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Thermic stress response of three lettuce cultivars (*Lactuca sativa* L.) in mulched fields. An overview of the region's climate

Respuesta al estrés térmico de tres cultivares de lechuga (*Lactuca sativa* L.) en campo acolchado. Una aproximación a la realidad de la zona

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Article Data

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Abstract

In order to evaluate the agro-morphological and productive response to thermal stress of 3 lettuce cultivars (*Lactuca sativa* L.) in mulched field, an experimental plot was implemented in the community of Puerto La Boca, Puerto Cayo, Ecuador. The study factor was the effect of heat stress on 3 cultivars of curly lettuce; were housed in an experimental design of completely randomized blocks with 3 treatments (T_1 Patagonia, T_2 Starfighter, T_3 Batavia BG) and 4 replications, the effect of transplant time was blocked. Each experimental unit had 45 plants planted in 3 rows at 0.20 m between plants and 0.80 m between rows and 1.80 m between platforms. Variables such as: number of leaves, percentage of leaf area, leaf area index, head diameter, plant height, root size, weight of plant with root, weight of plant without root and flavor were evaluated. The results were; Starfighter was outstanding in the number of leaves, non-spicy flavor for consumption, while Batavia BG was notorious in the percentage of leaf area and leaf area index, root size and weight of plant with root; but its leaves were spicy. Patagonia stood out in the diameter of the head and height of the plant. It was possible to select the Starfighter cultivar as the one with the best behavior and leaf quality.

Resumen

Con el objetivo de evaluar la respuesta agro-morfológica y productiva al estrés térmico de 3 cultivares de lechuga (*Lactuca sativa* L.) en campo acolchado, fue implementada una parcela experimental en la comunidad de Puerto La Boca, Puerto Cayo, Ecuador. El factor de estudio fue el efecto del estrés térmico en 3 cultivares de lechuga crespa; fueron alojados en un diseño experimental de bloques completamente aleatorios con 3 tratamientos (T_1 Patagonia, T_2 Starfighter, T_3 Batavia BG) y 4 repeticiones, se bloque el efecto del tiempo de trasplante. Cada unidad experimental tuvo 45 plantas sembradas en 3 hileras a 0.20 m entre plantas y a 0.80 m entre hileras y 1.80 m entre platabandas. Se evaluó las variables como: número de hojas, porcentaje de área foliar, índice de área foliar, diámetro de la cabeza, altura de planta, tamaño de raíz, peso de planta con raíz, peso de planta sin raíz y sabor. Los resultados fueron; Starfighter, fue sobresaliente en el número de hojas, sabor no picante para el consumo, en cambio Batavia BG fue notorio en el porcentaje de área foliar e índice de área foliar, tamaño de raíz y peso de planta con raíz; pero sus hojas fueron picantes. Patagonia resaltó en el diámetro de la cabeza y altura de planta. Se logró seleccionar al cultivar Starfighter como la que tuvo mejor comportamiento y calidad de hojas.

Introduction

Lettuce (*Lactuca sativa* L.) is considered the most important leafy vegetable worldwide due to its wide acceptance in human food and its nutritional value[1,2]. Its consumption is closely linked to the preparation of salads, in which the various flavors and textures of its leaves are used. From a nutritional point of view, it stands out for its high fibre content and low energy intake[1,2]. This species originates in Europe and Southwest Asia, and develops optimally in ranges of (temperature between 7 and 25° C)[3-6].

However, when the temperature exceeds 30° C, morphological alterations are observed such as the thinning of its leaves, and elongation of internodes; reducing its commercial value. In addition, heat stress can lead to discoloration of the veins, burns at the tips, premature heading and leaf deformations[7-9]. It has been reported that, under extreme heat conditions, plant biomass and leaf morphology change significantly: leaves and stems become elongated, and leaves become narrower[7]. Variations in the content of chlorophyll and osmoprotective compounds (proline and soluble sugars) were also evidenced, which decrease in initial stages and increase later. At the same time, the activity of antioxidant enzymes such as catalase, peroxidase and superoxide dismutase, as well as the levels of malondialdehyde, followed similar patterns[10].

Beyond the physiological aspects, global warming represents a growing threat to lettuce crops. Among the most damaging effects are extreme heat and drought, which compromise agricultural productivity[11]. The Intergovernmental Panel on Climate Change (IPCC) projects an increase of 0.2° C per decade in global air temperature, reaching an additional (1.8 to 4.0° C by 2100)[12]. In this scenario, the development of improved and heat-tolerant cultivars becomes essential to maintain production in increasingly demanding environments.

Photosynthesis, a highly complex system whose purpose is to provide energy, organic matter for growth, development and determines the yield of a crop; however, light and temperature are the vital factors that affect this process, such as the activities of carbon assimilation enzymes, opening of stomata, accumulation of metabolites and the pigment composition of cells[13-17].

