA	21 eB	13 dB	8 bC
Belíssima	74 bA	32 dB	7 eC	0 cC	75 aA	49 cB	7 eC	1 bC
Everglades	92 aA	87 aA	74 aB	51 aC	93 aA	90 aA	72 aB	53 aC
Verônica	68 cA	61 cA	2 eB	0 cB	72 aA	61 bA	2 eB	0 bB
CV (%)								27.69

*Mean values followed by the same uppercase letter in the row and lowercase letter in the column do not differ from each other according to the Scott-Knott test at 5% probability.

At 30°C, regardless of the defoliation treatments, some genotypes maintained germination, such as UFU-86#2#1#1, UFU-75#3#2#1, UFU-206#1#6#1, UFU-125#2#2#1, and UFU-206#1#5#1. A more detailed study of these genotypes is recommended. The seeds of most of the lettuce cultivars are susceptible to thermoinhibition or failure of germination at temperatures higher than 28°C (Yoong et al., 2016), particularly in the UFU MC BIOFORT1, Grand Rapids, Uberlândia 10000, Belíssima, and Verônica varieties. According to Gonai et al. (2004), the maximum germination temperature in lettuce seeds is primarily determined by antagonistic interactions between gibberellin (GA) and abscisic acid (ABA). Studies conducted on lettuce have shown that the ABA concentration is maintained at very high levels when seeds are soaked at high temperatures, but rapidly decreases when imbibition occurs at optimum temperatures for germination (Argyris et al., 2008). GA is required for lettuce seed germination at high temperatures, even when ABA biosynthesis is inhibited (Argyris et al., 2011).

At 30 and 35°C, most of the seeds exhibited thermoinhibition of germination, causing a reduction in physiological potential. Almeida et al. (2019) suggested that thermoinhibited seeds were evaluated 11 days

after setting up the germination test. However, upon analyzing the data of these authors, significant increases in germination would justify prolonging the test beyond that recommended by the Rules for Seed Analysis (Brasil, 2009). The greatest increase observed was 9% in the Grand Rapids cultivar. In the other cultivars, this percentage increase was not greater than 2%. In addition, Catão et al. (2014) and Catão et al. (2018) considered the Grand Rapids cultivar to be thermosensitive, based on the reduction in physiological potential and low activity of the endo-β-mannanase enzyme at high temperatures. Thermoinhibition is not a condition that can be overcome over time, but rather through a reduction in the temperature to which seeds are exposed.

Light is another major environmental factor that affects seed germination, and there is evidence that light and temperature interact through phytochromes in regulating germination (Heschel et al., 2007). In lettuce seeds, the active phytochrome requirement for germination increases as the temperature increases and the seeds become more sensitive to thermoinhibition (Gonai et al., 2004). Therefore, further studies are required.

In addition to evaluating germination of the seeds under high temperatures, the genotypes were also separated into different groups using dissimilarity measurements (Figure 1), which may be useful for breeding of tropicalized lettuce (Araujo, Telhado, Sakai, Ledo, & Melo, 2016). Mahalanobis generalized distance showed that the genetic dissimilarity measurements among the 41 genotypes ranged from 2.07 (UFU-189#3#4#1) to 959.85 (Everglades), also indicating high diversity of the Everglades cultivar compared to the others. Thus, Everglades is dominant over the other genotypes apart from germination temperature or the practice of defoliation. In contrast, the UFU-189#3#4#1 genotype and the Verônica cultivar prove to be thermosensitive, unlike Everglades. Marostega, Araujo, Luz, Neves, and Barelli (2017) determined that the identification of genetic variability in relation to the characteristics of seed germination allows for a better distinction and selection of the genotypes. Souza, Martins, Azevedo, and Pereira (2012) found greater contributions to genetic divergence based on the characteristics of seedlings in Passiflora species in the germination percentage and germination speed index variables.

