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Adaptation of Lettuce Cultivars to High Temperatures and Different Types of Fertilization: Anticipating Management Strategies to Mitigate the Adverse Effects of Global Climate Change

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Abstract:
Global climate change (GCC) is already a reality and has the potential to cause significant damage to Brazilian and world agriculture. One of the most important aspects for tropical countries is the possibility of a significant increase in average air temperature which, according to the Intergovernmental Panel on Climate Change (IPCC), could vary between 1.4 oC and 4.4 oC by the end of this century. Increases in the occurrence of heat waves, extreme rainfall and droughts are also projected. The average annual air temperature in Brazil is projected to exceed 30°C. Lettuce (Lactuca sativa L.) is the most widely consumed leafy vegetable in Brazil and worldwide and originates from regions with a moderate climate and is therefore vulnerable to very high temperatures. The aim of this study was to evaluate the performance of commercial lettuce cultivars in a simulated environment with projected temperatures like those at the end of the 21st century. It also aimed to assess the positive effects of using a biofertilizer, called Hortbio®, as a strategy to mitigate the negative impacts of high temperatures. To this end, two experiments were conducted in a factorial scheme of 11 (eleven lettuce cultivars) x 2 (two types of fertigation: Hortbio® = organic and mineral fertilizers). The first experiment was conducted at a temperature of 25 oC/20 oC (day/night), representing the historical average air temperature in Brazil. The second experiment was conducted at an average air temperature of 30 oC/25 oC, representing the projected temperature for the end of the 21st century. The following morpho-agronomic attributes were assessed: total mass, commercial mass, number of leaves and plant height. The occurrence of morphophysiological disorders was also assessed, including days to setting, occurrence of leaf chlorosis and tipburn. The results showed that, although well adapted to the country's historical tropical temperatures, most of the cultivars showed low levels of adaptation to the environmental conditions projected in the GCCs scenarios. Only three cultivars showed good results when grown at 30 oC/25 oC. The main morphophysiological disorders observed were early setting, tipburn and leaf chlorosis, although leaf necrosis and even plant death were also observed when the plants were grown in the worst-case scenario. The total mass showed a tendency to increase at 30 oC/ 25 oC regime. However, this result was not reflected in the commercial mass due to the simultaneous occurrence of morphophysiological disorders. The main characteristic that differentiates the three cultivars that showed the best results under these conditions was the earliness of the production cycle. The use of biofertilizer apparently has the potential to mitigate part of the negative impacts of high temperatures on the development of cultivated lettuce plants. This work indicates lettuce germplasm with potential use in genetic breeding

programs aimed at adapting to GCCs, as well as presenting alternative management strategies aimed at adapting lettuce cultivation in GCCs scenarios.

Keywords: Global Warming, Climate Emergence, Horticulture, Leafy Vegetables, Biofertilizers, Bioinputs, Plant Genetic breeding, Climatic Justice.

INTRODUCTION

Human activities are undoubtedly the main cause of global warming observed in recent decades. This rise in average air temperature is mainly due to the increase in atmospheric emissions of greenhouse gases (GHGs) since the industrial revolution (IPCC, 2023). The increase in the global average surface temperature observed between 2011 and 2020, when compared to the period from 1850 to 1900, was 1.1 °C (UN Intergovernmental Panel on Climate Change, IPCC, 2023), a figure close to that established by the Paris Agreement as capable of preventing further damage from global warming to the planet's climate system, which was 1.5 °C (IPCC, 2018). In February 2024, the European Climate Service pointed out that for the first time the 1.5 °C global warming limit had been exceeded for 12 consecutive months. During this period, the average global temperature was estimated at 1.52 °C above the 1850-1900 average (Poynting, 2024). Between January and September 2024 this figure already reached 1.54 °C above the historical average according to data from the World Meteorological Organization (WMO) (Eldiario, 2024). There is high confidence that the average global temperature since 1970 has risen faster than in any previous 50-year period in the last 2000 years. Unequivocally, there is a strong relationship between the increase in the atmospheric concentration of GHGs and human activities, with atmospheric concentrations of CO_2 peaking in 2019 at 410 ppm, and there is high confidence that this value is higher than any observed in the last 2 million years. The levels of CH_4 and N_2O, in turn, reached values of 1866 ppb and 332 ppb that same year, which represent, with a very high level of confidence, values higher than those observed in the last 800,000 years (IPCC, 2023).

Several GCCs are expected because of global warming. Among them, there is a virtual certainty that heat extremes (or heat waves) will become more frequent and more intense in most regions. On the other hand, cold extremes have become less frequent and less intense (IPCC, 2023). Projections show that limiting the increase in global average temperature to 1.5 or 2 °C will only be achieved with a deep and rapid reduction in atmospheric GHG emissions in the coming decades (IPCC, 2021), a scenario that has so far proved unlikely. The 6th IPCC Report (IPCC 2021; IPCC 2023) used an index that predicts human influence and makes projections of temperature and its associated impacts. These are the so-called SSP scenarios. The most optimistic scenarios point to better estimates of temperature growth of between 1.4 and 1.8 °C, compared to the period between 1850 and 1900, if drastic reductions in atmospheric GHG emissions are achieved quickly. The pessimistic scenario considers that atmospheric GHG emissions will continue to grow at high rates for much of the 21st century, resulting in a greater increase in the global average temperature, with a best estimate of 4.4 °C. The intermediate scenarios would lead to estimated average temperature increases of between 2.7 and 3.6 °C (IPCC, 2021). It is therefore clear that agricultural activities need to adapt to this new scenario.

Regionalized projections have shown a very challenging situation for the Brazilian territory. The average temperatures projected for the end of the 21st century, considering an additional energy storage of 4.5 $W.m^{-2}$ and 8.5 $W.m^{-2}$ (RCP 4.5 and RCP 8.5 scenarios), respectively, could result in a temperature increase of between 2 °C and 8 °C, respectively, during the summer. The highest temperatures should be observed in the Midwest region, followed by the Northern, Northeastern, Southeastern and Southern regions. In winter, a similar temperature increase is also projected (Brasil, 2016a).

In turn, Hamada (2015) points out that the average air temperature for the reference period (1961-1991) in Brazil is around 25.5 °C and points to the possibility of reaching, by the end of the century, an average temperature that could reach 31.3 °C during spring in the Northern region of the country, in a pessimistic scenario. Regionally, this author found average temperature values for two scenarios, one optimistic and the other pessimistic, ranging from: North - 29.5 °C (spring) to 31.3 °C (spring); Northeast - 28.5 °C (spring) to 30 °C (spring); Midwest - 28.6 °C (spring) to 30.4 °C (spring); Southeast - 26.0 °C (spring) to 27.3 °C (summer); South - 25.0 °C (summer) to 26.1 °C (summer); Brazil - 27.1 °C (summer) to 28.4 °C (summer).

The data from Soares (2015), in turn, considering the average temperatures projected for scenarios of high GHG emissions, show projections of an increase in the average air temperature at the end of the century for different regions within the Brazilian territory, as well as the averages obtained for the country, similar to those resulting from the work carried out by Hamada (2015), with projected values for the Brazilian territory described as follows: for the São Francisco basin - 26.42 °C; for the Paraná basin - 28.16 °C; for the Amazon basin - 31.06 °C and; for the whole Brazilian territory - 29.25 °C.