Lettuce, like other vegetables, is affected by environmental changes, which makes them unprofitable, hence abiotic stress due to high temperatures (thermal stress ST), limits their quantity, quality, nutritional status, and production[18,19].

TS also affects the quality of vegetables, due to a change in color, texture of the fruit, for example in cucumber, pepper and tomato, affecting the nutritional status of vegetables, such as the reduction of lycopene in tomatoes, and β-carotene in spinach and lettuce, and increases the levels of nitrates, which are harmful for human consumption[20-22].

In Ecuador, various types of lettuce are grown, such as Batavia, mullet, romaine, Baby leaf and smooth and curly foliar varieties in green, red and purple tones[23-28]. The main producing areas include the provinces of Azuay, Tungurahua, Pichincha and Loja, where temperatures range between 15 and 20° C, ideal conditions for their development[29]. However, it is necessary to evaluate new lettuce cultivars

at different planting times and production systems, especially in coastal areas such as Puerto la Boca, in southern Manabí, where average temperatures exceed 20° C and genetic material with greater tolerance to heat is required[30].

In this context, the present research aimed to evaluate the agro-morphological and productive response of three lettuce cultivars to heat stress in the commune of Puerto la Boca, Jipijapa, Ecuador.

Materials and methods

Geographical location. The research was carried out in the Puerto La Boca Campus of the Puerto Cayo Parish of the Jipijapa Canton, located at 1°18'20" South latitude and 80°45'42" West longitude, at an altitude of 53 meters above sea level. The average temperature was 24.8° C/year and the average rainfall is 298 mm/year, with rainfall concentrated in the month of February and the driest month in August[31]. This research was carried out between the months of July and August 2022.

Study factors. The study factor was the response to TS in three cultivars of curly lettuce. The seeds were acquired from the company Agroser, Ecuador of the Rijk Zwaan brand[32].

Treatment. The treatments applied are indicated in Table 1 below.

Table 1 Treatments applied to three hybrid lettuce cultivars

Code	Treatment
T_1	Patagonia
T_2	Starfighter
T_3	Batavia BG V3

Experimental design. The experimental plot was implemented in an experimental design of completely randomized blocks (DBCA) with 4 replications and 3 treatments[33], the effect of transplant time was blocked. Each experimental unit (UE) had 45 plants planted in 3 rows at 0.20 m between plants and 1.80 m between platforms. There were 180 plants for the 4 repetitions. The number of plants for the entire experiment was 540 plants. The central lines were evaluated, which corresponded to 15 plants of each cultivar by repetition.

Statistical analysis. Once the assumptions of normality and homogeneity of variances were met, and based on the defined model, analyses of variance (ANVA) were performed to test hypotheses about the fixed effects, as well as comparisons of means of the treatments using the Tukey test ($P<0.05$). The ANVA was also used to estimate the variance components for random effects. All analyses were performed with infoStat software[34].

Response variables. i) *Number of sheets* (NDH). The total per plant was evaluated. ii) *Percentage of leaf area* (PAF). It was evaluated every 15 days using the Canopy Cover Free application from the Play Store[35]. iii) *Leaf area index* (FAI). It was calculated using the formula suggested by Hunt[36]. iv) *Head diameter* (cm) (DDC). This variable was measured every 15 days from head formation to harvest day with a caliper or caliper[37]. v) *Plant height* (cm) (AP). It was started at 15 days after transplanting until harvest, measured from the base to the top of the head with a tape measure[38]. vi) *Root size* (cm) (TDR). It was evaluated with a tape measure in cm. vii) *Weight of plant with root* (kg) (PPCR). Once the harvest was done, when the head of the lettuce was compact, each of the heads with their roots was weighed using an analytical balance in kg[37]. viii) *Weight of unrooted plant* (kg) (PPSR). When the head of the lettuce was compact, each of the heads was weighed, without their roots, using a gram scale in kg[37]. ix) *Taste*. This qualitative variable was determined by consuming the lettuces fresh, determining whether it

was spicy or not.

Management of the investigation. The experimental plot was implemented in the field in an area of 134 m^2. The soil was removed using a rototiller, then crumbled to obtain finer particles for the development of seedlings on the platforms. Organic matter (biocompost) was applied to give the plants adequate soil at the time of transplanting. Biocompost was applied at a rate of 75 kg per 33 m row. The ground was measured with the help of a tape measure, for the formation of the platforms of 0.80 m wide by 33 m long and a height of 0.15 m, finally, the leveling of the platforms was carried out.

The substrate was prepared with biocompost, guava leaf and local soil, in a 2:1:1 ratio. 10 kg of humus and 1 bag (10 g) of mycorrhiza were put in to prevent the attack of pathogens that cause *damping off*. Once the substrate was prepared, the holes were filled with it, taking care to moisten it. The seeds of the cultivars were then sown in these trays. The trays were watered two times a day to maintain humidity.