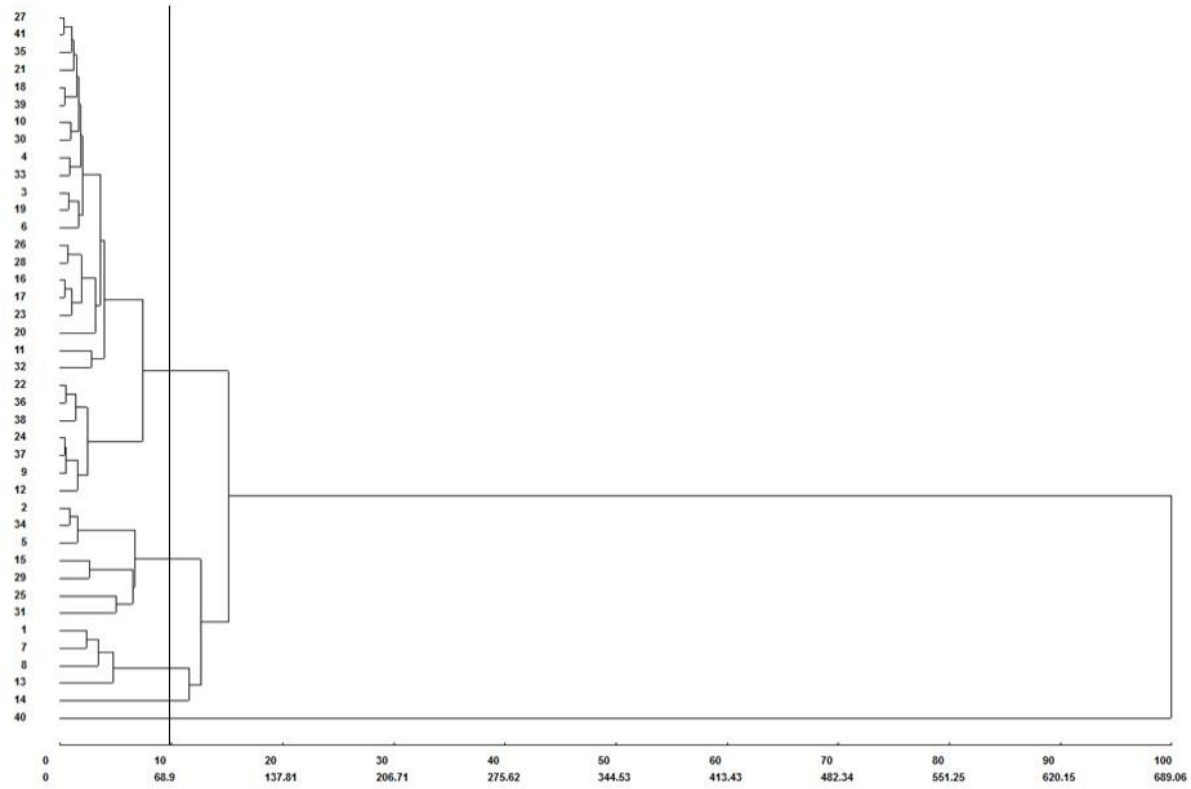

Figure 1. Dendrogram developed from quantitative data with the Mahalanobis generalized distance (D^2ii') and by the UPGMA clustering method among 41 lettuce genotypes. 1 = UFU-117#1#3#1; 2 = UFU-206#1#6#8; 3 = UFU-86#1#2#1; 4 = UFU-75#1#1#1; 5 = UFU-189#3#1#1; 6 = UFU-197#3#1#1; 7 = UFU-125#1#1#1; 8 = UFU-7#1#2#1; 9 = UFU-155#1#1#1; 10 = UFU-206#3#2#1; 11 = UFU-189#2#3#1; 12 = UFU-184#2#5#1; 13 = UFU-107#1#2#1; 14 = UFU-86#2#1#1; 15 = UFU-75#3#2#1; 16 = UFU-120#1#1#1; 17 = UFU-189#1#2#1; 18 = UFU-206#1#1#1; 19 = UFU-75#3#1#1; 20 = UFU-197#1#1; 21 = UFU-189#2#2#1; 22 = UFU-197#2#2#1; 23 = UFU-189#2#1#1; 24 = UFU-199#2#1#1; 25 = UFU-206#1#6#1; 26 = UFU-206#1#3#1; 27 = UFU-189#3#4#1; 28 = UFU-206#1#4#1; 29 = UFU-125#2#2#1; 30 = UFU-206#1#2#1; 31 = UFU-117#1#1#1; 32 = UFU-189#3#2#1; 33 = UFU-199#1#1#1; 34 = UFU-206#1#5#1; 35 = UFU-040#5#5#1; 36 = UFU MC BIOFORT1; 37 = Grand Rapids; 38 = Uberlândia 10000; 39 = Belíssima; 40 = Everglades; 41 = Verônica.

The groups formed in the UPGMA dendrogram (Figure 1) exhibited a cophenetic correlation coefficient of 83% (t-test, $p < 0.05$). Lopes, Franke, Souza, Bertoncelli, and Graminho (2017) suggest cophenetic correlation values above 0.80 to ensure a good fit among the original matrix distances and the graphic. However, lower values do not make the dendrogram ineffective, but indicate distortion, which means that it can still be used to evaluate the cluster formed.

Group I was composed of 28 genotypes, group II had seven, group III had four, group IV contained one genotype (UFU-86#2#1#1), and group V held only the Everglades cultivar. This information showed that the genetic diversity of Everglades differed significantly from that of the other genotypes.

To confirm the genetic variability of the 41 lettuce genotypes, a second methodology was used (Figure 2). The Tocher optimization method can be used to show miniscule genetic dissimilarities between the two genotypes. Values near zero indicate greater similarity (yellow), whereas values near 1 indicate greater genetic dissimilarity (black). The results in Figure 2 show substantial genetic variability among the genotypes. The Everglades cultivar is highly dissimilar from the other genotypes. However, the UFU-86#2#1#1 genotype was the most similar to Everglades, with germination greater than 50% at 30°C and 20% at 35°C (Table 3), showing potential for assisting future breeding programs. Cardoso, Silva, Pereira, Viano, and Araújo (2009), working with papaya genotypes, found that the identification of genetic variability in relation to seed germination characteristics allowed for better distinction of genotypes, with strong prospects for selection.