The number of days with maximum temperatures above 34 °C has also shown an upward trend in Brazil (Lima et al., 2015). Extreme average air temperature events are expected to become more frequent, longer and more intense (de Moraes et al, 2022). The greater frequency and intensity of heatwaves in Brazil has been supported by data obtained in various studies (Marengo et al, 2008; Ceccherini et al, 2016; Geirinhas et al, 2018; dos Reis et al, 2019; Bitencourt et al, 2020; Marengo et al, 2021).

The issue of global warming is even more important for vegetables, which includes several plant species that are better adapted to cold and mild climates. Among these, lettuce (*Lactuca sativa L.*) stands out because it originated in the Mediterranean region (Sala & Costa, 2012) and is therefore better adapted to regions with mild climates.

Since the 1990s, new lettuce cultivars have been developed to adapt to tropical and subtropical climates (Sala & Costa (2012). Excessive heat causes important morphophysiological changes in the lettuce crop, such as early setting and tipburn. Tipburn is understood to be a phenomenon of localized Ca deficiency, leading to the development of necrosis on the tips and margins of developing leaves. Symptoms appear when the laticifers swell, rupture and release latex into the surrounding tissue, causing parenchyma collapse, occlusion of xylem elements and, finally, coagulation of the latex. Tipburn occurs even in calcium-rich soils and is probably favored by situations in which there is a decrease in transpiration from the leaf surface, with the increased intensity, frequency and duration of high temperature events being some of the possible conditions for this (Misaghi & Grogan, 1978; Jenny & Hayes, 2010; Jenni et al., 2013; Uno et al., 2016; Macias-Gonzalez et al., 2019). Other negative impacts observed when high temperatures occur are poor seedling formation, uneven stands, low seed germination potential, seedling death, leaf bitterness due to latex accumulation, loss of the apical meristem, symptoms of boron (B) deficiency and, under extreme conditions, necrosis and even plant death (Kobori et al., 2011). Genetic breeding aimed at adapting to tropical and subtropical conditions has included replacing the "head lettuce" type with the smooth and curly types. The advantage of these two types of lettuce can be attributed to the absence of head formation. Another positive characteristic of curly lettuce for adaptation to summer cultivation is its slow setting. Although they are showing growth in consumer preference, American-type lettuces, which form heads, are more difficult to grow in conditions of high temperatures and rainfall (Sala & Costa, 2012). However, these studies do not yet take into account the possible temperature anomalies resulting from global warming. Continuing this work, seeking to select genotypes that are tolerant of the temperatures projected for the Brazilian territory in climate change scenarios, is a fundamental step towards supplying genetic breeding programs with top quality accessions, contributing to the adaptation of Brazilian lettuce to the GCCs

Another strategy for adapting to heat stress, which has seen increased use recently, is the use of bioproducts. Biofertilizers based on Arbuscular Mycorrhizal Fungi (AMFs), for example, have shown potential to mitigate the negative effects of high temperatures on soybean plants, improving their development and productivity. AMFs mitigate heat stress in cultivated plants by improving nutrient absorption capacity and optimizing photosynthetic and photochemical ratios, improving osmotic adjustment, promoting superior antioxidant activity and improving reproductive capacity (Junrami et al., 2022). Biofertilizers with other classes of microorganisms in their composition, such as plant growth-promoting rhizobacteria and endophytic microorganisms, for example, have also shown potential for mitigating the adverse effects of abiotic stresses, and are therefore an important strategy for adapting to extreme environmental events. Plant growth-promoting rhizobacteria, for example, are capable of regulating plant hormones to boost plant growth by improving nutrient absorption and antioxidant biosynthesis, as well as numerous osmolytes (Iqbal et al., 2023).

The Hortbio® biofertilizer was developed by Embrapa Hortaliças using agro-industrial waste as raw material (Cajamarca et al., 2019a) and has a variety of microorganisms in its composition, most of which are listed in the literature as possible plant growth promoters, namely: *Klebsiella*, *Pseudomonas*, *Bacillus*, *Streptomyces*, *Artrobacter*, *Lactococcus*, *Kurthia*, *Sporosarcina*, *Alcaligenes*, *Acinetobacter*, *Enterobacter*, *Gluconobacter*, *Stenotrophomas*, *Corynetobacterium*, *Pichia*, *Aspergillus*, *Penicillium* and *Trichoderma* (Bomfim et al., 2024). Given its microbiological composition, it contains indole- acetic acid (IAA-auxin), in a concentration that is potentially beneficial for plant development (Bomfim et al., 2024). IAA, as well as other molecules that normally accompany it, such as gibberellin, cytokinins, abscisic acid and ethylene, can reduce the negative impacts of abiotic stresses (Li et al., 2011; Mitler et al., 2012; Peleg & Blumwald, 2011; Bouzroud et al., 2018; Androcioli, 2019). The peak production of IAA-Auxin in Hortbio® biofertilizer occurs 10 days after its production, coinciding with the greatest diversity of microorganisms present (Fontenelle et al., 2017). IAA are recognized for their role in various processes linked to plant development, such as rooting, apical dominance, plant growth, geotropism, phototropism and fruit development (Alatzas, 2013).

The aim of this study was to evaluate the performance of commercial lettuce cultivars available on the Brazilian market when produced in a simulated environment with temperatures projected according to a GCC scenario compatible with the temperature projected for the Brazilian territory in intermediate and pessimistic environments, as well as to select those that could become promising accessions for conducting a genetic breeding program aimed at adapting to GCCs. It also aimed to assess the existence of positive effects from the use of a biofertilizer (Hortbio®) as a strategy to mitigate the negative impacts of projected high temperatures.

MATERIAL AND METHODS

Location

This work was carried out in the Soils and Plant Nutrition laboratory at Embrapa Hortaliças (latitude: 15º56'; longitude: 48º08'), located in the rural area of the Gama administrative region, Brasília, Federal District, Brazil. A plant growth chamber was used to simulate the following parameters: air temperature, relative humidity, radiation potential, atmospheric CO_2 concentration, shading and irrigation rate.

Setup of the Plant Growth Chamber

This study only assessed the effect of temperature on the development of lettuce plants. Two representative air temperature regimes were used, the aim of which was to cover the historical average observed and projected in intermediate and pessimistic MCG scenarios for the Brazilian territory, namely: 25 °C/20 °C (temperature maintained during the day / temperature maintained during the night) and 30 °C/25 °C. The relative humidity was kept constant between 60 and 70 % and the CO_2 atmosphere was maintained according to the environment in which the plants were placed. Irrigation was managed uniformly, applying a total of 200 mL of water per day, divided into two daily applications, throughout the plants' life cycle.

Constant artificial lighting was also used during the day (12-hour photoperiod) using 1200 C3 Full Spectro LED (Light-Emitting Diode) lamps in a module for plant growth. According to the manufacturer's manual, the composition of wavelengths in this module is: 400 to 500 nm (blue) - 9%; 500 to 600 nm (green) - 2%; 600 to 700 nm (red) - 64%; 700 to 800 nm (red/infrared) - 25%. The final color is pink and the radiation intensity used was 800 Umol/m^2/s^{-1}. The luminous flux at 1.50 m (height of the lamp in relation to the plants) from the plant was 800 lux.

Figure 1: Experiments carried out in a plant growth chamber under artificial LED lighting, forming a pink color.

Experimental Design

The experiments at each temperature were conducted individually. For each of them, at 25 °C/20 $^{(o)}$C and at 30 °C/25 °C, a completely randomized design was used with three replications and a factorial scheme of 11 x 2, with 11 commercial lettuce cultivars and two forms of fertilization (mineral and organic). Each experimental plot consisted of a pot containing soil duly corrected with dolomitic limestone, and a lettuce plant, making a total of 66 plots for each experiment.

