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INFLUENCE OF THE CULTIVAR, FERTILISER, AND IRRIGATION ON ICEBERG LETTUCE MORPHOLOGY - SINGLE FACTOR VS INTERACTION EFFECTS

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Abstract

This study aimed to examine the effect of cultivar, fertiliser and irrigation, and their combined effect, on different lettuce agronomic parameters and the possible occurrence of tipburn. Two iceberg lettuce cultivars ('Umbrinas' and 'Kavir') were cultivated during summer in the open field, in the black marsh soil containing a wide calcium-to-magnesium ratio. Plants were grown at a density 32×32 cm with regular agricultural practices. Three different levels of magnesium sulfate fertiliser (100 ml, 300 ml, 500 ml solution) were added twice during the growing cycle. Plants were irrigated using a drip irrigation system and sprinklers. After harvest morphological parameters were estimated (head weight, height, diameter, number of leaves, fresh leaf weight, stem height, diameter, and fresh weight). Cultivar 'Umbrinas' showed the highest value of all parameters: head weight (557.15 g), head height (13.91 cm), head diameter (16.90 cm), number of leaves (18.73), fresh leaf weight (527.50 g), stem height (65.73 mm), stem diameter (20.32 mm), and stem fresh weight (29.65 g). Application of all levels of magnesium sulfate fertiliser did not affect observed parameters compared to the control. Drip irrigation led to significantly higher head height, diameter and number of leaves, while sprinkler irrigation significantly enhanced the values of all stem parameters. Tipburn was noticed mainly in the 'Umbrinas' cultivar in the sprinkler irrigation treatment, whereas drip-irrigated plants showed no signs of tipburn. Interaction of all three factors showed a statistically significant impact on head weight, number of leaves, fresh leaf weight, and stem height.

Keywords: *Lactuca sativa*, *Magnesium sulfate fertiliser*, *Drip irrigation*, *Sprinkler irrigation*, *Morphology traits.*

Introduction

Lettuce (*Lactuca sativa*, L.) is an annual leafy vegetable crop from the Asteraceae family. It has wide morphological viability, classified into several types (butterhead, iceberg, leaf, romaine, Batavia, and stem). It is rich in health-beneficial secondary metabolites, mainly phenolic compounds, carotenoids, and folate, even though it has low nutritional value (Ćavar Zeljković et al., 2023). Iceberg lettuce is usually used as a fresh-cut or minimally processed leafy vegetable, with thick leaves, a specific crisp texture, and neutral to a slightly bitter flavour. The advantage of growing lettuce is quick and all-year-round production in different systems (open field, covered systems or hydroponics) with the possibility of multiple growing cycles. It is a cool season crop with optimal temperatures of 21-23 °C (day) and 15-18 °C

(night). During the summer, daily temperatures usually exceed 30 °C which can raise the risk of bolting (Ilić et al., 2017). Early bolting leads to a shortened vegetative phase, decreased biomass, poor head formation, bitter taste, and consequently plants lose marketable value (Lafta et al., 2021). Magnesium (Mg) as a secondary nutrient is important for a wide range of processes in plants such as photosynthesis, nutrient metabolism, cell membrane stability, enzyme activation, and resilience against various stressors (Ahmed et al., 2023). The BCSR (Basic cation saturation ratio) concept is based on the existence of an optimum saturation of the soil cation exchange capacity, particularly cations Ca, Mg, and K, and that optimum ratio will lead to maximum yields (Bear et al., 1945). On the contrary, research by McLean et al. (1983) suggested that for maximum crop yields emphasis should be undertaken on providing adequate levels of each basic cation rather than trying to modulate the optimal ratio. Antagonism between Ca and Mg can occur both on a soil and plant tissue level, and high concentrations of each of the elements can interrupt the acquisition of the other. Calcium (Ca) is considered a relatively immobile element within plant tissue. The transpiration rate usually regulates the uptake and movement of water and Ca where rapidly transpiring outer leaves gain more water and Ca compared to inner leaves. Environmental factors that promote rapid growth, modify the transpiration rate and change the balance of Ca distribution favour the occurrence of tipburn (Sago, 2016). Tipburn is mainly recognised as a calcium-associated physiological disorder visually seen as the browning of leaf margins which reduces the quality and shelf life of fresh and processed products. Proper irrigation management can have an impact on lettuce yield, head size and uniformity, chemical content, and browning-related enzymes (Luna et al., 2012). Drip irrigation has several advantages compared to sprinkler irrigation in a manner that it reduces water usage and loss caused by evaporation, decreases soil erosion, leakage of fertilisers and pesticides, and availability of water for the weeds.
Therefore, this study aimed to examine the effect of cultivar, fertiliser and irrigation, and their combined effect, on different iceberg lettuce agronomic parameters, and the possible occurrence of tipburn during summer production.