The final transplant was carried out in rows, for which holes were made with a depth of 0.15 m at a distance of 0.20 m between plants within the row, then one plant per hole was transplanted. A coat of worm humus (50 g) was added to the transplant to encourage root development. The platforms were covered with a plastic mulch in order to keep the soil temperature stable, control weeds, conserve moisture and prevent the loss of nutrients through leaching and evaporation.

Disease control was preventive according to the history of disease presence in the area. To control mildew caused by oomycete *Bremia lactucae* and other leaf spots, Metalaxyl + Mancozeb (Ridomil) (2.5 g L^{-1}) was applied alternated with Chlorothalonil (2.5 mL L^{-1}), *Trichoderma* (3 mL L^{-1}) and *Bacillus subtilis* (3 mL L^{-1}) from the eighth day after transplantation[39].

Pest control was carried out according to the monitoring and application of the damage threshold for the control of insect pests such as whiteflies (*Bemisia tabaci*), bold (*Prodiplosis longifila*) and aphids (*Myzus persicae*), the application of Thiamethoxan (0.25 mL L^{-1}) was used, alternating with abamectin (2.25 mL L^{-1}), Confidor (0.60 g L^{-1}) and Neen (4 mL L^{-1}).), starting 10 days after transplantation[40].

The plants were watered using the drip irrigation system and the frequency was 2 to 3 times a day, depending on the temperature.

Harvesting was done from age to 40 after transplanting.

Results

Table 2 Analysis of variance for cultivars

FV	gl	Medium squares				
		NDH	PAF	IDAF	DDC (cm)	AP (cm)
Rep	3	4.81**	218.29**	110.46ns	2.00ns	12.86*
Cultivar	2	12.14**	334.60**	358.67**	17.64ns	19.49**
Date	2	91.26**	4055.05**	7449.69**	806.29**	418.77**
Cultivate*Date	4	1.07ns	45.49ns	4.62ns	9.48ns	5.09ns
Error	24	.62	30.39	57.35	5.98	3.44
Total	35					
CV		7.74	10.54	12.14	8.48	10.02

*: significant at P<0.05, **: highly significant at P<0.01. ns: not significant, NDH: number of leaves, PAF: percentage of leaf area, IDAF: leaf area index, DDC: head diameter, AP: plant height, IDAF: índice de área foliar, DDC: diámetro de cabeza, AP: altura de planta

The analysis with the Shapiro - Wilks test (P<0.05) denoted normal distribution of the evaluated data of the variables NDH, PAF, IAF, DDC and AP, TDR, PPCR, and PPSR. The analysis of homogeneity of variances using the Levene test (P<0.05) indicated that the variables NDH, PAF, IAF, DDC, AP, TDR, PPCR and PPSR were homogeneous.

These analyses suggested the continuation of ANVA and the comparison of means of treatments.

Analysis of agro-morphological and productive variables. The ANVA for cultivars presented highly significant differences (P<0.01) (Table 2), for the variables NDH, PAF, IDAF, DDC, AP, with coefficients of variation (CV) of 8 to 12 %, which are within the ranges allowed for this type of research. The interaction Cultivate * Date was not significant (P<0.05).

The ANVA for cultivation of TDR, PPCR and PPSR, indicated that there were highly significant (P<0.01) and significant (P<0.05) differences, with CV between 7 and 12 %.

Table 3 Analysis of variance for productive variables

FV	gl	Medium squares		
		TDR (cm)	PPCR 8 (g)	PPSR (g)
Rep	3	0.07ns	38.44ns	2316.50ns
Cultivar	2	2.86**	10823.56*	5666.64*
Error	6	.19	1101.46	1122.68
Total	11			
CV		6.97	9.72	11.76

*: significant at P<0.05, **: highly significant at P<0.01, ns: not significant, TDR: root size, PPCR: weight of plant with root, PPSR: weight of plant without root.

The comparison of means using Tukey's test (P<0.05) (Table 4), indicated that the Starfighter cultivar was outstanding for the NDH, while Batavia BG was notorious in the PAF and IDAF and Patagonia stood out in the DDC and AP.

Tukey's test (P<0.05) indicated that Batavia BG was outstanding for TDR and PPCR (Table 5); and all cultivars showed the same behavior for PPSR. Batavia BG developed well, but when the leaves were consumed, they were spicy, which gave it an unpleasant taste to the palate. The Starfighter cultivar had better behavior and leaf quality (not spicy).