Seedling Production and Characteristics of the Cultivars Used

The seedlings were grown in a greenhouse in 128-cell polystyrene trays containing commercial Bioplant® substrate. Eleven commercial lettuce cultivars were used and, for ethical reasons, their names will not be mentioned. Table 1 shows the main characteristics of each cultivar used. Transplanting took place 21 days after sowing. After this period, the seedlings were transplanted into three-liter plastic pots, which were then placed entirely at random in a plant growth chamber.

Table 1: Code and main characteristics of the cultivars used in the trials.

Cultivar code	Adaptation to tropical conditions?	Adapted to summer cultivation?	Tolerance to early setting	Tipburn tolerance	Production cycle (days)	Precocity	Types of lettuce
1	Yes	Yes	No	No	58	Normal	Smooth
2	Yes	Yes	No	No	60	Normal	Smooth
3	Yes	Yes	Yes	No	35 a 45	Very early	Curly
4	Yes	Yes	Yes	No	35 a 45	Very early	Curly
5	Yes	Yes	Yes	No	35 a 45	Very early	Curly
6	Yes	Yes	No	No	80	Too late	American
7	Yes	Yes	No	No	60 a 80	Late to very late	Curly
8	Yes	Yes	No	Yes	55	Normal	Curly
9	Yes	Yes	No	No	70 a 90	Late to very late	Curly
10	Yes	Yes	Yes	Yes	56	Normal	Curly
11	Yes	Yes	No	Yes	75 a 90	Too late	American

The information in Table 1 was taken from publicity materials produced by the companies responsible for developing and/or marketing each of the cultivars used. The cultivars chosen are the result of breeding programs aimed at tropicalizing the lettuce crop and, for this reason, they are adapted to growing in hot regions and, therefore, have heat tolerance to a greater or lesser extent. The information provided by the companies indicates that the Code 3, 4, 5 and 10 cultivars are tolerant to early setting and that the Code 8, 10 and 11 cultivars are tolerant to tipburn. Cultivars with a variable production cycle were also evaluated, ranging from very early (production cycle between 35 and 45 days) to very late (cycles of more than 70 days). The types of lettuce evaluated include cultivars of Smooth, Curly and American lettuce-types. However, it is important to note that the information contained in Table 1 also reflects the conditions in which the cultivars were evaluated and validated, which in turn may reflect different heat intensities. This fact, as well as leading us to the obvious conclusion that these cultivars are better adapted to the current climate, may also indicate that they may have different levels of heat tolerance, depending on the climatic conditions of the locations and times of year where they were developed and validated.

PREPARATION OF HORTBIO BIOFERTILIZER®

Preparation of Effective Microorganisms (EM)

The efficient microorganisms (EM) were captured using cooked rice "bait". The cooked rice, unsalted and unseasoned, was placed in a plastic tray covered with an antiophidic screen to prevent insects from entering. Once covered, it was buried with a thin layer of soil in an area of secondary vegetation of a well-preserved Cerradão, located in the Organic Agriculture Sector, a sub-area of the Experimental Fields Sector of Embrapa Hortaliças. After seven days, the tray was removed from the site and the dark colonies, which in theory constitute non-beneficial microorganisms, were segregated and discarded, while the colonies with other different colorations were separated for later use, according to the instructions defined in Bonfim et al., 2011. The usable colonies (with different colors, except for the dark ones) were then added to a plastic container containing a mixture of 1 L of sugarcane juice and 10 L of water. The suspension obtained was then aerated for 15 minutes every 1 hour for seven days. At the end of this process, the inoculant (EM) was ready for use.

Preparation and Characterization of Hortbio®

The biofertilizer used in the experiment was Hortbio®, which was applied in the form of an aqueous suspension, diluted to 5% in water. The biofertilizer was produced in a plastic tank with a capacity of 100 liters. The raw materials used and their respective quantities are blood meal (1.1 kg), rice bran (4.4 kg), castor bean meal (1.1 kg), bone meal (2.2 kg), crushed seeds (1.1 kg), wood ash (1.1 kg), "rapadura" (concentrated sugar cane extract) (0.55 kg) and corn bran (0.55 kg), enriched with 1 L of efficient microorganisms (EM). At the end, non-chlorinated water was added up to a final volume of 100 L. The final mixture was stored in a shaded, cool place, aerated for 15 minutes every hour using an air compressor and a stopwatch (Cajamarca et al., 2019a).

The ingredients were then mixed in a 200 L plastic drum. The drum was then sealed to prevent contamination. Hortbio® was then aerated for 15 minutes every hour, using an air compressor as follows: one end of the hose was connected to the compressor and the other was submerged in the aqueous suspension that would make up the biofertilizer.

The chemical composition of the biofertilizer used is described in Table 2, as determined by Cajamarca et al. (2019). Bomfim (2016) evaluated the microbiological composition of Hortbio® and found a total of 217 isolates, of which 120 were bacteria, 61 were yeasts and 36 were fungi. Genera listed in the literature as responsible for carrying out processes that can result in plant growth promotion by various means were found and are listed below: *Klebsiella*, *Pseudomonas*, *Bacillus*, *Streptomyces*, *Artrobacter*, *Lactococcus*, *Kurthia*, *Sporosarcina*, *Alcaligenes*, *Acinetobacter*, *Enterobacter*, *Gluconobacter*, *Stenotrophomas*, *Corynetobacterium*, *Pichia*, *Aspergillus*, *Penicillium* and *Trichoderma* (Bomfim et al., 2024). This composition also results in the production of substances capable of mitigating the negative effects of high temperatures on the development of cultivated plants, such as Indole-Acetic Acid (IAA), also known as Auxin, which, in the case of Hortbio®, is present in an adequate concentration to promote positive effects on the development of plant species (Bomfim, 2024).

Table 2: Chemical characterization of Hortbio® biofertilizer.

pH	EC	C	N	C/N	P	K	S	Ca	Mg	Fe
	$dS.m^{-1}$	$g.L^{-1}$								
6	5,9	200,3	4,5	4,5	0,81	2,93	0,46	3,91	1	121,15

Source: Cajamarca et al. (2019) and Cajamarca et al. (2020)

Mineral Fertilization

For mineral fertilization, a solution containing four liters of a mixture of the following fertilizers was applied weekly: 300 mg of urea; 370 mg of calcium nitrate; 470 mg of potassium chloride; 400 mg of magnesium sulphate and 50 mg of simple superphosphate. These values were calculated according to the crop's needs and, above all, taking into account the maximum limits of nutrient availability in the biofertilizer used. This solution was then added to the plants receiving this treatment five times, once a week for five weeks. These values were added in such a way as to approximate, as far as possible, the concentration of the nutrients N, P, K, Ca, Mg and S provided by the Hortbio biofertilizer®, to minimize the effects of the different availability of these nutrients in the mineral and organic nutrient solutions. Even so, given the complexity of the chemical composition of Hortbio®, some elements remained unbalanced. N and P were used as the basis for the similarity of the concentrations applied.

Application of Hortbio Biofertilizer® and Mineral Fertilization
The amount of Hortbio® used for the entire cycle was 410 mL, diluted in 8 L of water. This amount was distributed among the treatments that used this biofertilizer as a nutrient source in five stages, one every week for five weeks.