Materials and methods

Two iceberg lettuce cultivars were studied 'Umbrinas' (Rijk Zwaan, the Netherlands) and 'Kavir' (Meridiem Seeds, Spain). Lettuce seeds were sown on July 23 in peat cubes size 4 cm in the substrate Potgrond H (Klasmann-Deilmann, Germany) in a glasshouse condition in the company Grow Rasad (Serbia). Plants were cultivated in the summer open-field experiment from August to October 2018 in the company Iceberg Salat Centar (Surčin, Serbia). Seedlings were transplanted on August 11, manually in the black marsh soil. Before the experiment, randomised soil samples were taken at 0-30 cm depth. A pH was determined potentiometrically (SRPS EN ISO 10390, 2022), Ca and Mg using extraction with molar ammonium acetate solution (ISO/TS 22171, 2023), total nitrogen was measured with dry combustion (SRPS ISO 13878, 2005), readily available phosphorus and potassium were determined using AL method (Egnér et al., 1960), and volumetric method for $CaCO_3$ (SRPS ISO 10693, 2005). Regular agricultural measures were applied (weed hoeing, irrigation, and preventive protection against diseases and pests). The experiment was arranged in a complete block design using Mg sulfate fertiliser (Yara Tera KRISTA, MgS, Norway) where 30 g was dissolved in 15 l of water and added twice during the growing cycle. Fertiliser was applied in three different levels (100 ml, 300 ml, 500 ml), and control (without fertilisation). Plants were irrigated using a drip irrigation system (T-Tape 506-10-1350, Rivulis, Israel) and sprinklers (type 501-U, NaanDan Jain, Israel) 11 times during the vegetation period. After transplanting, firstly we used sprinklers, and after successful rooting, plants were irrigated using two systems with the same quantity of water. The dimension of the plots was 1.3 × 2 m, in three

replications, the distance between plants was 32 × 32 cm, 50 cm among repetitions, and 100 cm among treatments. Lettuce plants were harvested 55 days after transplanting when heads were fully matured and reached commercial size. Agronomic parameters were measured by taking 6 plants per treatment using a scale, digital caliper, and ruler. The presence of the leaves affected by the tipburn was analysed during the morphological measurements. Statistical analysis was performed using a three-way ANOVA with a Tukey's test for post hoc comparison. All results were calculated at a significance level α of 0.05 using the software SPSS Statistics (Version 25.0.; Armonk, NY, USA: IBM Corp) and Microsoft Office Excel 2019 (Microsoft Corp., USA). During the growing cycle air temperature, air relative humidity, and precipitation were collected using RC-4HC Data Logger (Elitech Technology Inc., USA) and rain gauge (TFA Dostmann GmbH & Co. KG, Germany**)** (Table 1). Plants were exposed to different photoperiods from 14 h 16 min to 11 h 35 min.

Table 1. Climatic data during the iceberg lettuce growing cycle

	Average air temperature (°C)	**Maximum air temperature (°C)**	**Minimum air temperature (°C)**	**Average air humidity (%)**	**Total precipitation (mm)**
August 2018	23.7	34.7	14.2	66.0	19.0
September 2018	19.2	33.5	2.9	62.9	12.1
October 2018	12.4	23.3	3.0	71.2	1.0

Results and discussion

Cultivar showed significant influence on all tested morphological parameters except head diameter (Table 2).