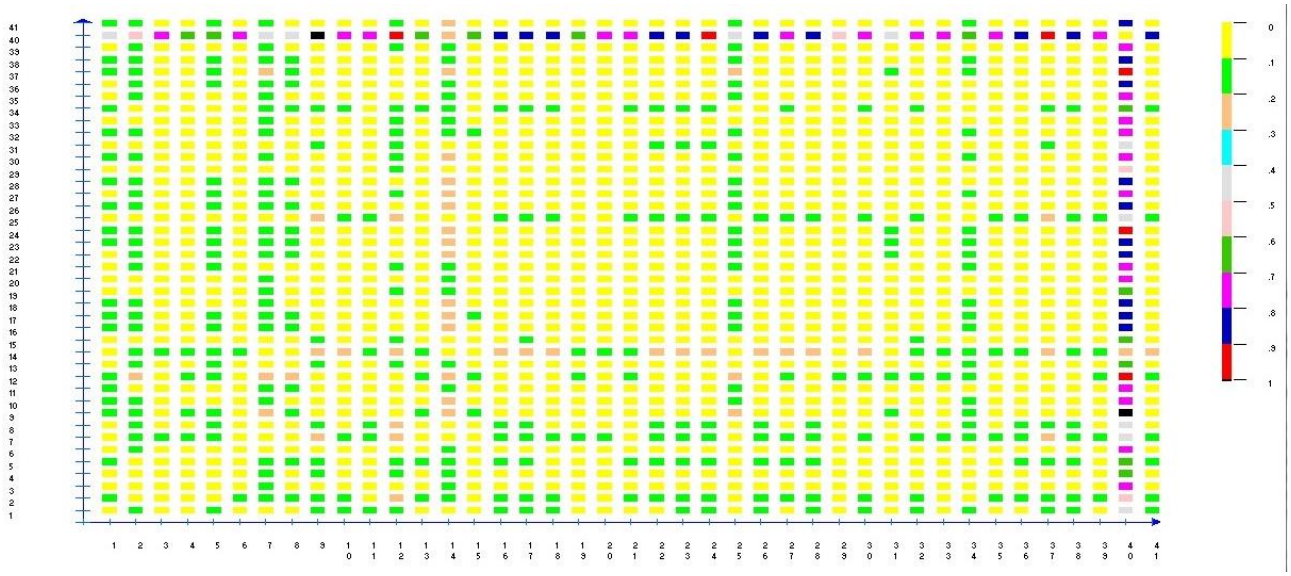

Figure 2. Clustering by the Tocher optimization method, based on eight physiological traits of germination of 41 lettuce genotypes. 1 = UFU-117#1#3#1; 2 = UFU-206#1#6#8; 3 = UFU-86#1#2#1; 4 = UFU-75#1#1#1; 5 = UFU-189#3#1#1; 6 = UFU-197#3#1#1; 7 = UFU-125#1#1#1; 8 = UFU-7#1#2#1; 9 = UFU-155#1#1#1; 10 = UFU-206#3#2#1; 11 = UFU-189#2#3#1; 12 = UFU-184#2#5#1; 13 = UFU-107#1#2#1; 14 = UFU-86#2#1#1; 15 = UFU-75#3#2#1; 16 = UFU-120#1#1#1; 17 = UFU-189#1#2#1; 18 = UFU-206#1#1#1; 19 = UFU-75#3#1#1; 20 = UFU-197#1#1; 21 = UFU-189#2#2#1; 22 = UFU-197#2#2#1; 23 = UFU-189#2#1#1; 24 = UFU-199#2#1#1; 25 = UFU-206#1#6#1; 26 = UFU-206#1#3#1; 27 = UFU-189#3#4#1; 28 = UFU-206#1#4#1; 29 = UFU-125#2#2#1; 30 = UFU-206#1#2#1; 31 = UFU-117#1#1#1; 32 = UFU-189#3#2#1; 33 = UFU-199#1#1#1; 34 = UFU-206#1#5#1; 35 = UFU-040#5#5#1; 36 = UFU MC BIOFORT1; 37 = Grand Rapids; 38 = Uberlândia 10000; 39 = Belíssima; 40 = Everglades; 41 = Verônica.

In this context, analyses performed to evaluate the genetic diversity among lettuce genotypes suggested that gathering knowledge regarding aspects of seed germination under high temperatures is fundamental for breeding programs. In this sense, Cruz et al. (2012) reported that although the volume of genetic information from molecular markers has increased considerably in genetic diversity studies, diversity studies are still defined by phenotypic characteristics, mainly those of a quantitative nature.

The analyses of the clustering methods allowed for the identification of promising genotypes (UFU-86#2#1#1 and Everglades), confirmed by the grouping methods, as well as the recognition of those with greater genetic dissimilarity. The most divergent genotypes can be used in directed hybridization, allowing the number of desirable recombinations to be increased and the superior genotypes for the production of seeds tolerant to thermoinhibition to be obtained.

Conclusion

The genotypes UFU-189#3#1#1, UFU-189#1#2#1, UFU-189#3#4#1, UFU-206#1#6#1, and Grand Rapids had high seed yield. With removal of the basal leaves, there was a higher yield of seeds per plant, and a change in seed physiological quality. The genotypes have genetic variability for thermoinhibition, and the genotypes UFU-86#2#1#1 and Everglades have similar performance.

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