The final concentration of each nutrient applied was: for mineral fertigation - 233.35 mg/L of N, 39.38 mg/L of P, 305.5 mg/L of K, 84 mg/L of Ca, 75 mg/L of S; for organic fertigation - 230.63 mg/L of N, 41.25 mg/L of P, 150.16 mg/L of K, 200.39 mg/L of Ca, 23.58 mg/L of S;

Statistical Analysis
At 45 days after transplanting, the crop was harvested by cutting the plant close to the ground. The characters assessed were plant height (HL), number of leaves (NF), total mass (MT) and commercial mass (MC). These data were submitted to analysis of variance ($p<0.05$) and then to the Scott & Knott (1974) mean test ($p<0.05$).

In order to verify the occurrence of physiological disorders when cultivated at 30 °C, the number of days to the occurrence of early setting was quantified, as well as the occurrence of tipburn and chlorosis was reclassified. For tipburn, the symptoms were reclassified as weak, moderated and severe, receiving the values of 1, 2 and 3, respectively. Chlorosis, when it occurred, showed a very similar pattern between plants and, for this reason, was reclassified only as: non-occurrence - 0 and occurrence - 1. The mean and standard deviation of each of the aforementioned disorders were then calculated.

RESULTS AND DISCUSSION

There were no significant interactions between the factors evaluated for any of the variables, but there were significant statistical differences for several of the attributes, either when analyzing the cultivar or the type of fertilization used. Table 3 shows the results of the agronomic evaluations. It can be inferred that there is a tendency for the MV averages to show higher values at 30 °C/25 °C than at 25 °C/20 °C. However, this trend was not maintained for the other three attributes evaluated, mainly due to the negative effects of high temperatures, resulting in plant and leaf patterns that reduced the averages for MC and NF, as well as an increase in plant height due to the emission of the floral pendant.

At 25 °C/20 °C, cultivars 1, 4, 5, 6, 8, 9 and 11 had the highest MT and MC values, followed by all the others. The NF attribute was the most uneven, with cultivar 1 showing the highest value, followed by cultivars 2 and 3, then cultivars 4, 5, 7 and 10, and finally cultivars 6, 8, 9 and 11. The data obtained for the plant height attribute was, like the MT and MC attributes, very uniform, with the plants of cultivars 1, 5, 6, 7, 8 and 10 showing the highest averages, while cultivars 2, 3, 4, 9 and 11 showed the lowest. These results suggest that the 11 cultivars evaluated are well adapted to the historical average air temperature for Brazil, which is 25.5 °C (Hamada, 2015) and result in satisfactory MT and MC values. The uniformity of the height of the plants grown at this temperature reinforces the previous argument since it points to the non-emission of the floral pendant, as seen in Figure 2. Early setting is one of the biggest problems related to alfaculture in tropical environments, especially in warmer regions and seasons (Sala & Costa, 2012). Apparently, the NF attribute's behavior was probably more influenced by the genetic characteristics of the cultivars tested than by temperature.

Table 4: Morpho-agronomic attributes of 11 lettuce cultivars subjected to two temperatures and fertilized with mineral or organic fertilizer.

	MT		MC		NF		AL	
	25 ºC/20 ºC	30 ºC/25 ºC	25 ºC/20 ºC	30 ºC/25 ºC	25 ºC/20 ºC	30 ºC/25 ºC	25 ºC/20 ºC	30 ºC/25 ºC
	g				cm			
	Genotypes							
1	93,25 a	122,52 a	89,48 a	0,00 c	28,50 a	15,00 a	27,49 a	31,58 b
2	63,66 b	115,74 a	62,36 b	14,57 c	21,17 b	15,50 a	18,79 b	23,08 c
3	62,31 b	108,31 a	58,44 b	88,59 a	13,00 d	16,66 a	15,63 b	18,25 c
4	94,28 a	131,48 a	84,20 a	125,03 a	16,17 c	13,50 a	20,66 b	21,67 c
5	88,85 a	108,71 a	83,94 a	107,91a	17,17 c	14,67 a	29,08 a	20,92 c
6	94,76 a	102,49 a	85,72 a	20,06 c	14,50 d	13,67 a	23,65 a	23,17 c
7	72,98 b	79,40 b	68,29 b	0,00 c	15,67 c	11,66 b	27,08 a	29,33 b
8	86,00 a	96,02 b	78,71 a	20,58 c	14,00 d	11,00 b	24,77 a	20,75 c
9	92,12 a	77,75 b	87,14 a	15,34 c	12,67 d	12,33 b	21,03 b	23,25 c
10	60,12 b	74,23 b	58,86 b	0,00 c	17,33 c	16,16 a	22,52 a	37,08 a
11	91,35 a	58,97 b	83,00 a	47,09 b	12,17 d	9,50 b	16,17 b	15,58 c
Fertilizers								
FM	80,73 a	116,68 a	80,73 a	27,10 a	17,30 a	16,36 a	23,95 a	26,62 a
Hortbio®	77,75 a	78,90 b	72,02 a	38,79 a	15,85 b	10,85 b	20,93 b	21,50 b

MT - Total mass; MC - Commercial mass; NF - Number of leaves; AL - Plant height; FM - Mineral fertilizer.

Figure 2: Lettuce plants grown at 25 °C/20 °C at the end of their growing cycle.

Changes in the order of the cultivars were observed for the attributes MT, MC, NF and AL when the lettuce plants were grown under simulated temperature conditions at 30 °C/25 °C. For the MT attribute, cultivars 1, 2, 3, 4, 5 and 6 had the highest averages, while cultivars 7, 8, 9, 10 and 11 had the lowest. Contrary to what was observed when the experiment was conducted at a temperature of 25 °C/20 °C when the behavior of the data obtained for MC followed that observed for MT, when the setup temperature was 30 °C, a very different behavior was observed. At 30 °C/25 °C, the only cultivars that showed MC values close to those of MT were 3, 4 and 5, which also had the highest MC averages. Cultivar 11 followed as those with the highest MC at this temperature. The values for the latter, however, were already much lower than those observed when the experiment was conducted at 25 °C/20 °C, with the average measured at 30 °C/25 °C corresponding to 56.73 % of those obtained for the first temperature. All the other cultivars had statistically similar MC averages, which were lower than those mentioned above. Cultivars 1, 7 and 10 showed such an intense combination of physiological disorder symptoms, consisting mainly of symptoms such as early setting, tipburn, chlorosis and leaf necrosis, when grown at 30 °C/25 °C, that they did not allow any part of the plants to be used, resulting in zero MC values. Reinforcing this perception, these cultivars (1, 7 and 10) were the ones that also had the highest average plant heights (cultivar 7, followed by cultivars 1 and 10), these values being related to the process of issuing the floral pendant. An overview of the lettuces grown at 30 °C/25 °C throughout their cultivation cycle, now at the end of the production cycle, can be seen in Figure 3.

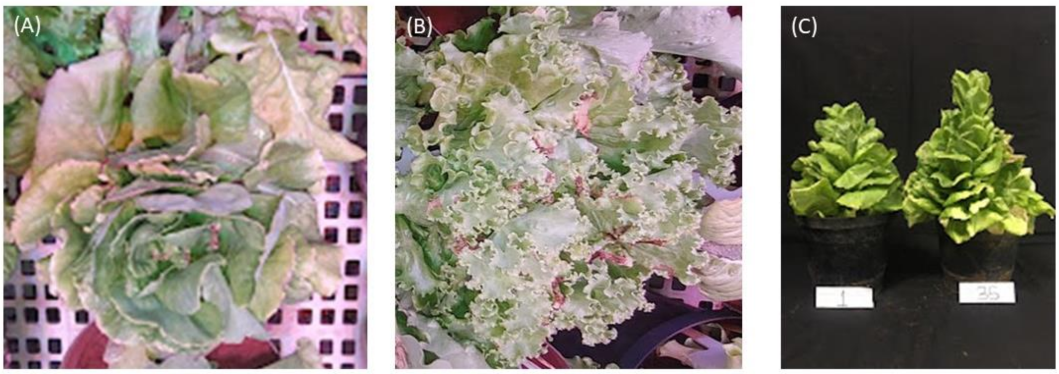
Figure 3: Overview of the experiment, focusing on the lettuce plants and their unevenness at the end of the development cycle when subjected to a temperature of 30 °C/25 °C.