Table 2. Main and interaction factors effects affecting morphological traits in iceberg

	Head height (cm)	**Head diameter (cm)**	**Head weight (g)**	**Number of leaves**	**Fresh leaf weight (g)**	**Stem height (mm)**	**Stem diameter (mm)**	**Fresh stem weight (g)**
Main factors								
Cultivar								
Umbrinas	13.91±0.38 **b**	16.90±0.37	557.15±18.99 **b**	18.73±0.78 **b**	527.50±18.14 **b**	65.73±4.81 **b**	20.32±0.80 **b**	29.65±2.62 **b**
Kavir	12.07±0.36 **a**	16.76±0.42	510.83±16.68 **a**	14.38±0.66 **a**	493.00±16.31 **a**	37.79±3.47 **a**	18.60±0.68 **a**	17.83±2.25 **a**
Fertiliser								
Control	12.77±0.32	16.72±0.42	542.71±13.07	16.50±0.91	519.96±12.12	48.90±3.31	20.03±1.10	22.75±1.79
100 ml	13.15±0.39	16.96±0.45	543.88±23.08	15.79±0.68	519.17±22.41	53.47±3.95	19.12±0.73	24.71±2.67
300 ml	12.95±0.34	16.74±0.32	521.33±15.10	17.04±0.66	498.96±15.21	48.88±3.99	19.24±0.57	22.38±2.38
500 ml	13.09±0.41	16.90±0.39	528.04±20.09	16.88±0.65	502.92±19.16	55.79±5.32	19.45±0.55	25.13±2.91
Irrigation								
Sprinklers	12.73±0.40 **a**	16.49±0.38 **a**	538.27±17.32	12.63±0.62 **a**	510.19±17.37	55.03±4.16 **b**	20.20±0.89 **b**	28.08±2.86 **b**
Drip irrigation	13.25±0.34 **b**	17.17±0.41 **b**	529.71±18.35	20.48±0.82 **b**	510.31±17.08	48.49±4.13 **a**	18.72±0.59 **a**	19.40±2.02 **a**
Significance								
Cultivar (C)	***	ns	***	***	***	***	**	***
Fertiliser (F)	ns	ns	ns	ns	ns	ns	ns	ns
Irrigation (I)	**	**	ns	***	ns	**	**	***
Interaction factors								
C × F	ns	ns	***	ns	***	**	***	*
C × I	***	ns	*	*	*	ns	ns	ns
F × I	*	*	**	***	**	ns	ns	ns
C × F × I	ns	ns	*	***	*	**	ns	ns

The data show the means (n = 6) ± SE. Values followed by the same letter are not significantly different at the 0.05% level of probability according to Tukey's test. Groups of data within the same parameter and factor with no letters are not different from each other. Asterisks indicate significant differences at * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns, non-significant.

The results showed the highest value of head weight (557.15 g), head diameter (16.90 cm), head height (13.91 cm), number of leaves (18.73), fresh leaf weight (527.50 g), stem height (65.73 mm), stem diameter (20.32 mm), and stem fresh weight (29.65 g) in cultivar 'Umbrinas'. Head weight is one of the most important traits impacting the lettuce yield. Although 'Umbrinas' was suggested as a cultivar for spring cultivation, during our trial this cultivar exhibited the highest head fresh weight compared to the summer cultivar 'Kavir'. On the other side, 'Kavir' showed lighter and smaller stems, which is a crucial point for the processing industry, by obtaining a higher proportion of edible parts and less waste. Research by Rouphael et al. (2017) showed a significant genotypic influence of different iceberg cultivars on the head fresh mass. Furthermore, in the same study cultivar 'Umbrinas' obtained a lower head fresh weight (485 g) compared to our trial (557.15 g). The head size is a considerable parameter for the consumer's choice since cultivars with longer and larger heads are preferred and recognised as good marketable heads of high quality (Maboko and Du Plooy, 2008). Our results for head diameter are in line with or even higher compared to 25 crisphead lettuce cultivars examined by Lafta et al. (2021). Number of leaves obtained in our experiment was lower compared to trial with iceberg lettuce in hydroponics (Maia et al., 2024).

Stem fresh mass is determined by stem length and diameter in the sense that longer and larger stems are heavier. According to Yuri et al. (2004) stems up to 6 cm are the most favorable for the processing industry, while values up to 9 cm are still acceptable, whereas values higher than that are usually not recommended. Our results for both cultivars showed acceptable values of stem height (3.78; 6.57 cm, Table 2). Cultivar 'Umbrinas' showed 1.7 times longer stem than 'Kavir' which could mean that this cultivar is susceptible to high temperatures during the growing cycle. Likewise, our results for stem diameter are in line with the results of Maia et al. (2024) under hydroponic cultivation in a conventional greenhouse in the summer.