Table 4 Analysis of means of agro-morphological variables

Variety	NDH	PAF	IDAF	DDC (cm)	AP (cm)
Starfighter	**11.36 a**	53.18 b	56.75 b	27.37 a	17.25 b
Batavia BG	9.61 b	**61.91 a**	**66.18 a**	28.75 a	17.91 ab
Patagonia	9.40 b	60.64 a	61.21 ab	**29.39 a**	**19.68 a**
DSH	.80	6.41	.94	2.50	1.89

Means with a common letter are not significantly different (P<0.05). NDH: number of leaves, PAF: percentage of leaf area, IDAF: leaf area index, DDC: head diameter, AP: plant height.

Table 5 Analysis of means of productive variables

Variety	TDR	PPCR (g)	PPSR (g)
Starfighter	5.92 b	304.11 b	255.13 a
Batavia BG	**7.17 a**	**394.11 a**	323.17 a
Patagonia	5.59 b	318.84 b	272.67 a
DSH	.94	72.00	72.70

Means with a common letter are not significantly different (P<0.05). TDR: root size, PPCR: weight of plant with root, PPSR: weight of plant without root.

Discussion

In Riobamba and Cotopaxi, many lettuce cultivars have been introduced[41-44], some of which adapted to the environmental and soil conditions of these sites. In this research we have evaluated crepe lettuce, which was not evaluated under the coastal conditions (South Zone of Manabí), particularly in the commune of Puerto La Boca.

Chacha Barba et al.[44], evaluated the morpho-physiological and productive behavior of two lettuce cultivars, in hydroponic and conventional field systems. The cultivars evaluated were Cherokee Rz (81 - 36) and Starfighter Rz (81 - 85). The results of this study indicated that Starfighter was outstanding with an average of 11 leaves in a hydroponic system, 724 cm^2 of PAF and 8272 kg ha^{-1} of yield. In our study, 11 leaves per plant were obtained, a PAF of 728 cm^2 (53 %) per plant, with a DDC of 27.37 cm per plant and 15.946 kg ha^{-1} yield. Rojas Hidalgo[30], applying fertilizers such as calcium phosphonate, increased the NDH in the cultivar Great Leakes 659, obtained at harvest 16.4 leaves on average. López Carhuanta[45], applied calcium-boron phosphonate achieving an increase of 22 leaves.

It was notorious that the Batavia and Patagonia cultivars had high yields, but when consumed they were spicy and not pleasant to the palate. This is possibly due to the water stress they suffered during the development of the crop[44,46], and the effects of high temperatures[47]. These conditions, however, did not affect the Starfigther cultivar, which was suitable for consumption, remaining fresh and green. Although severe abiotic stress damages plant growth and development[48], applied at moderate levels it can stimulate the synthesis of beneficial compounds for health without negatively affecting crop productivity[48]. There is evidence that plants subjected to moderate abiotic stress have a greater tolerance to subsequent stresses, such as those derived from postharvest storage[46,49]. This effect is due to the metabolic memory induced in the first stress inflicted. The deliberate application of moderate stresses, with the aim of tolerating subsequent stresses[49,50].

It should be mentioned that the average annual temperature in Puerto la Boca is 24° C, which indicates that species in general must be adapted to high temperatures[7,47]. The Starfighter cultivar has a wide range of adaptation[32], this was noticeable in this experiment, planted in summer; this cultivar had a behavior for fresh consumption. However, we consider that it should also be evaluated in the winter season to study its behavior and adaptation at another time.

Due to climate change, the limitation of natural water resources for food production has become a real problem for agriculture[10,11]. For this reason, various efforts have been made to seek technological strategies to reduce the use of water resources in food production [51.52]. In this study we have selected new cultivars of curly lettuce that tolerate heat stress and are a reason for the search for alternatives to changes in air temperature and respond in the generation of leaf foods, such as lettuce, and these can be extrapolated to other types of cultivars.

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Conflicts of interest

The authors declare that this research was carried out at the State University of the South of Manabí (Jipijapa Canton) and does not present conflicts of interest.

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Ethical considerations

The approval of the research by the Directorate of Research and Graduate Studies, the Ethics Committee, and the Research Committee of the Agricultural Career of the State University of Southern Manabí (UNESUM), (Jipijapa Canton), followed the guidelines established by these instances.

Limitations in the research

The authors point out that there were no limitations in the present research work.

Authors' contribution

Julio Gabriel-Ortega, planning of the experiment, statistical analysis, systematization, syntax, grammar and interpretation of the information. *Génesis Baque López*, data collection, systematization and interpretation of information. *Gema Burgos López*, transcription, systematization and revision of the document. *Bolívar Mendoza Marcillo*, syntax, spelling, and revision of the document.

Access to data

The data and information of this research are present in the article.

Consent for publication

The authors, after reviewing the document, are considered approved for publication.

Use of Artificial Intelligence

We assume that the entire document was written based on ethical and professional criteria, and AI was not used to make the images or text.

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