These results make it clear that the cultivars currently sold in Brazil are poorly adapted to the temperature conditions projected in the GCCs scenarios and reinforce the need to conduct genetic breeding programs based on these conditions. The Intergovernmental Panel on Climate Change (IPCC) clearly shows that there is a strong relationship between the increase in atmospheric concentrations of GHGs and global warming, with a possible increase in the planet's average temperature of between 1.4 °C and 4.4 °C. Considering the historical average air temperature for the Brazilian territory evaluated by Hamada (2015), which is 25.5 °C, and disregarding locally influential factors, the values reached at the end of the 21st century would be between 25.0 °C (South region) and 31.3 °C (North region), figures that are at the upper limit or higher than those that favor the development of lettuce plants, which are between 15 °C and 25 °C (Embrapa, 2019; Sanders, 2019), possibly resulting in negative effects on cultivated plants. Hamada (2015) herself, using regionalized models, projects the average air temperature at the end of the 21st century for the Brazilian territory (average for the country's total territory) ranging from 27.1 °C to 28.4 °C. Soares (2015), on the other hand, projected the average air temperature for the same territory and the same period to vary between 27.94 °C and 29.25 °C.

The projected temperatures mentioned above can lead to a series of negative effects on lettuce plants. The results obtained in the present work reinforce this statement, with damage such as early setting, tipburn, chlorosis and leaf necrosis, and even death being observed in lettuce plants grown at 30 °C/25 °C for a period of 45 days. None of these disorders were observed for plants grown at 25 °C/20 °C for the same length of time, reflecting the genetic breeding processes aimed at tropicalizing the commercial cultivars used in this study. Table 5 shows the results obtained for three of these disorders, which proved to be the most important, namely: early setting, tipburn and leaf chlorosis. Figure 4 shows examples of the three morphophysiological disorders most frequently observed in this study.

Table 5: Main physiological disorders observed when the 11 commercial lettuce cultivars were subjected to temperature regime of 30 °C/25 °C using organic or mineral fertilization.

Cultivars	Days to setting	Standard Deviation	Tipburning	Standard Deviation	Chlorosis	Standard Deviation
Hortbio						
1	40.0	5.5	1.3	1.5	0.7	0.6
2	37.3	5.8	1.7	1.2	1.0	0.0
3	40.7	5.8	0.7	0.6	0.3	0.6
4	44.0	0.0	0.7	0.6	0.0	0.0
5	43.0	1.7	1.0	0.0	0.3	0.6
6	43.0	1.7	1.7	0.6	0.7	0.6
7	38.7	4.0	1.7	1.2	0.7	0.6
8	43.0	1.7	1.7	1.2	1.0	0.0
9	44.0	0.0	3.0	0.0	0.3	0.6
10	36.0	3.5	2.3	1.2	0.7	0.6
11	42.7	2.3	2.0	1.0	0.3	0.6
Average	41.1	2.9	1.6	0.8	0.5	0.4
Mineral Fertilizers						
1	34.7	5.0	1.7	1.2	1.0	0.0
2	33.7	9.0	2.0	1.0	1.0	0.0
3	44.0	0.0	1.0	1.7	0.3	0.6
4	38.7	4.0	1.0	0.0	0.7	0.6
5	44.0	0.0	1.7	0.6	0.0	0.0
6	35.7	7.6	2.7	0.6	0.0	0.0
7	29.0	0.0	2.7	0.6	1.0	0.0
8	41.7	2.1	1.7	1.2	0.7	0.6
9	30.0	0.0	3.0	0.0	1.7	1.2
10	30.0	3.6	2.3	1.2	0.7	0.6
11	44.0	0.0	2.7	0.6	1.0	0.0
Average	36.8	2.9	2.0	0.8	0.7	0.3



(A) Lettuce plant presented chlorosis and tipburn symptoms; (B) Lettuce plant presented tipburn symptoms; (C) Lettuce plants presented bolting symptoms.

Figure 4: Lettuce plants grown at 30 °C, showing the three main morphophysiological symptoms observed, namely: (A) Chlorosis and tipburn; (B) tipburn and; (C) early setting (bolting symptoms).

As for the type of fertilization used, statistically significant differences were found for the following attributes: MT at 30°/25°; NF at 25 °C/20 °C and 30 °C/25 °C and AL at 25 °C/20 °C and

30 ºC/25 ºC. In general, the use of biofertilizer reduced all the attributes. This is a negative aspect as it indicates lower plant development and lower productivity. However, for the plant height (AL) attribute, this reflected a lower occurrence of early setting, which may be linked to the existence of reasonable concentrations of IAA in Hortbio® (Bomfim et al., 2024).

Figure 5 shows the graphical arrangement of the means and standard deviation for the occurrence of chlorosis in the lettuce plants of the different cultivars used in this work when grown at 30 ºC/25 ºC and fertilized with Hortbio® organic fertilizer. Figure 6 shows the same results when mineral fertilization was used. Figure 7, on the other hand, shows the comparison between the averages obtained for the two types of fertilizer used.

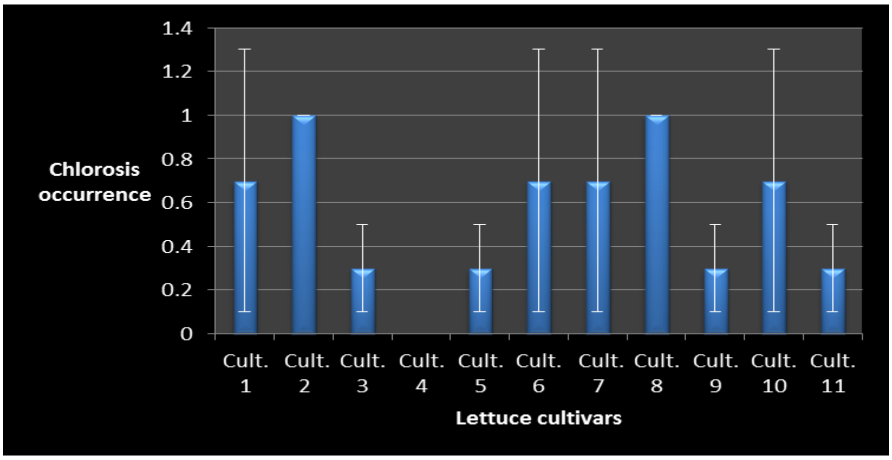


Figure 5: Average and standard deviation for the occurrence of leaf chlorosis symptoms in 11 lettuce cultivars grown under an average air temperature of 30 ºC/25 ºC and fertilized with Hortbio biofertilizer.