Application of all levels of Mg sulfate fertiliser did not affect any of the observed morphological parameters (Table 2). Nevertheless, the application of 100 ml solution of fertiliser obtained the highest head weight, height and diameter, while 500 ml solution contributed to the highest stem fresh weight and height, and number of leaves but without statistically significant difference. Preciado-Mongui et al. (2023) found an optimal fertilisation formula for lettuce using nitrogen and Mg fertiliser to optimise yield and balanced nutrition. Soil analysis in our experiment showed a neutral to slightly alkaline reaction (pH (H_2O)- 7.6, pH (KCl)- 7.1), very high concentration of Ca 12.375 mg/kg, and high concentration of Mg 726 mg/kg, wide range ratio Ca/Mg (10.23), and adequate levels of macronutrients (total nitrogen-0.19%; readily available phosphorus-26.67 mg/100 g; readily available potassium-18.13 mg/100 g, and 2.82% $CaCO_3$). Results of Ca and Mg, and their ratio, which means Mg ion deficiency, while pH was in the acceptable range for availability of various nutrients (Hazelton and Murphy, 2016). Mg fertiliser was applied twice but possibly with a low dosage of Mg. There is a possibility that with an increased quantity, applied during basic tillage and the vegetation period, continuously, it can be expected to lower the current Ca/Mg ratio to a more balanced level. Literature data are not consistent about the significance of the Ca/Mg ratio. Regression analysis showed no relationship between soybean and corn yields and soil Ca/Mg ratio (Leiva Soto et al., 2023). Research by Zhang (1999) showed that both soil Ca/Mg ratio and plant Ca/Mg ratio affected lettuce yield. The lower critical level of the soil Ca/Mg ratio was 0.5 and within a range from 0.50 to 7.70, no reduction of the yield was observed.

Drip irrigation system led to significantly higher head height and diameter, number of leaves, while irrigation with sprinklers led to significantly higher stem parameters. Type of the irrigation did not influence the head weight. According to Kaniszewski et al. (2017) drip irrigation system gave better results compared to sprinkler irrigation.
Tipburn was noticed mainly in the 'Umbrinas' cultivar in the sprinkler's irrigation treatment, whereas drip-irrigated plants showed no signs of tipburn (Table 3).

Table 3. Number of lettuce plants affected with tipburn after harvest

Cultivar	Umbrinas		Kavir	
Irrigation	Sprinklers	Drip irrigation	Sprinklers	Drip irrigation
Fertiliser	**Number of plants affected by tipburn**			
Control	2	0	0	0
100 ml	1	0	0	0
300 ml	1	0	0	0
500 ml	0	0	1	0

Application of 500 ml solution resulted in no tipburn in the 'Umbrinas', while in the 'Kavir', the same level of solution led to the appearance of tipburn in 1/6 analysed plants. Results of Kuronuma et al. (2022) with tipburn incidence and calcium acquisition in lisianthus showed that tipburn can be significantly decreased with increasing Mg level in two of three cultivars.
Even though the influence of fertilisation was not statistically significant on particular morphology traits, three main factors conjointly affected head weight, number of leaves, fresh leaf weight, and stem height, in a way that cannot be predicted by testing just a single factor.

Conclusion

The presented study showed that the cultivar influenced all tested parameters except head diameter. Cultivar 'Umbrinas' showed the highest value of all morphological parameters. On the other side, 'Kavir' showed more favourable values of the stem measurements compared to the head parameters relation which is important for the processing industry, especially during early and mid-summer cultivation to avoid raw material and economic losses. The application of magnesium sulfate fertiliser did not affect the tested parameters. Drip irrigation led to significantly higher head height, diameter and number of leaves, while sprinkler irrigation significantly enhanced the values of all stem parameters. Tipburn was noticed mainly in the 'Umbrinas' cultivar in the sprinkler's irrigation treatment.The application of 500 ml Mg solution resulted in no tipburn in the 'Umbrinas', while in the 'Kavir', led to the appearance in 1/6 analysed plants. Nevertheless, the interaction of all three factors showed a statistically significant impact on head weight, number of leaves, fresh leaf weight, and stem height.

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