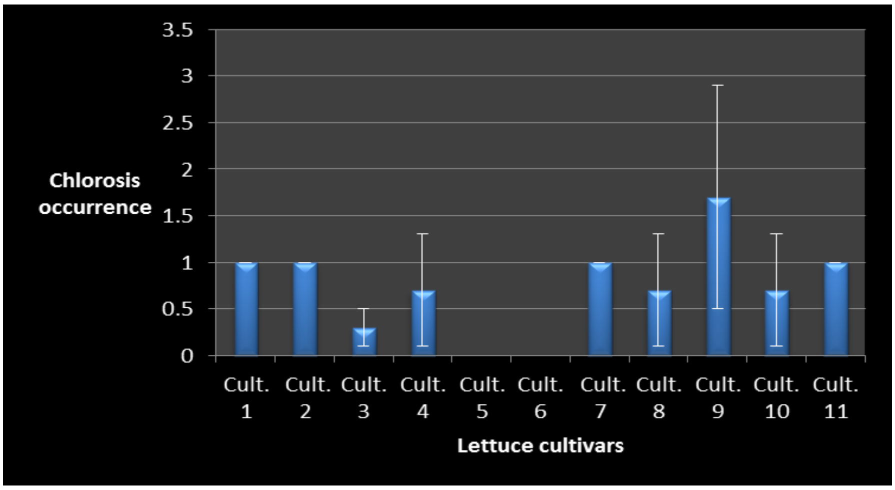


Figure 6: Average and standard deviation for the occurrence of leaf chlorosis symptoms in 11 lettuce cultivars grown under an average air temperature of 30 ºC/25 ºC and fertilized with mineral fertilizers.

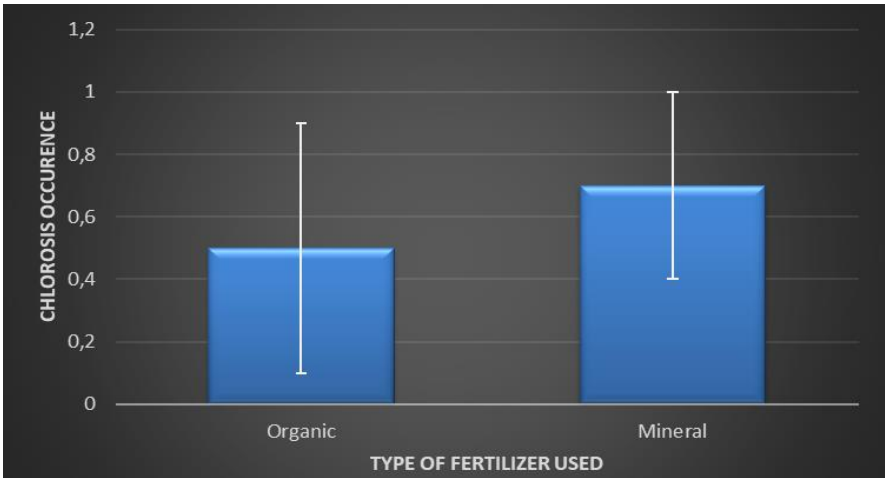


Figure 7: Mean and standard deviation for the occurrence of leaf chlorosis symptoms in lettuce plants grown at an average air temperature of 30 ºC/25 ºC and fertilized with Hortbio® biofertilizer or mineral fertilizers.

Although the mean ± standard deviation values coincide and there is therefore a great variation of data, it is possible to observe that there is a tendency for the mean values observed for the reclassification of the leaf chlorosis attribute to be lower when the plants were fertilized with Hortbio biofertilizer than when they were fertilized exclusively with mineral fertilizers. Parida & Das (2005) mention that leaf chlorosis can occur due to abiotic stress caused by salinity. Salinity can be a co-occurring factor with rising temperatures, causing damage to cultivated plants (Bolfagón et al., 2020) and, potentially, due to its greater saline potential, the use of mineral fertilizers can magnify this phenomenon. Leaf chlorosis has not been commonly cited as a response of lettuce plants to heat stress, but it was a widely occurring symptom in this study. The occurrence of leaf chlorosis in cultivated lettuce plants is also linked to nutritional deficits, especially about N deficiency (Tischer & Siqueira Neto, 2012). Both possibilities of occurrence can be indirectly linked to cultivation at high temperatures, since these can lead to an increase in water deficit which, in turn, can maximize the occurrence of saline and nutritional stresses, since these processes are highly dependent on water flow.

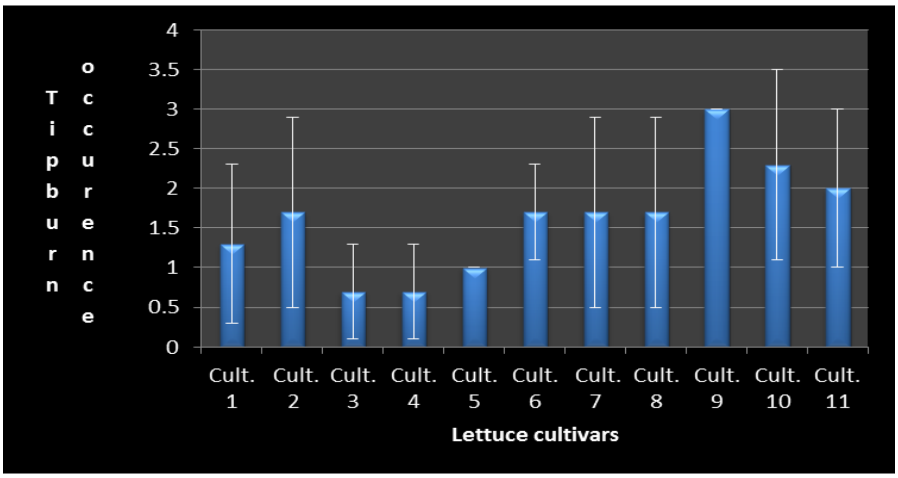


Figure 8: Average and standard deviation for the occurrence of tipburn symptoms in 11 lettuce cultivars grown under an average air temperature of 30 ºC/25 ºC and fertilized with Hortbio® biofertilizer.

Tischer & Siqueira Neto (2012) also report that the reduction or omission of N input can significantly impair the development of cultivated lettuce plants, resulting in losses in productivity, as well as the nutritional potential and yellowing of the leaves. These authors also report that, to a lesser extent, the occurrence of chlorosis can also be associated with a deficit in other nutrients, some of which are less mobile than N, such as K, Mg and S.

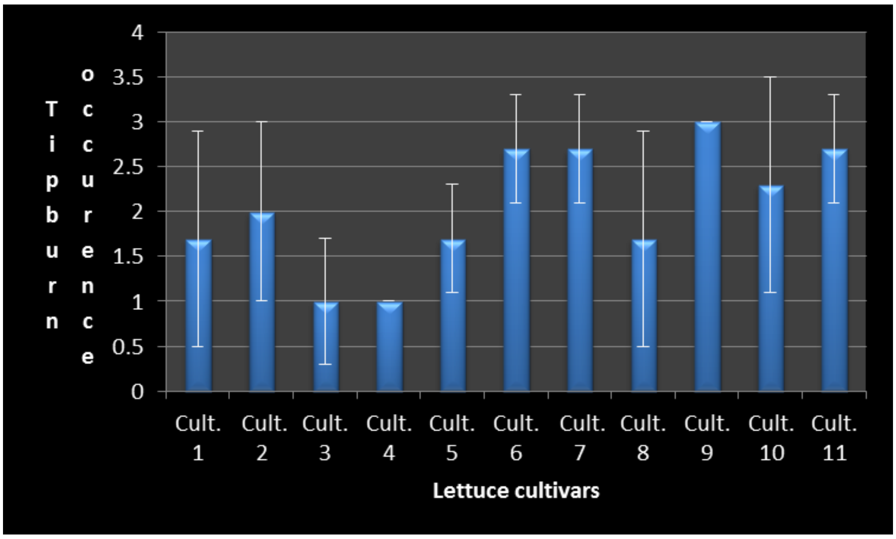


Figure 9: Average and standard deviation for the occurrence of tipburn symptoms in 11 lettuce cultivars grown under an average air temperature of 30 ºC/25 ºC and fertilized with mineral fertilizers.

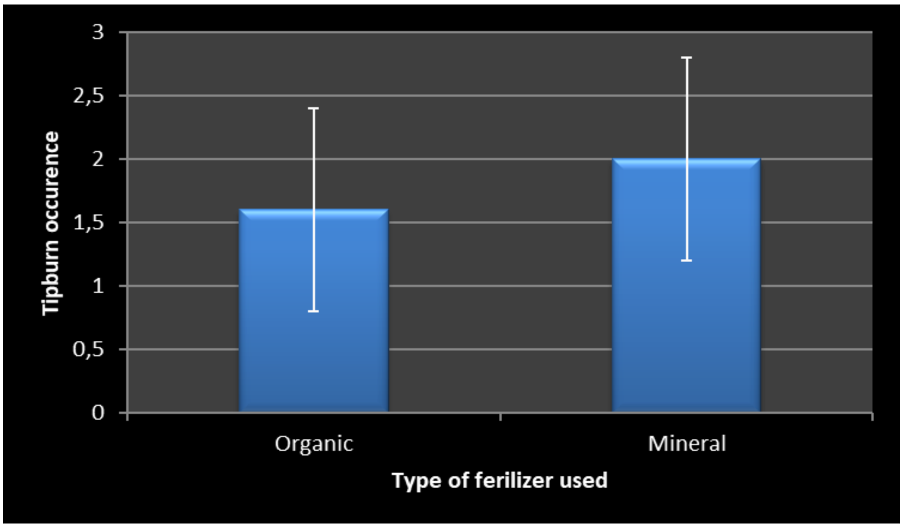


Figure 10: Average and standard deviation for the occurrence of tipburn symptoms in lettuce plants grown at an average air temperature of 30 ºC/25 ºC and fertilized with Hortbio biofertilizer or mineral fertilizers.

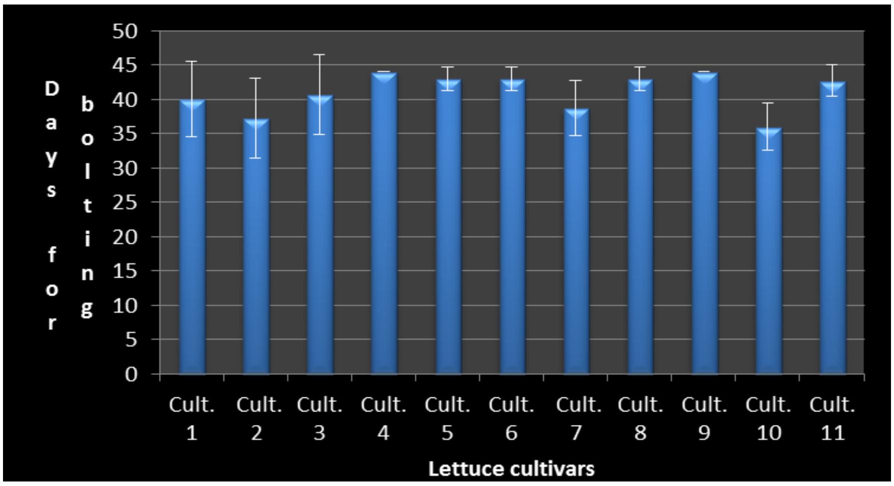


Figure 11: Mean and standard deviation for the attribute days to setting (bolting) in 11 lettuce cultivars grown under an average air temperature of 30 ºC/25 ºC and fertilized with Hortbio® biofertilizer.

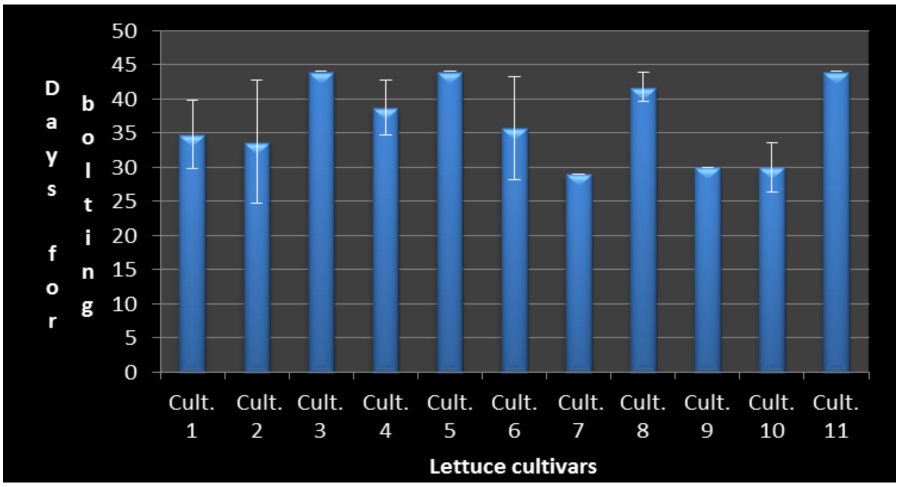


Figure 12: Mean and standard deviation for the attribute days to setting (bolting) in 11 lettuce cultivars grown under an average air temperature of 30 ºC/25 ºC and fertilized with mineral fertilizers.

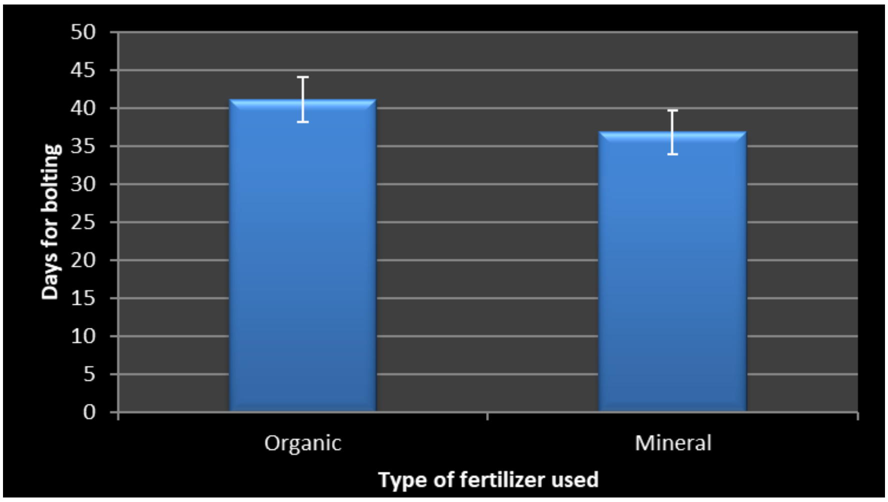


Figure 13: Mean and standard deviation for the attribute days to setting (bolting) in lettuce plants grown at an average air temperature of 30 ºC/25 ºC and fertilized with Hortbio biofertilizer or mineral fertilizers.

The same behavior observed for leaf chlorosis was also obtained for the tipburn and early setting. Figures 8, 9 and 10 show the results observed for the attribute tipburn, while Figures 11, 12 and 13 show the results obtained for the attribute days to setting, when the plants were grown under an average air temperature of 30 ºC/25 ºC. Tipburn is known to be one of the main symptoms that affect lettuce plants when they grow under high temperature conditions and occurs due to the difficulty plants have in absorbing Ca, even under conditions of high levels of this element in soils or nutrient solutions (Misaghi & Grogan, 1978; Jenny & Hayes, 2010; Jenni et al., 2013; Uno et al., 2016; Macias-Gonzalez et al., 2019). Tipburn occurs when there is localized Ca deficiency, leading to the development of necrosis at the tips and margins of the leaves. Under these conditions, the laticifers rupture with the consequent release of latex into the surrounding tissue, resulting in parenchyma collapse, occlusion of xylem elements and, finally, latex coagulation (Macias-Gonzalez et al., 2019).

Another process that commonly occurs when lettuce plants are subjected to high temperatures is early setting, causing a decline in quality and yield losses (Wang et al., 2022). Premature setting is characterized by the emission of the floral pendant before the end of the expected production cycle, and this process is very easily observed in tropical regions when non-adapted cultivars are used (Sala & Costa, 2012). Chen et al. (2018), evaluating the genetic mechanisms involved in the early setting of lettuce, found that these play a fundamental role in the occurrence of this process when affected by high temperatures, such as the role of the gene called LsSOC1, associated with the induction of flowering, in affecting the emission of the floral pendant. Han et al. (2016), on the other hand, evaluated the effects of a line with resistance to pollination (S24) and a line sensitive to pollination (S39) and found a total of 12,204 differentially expressed genes when comparing S39 x S24. These authors also found 30 differentially expressed proteins when comparing S39 x S24, as well as verifying the effect of exogenous gibberellin in promoting the setting of fruit in the two lines evaluated. These facts suggest that different genetic materials may show different resilience when subjected to the climatic conditions projected in GCCs scenarios and, therefore, could be a possible explanation for the different behavior between the cultivars evaluated. Overall, the data indicates that the earliness of the production cycle plays an important role in adapting to the temperature conditions projected in GCCs scenarios.

Early setting, as well as the occurrence of tipburn and leaf chlorosis, tended to have their negative effects mitigated when biofertilizer was used for most of the cultivars tested. Although there was no significant interaction between the factors, it seems that, for some cultivars, there is a relationship between the cultivar tested and the deleterious effects of temperature, as can be seen with cultivar 9 (Figures 8 and 9), which had no change in the presence of tipburn related to the type of fertilizer, unlike the other cultivars which showed lower values in Hortbio. Although Han et al. (2016) found linear effects between the addition of exogenous gibberellin and the increase in the occurrence of early fruit set, many other studies have pointed to the occurrence of a mitigating effect of abiotic stresses when using organic compounds, which can contain molecules that potentially promote plant growth such as auxins, gibberellins, cytokinins, abscisic acid and ethylene (Li et al., 2011; Mitler et al., 2012; Peleg & Blumwald, 2011; Bouzroud et al., 2018; Androcioli, 2019). This mitigating effect has been attributed to the presence of these molecules and also to effects such as improving water and nutrient absorption capacity, optimizing the photosynthetic and photochemical ratio, improving osmotic adjustment, promoting superior antioxidant activity (Junrami et al., (2022), as well as improving reproductive capacity, regulating plant hormones to boost their growth by improving nutrient absorption and antioxidant biosynthesis, as well as numerous osmolytes (Iqbal et al., 2023). Figure 14 shows the effect of the application of Hortbio biofertilizer® on the fruit set of lettuce plants grown at 30 °C compared to treatments that used fertigation with mineral fertilizers.

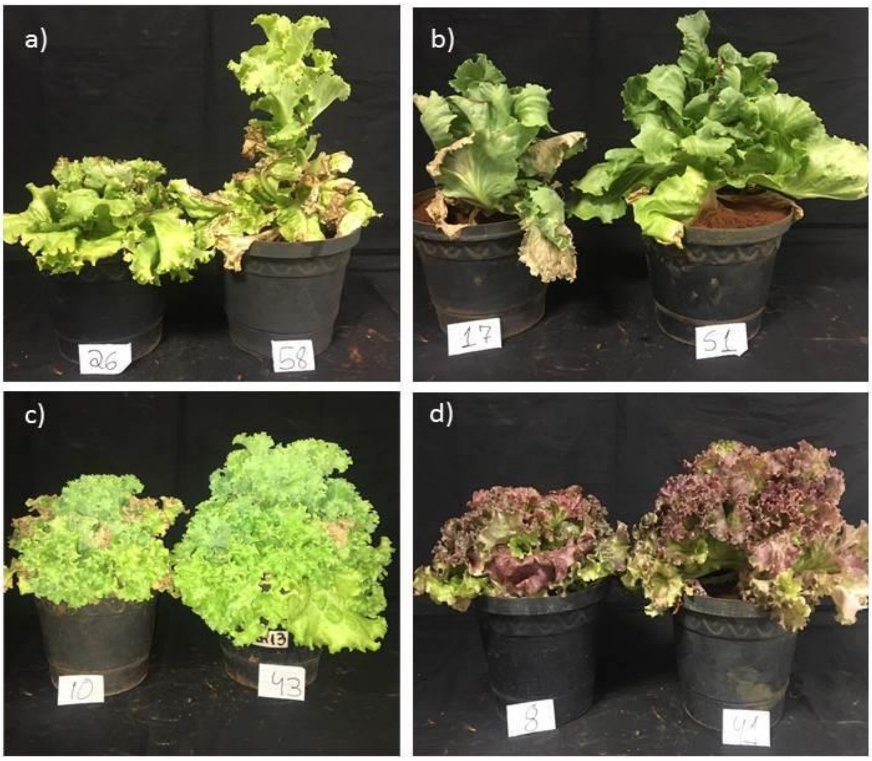


Figure 14: Examples of the response of plants grown at 30 °C/25 °C and fertigated with Hortbio® (on the left of each photo) and with mineral fertilizers (on the right of each photo). In photos a) c) and d) you can see that the plants fertigated with Hortbio® doesn't present early setting process and those fertigated with mineral fertilizers does. In contrast, b) shows a cultivar that is very susceptible to early setting, in which both treatments resulted in the emission of floral pendant.

CONCLUSIONS

Most of the commercial lettuce cultivars evaluated, which are currently available on the Brazilian market, are not adapted to the climatic conditions projected for the country. Only three of the eleven cultivars studied showed good yields when grown at 30 °C/25 °C. These cultivars contain valuable genetic material for conducting future studies, whether genetic or classical breeding, to support breeding programs aimed at developing lettuce cultivars adapted to the high temperatures projected in GCC scenarios. It seems that the earliness of the production cycle plays a fundamental role in adapting lettuce to the projected temperatures. There were indications that the use of Hortbio® biofertilizer has potential to mitigate the effects of high temperatures on the development of lettuce plants, but further evaluation is needed to better understand the mechanisms involved in this process.

Conducting work like this, which is essentially aimed at adapting agricultural crops mostly linked to small holder farming, is still a way of practicing Climate Justice, which can be seen as a concept that recognizes the unequal effects of the climate crisis on populations, often exacerbating existing social, economic and environmental inequalities. This concept is linked to historically marginalized communities, such as low-income populations, and assumes that such communities are potentially more vulnerable to the negative effects of the climate crisis. As a result, it seeks actions and public policies that can help mitigate these negative impacts and adapt to the projected scenarios, which is the aim of this work.

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