

Article

EC Sensitivity of Hydroponically-Grown Lettuce (*Lactuca sativa* L.) Types in Terms of Nitrate Accumulation

Noémi Kappel^{1,*}, Ildikó Fruzsina Boros^{1,2}, Francia Seconde Ravelombola³ and László Sipos²

¹ Department of Vegetable and Mushroom Growing, Institute of Horticultural Science, Hungarian University of Agriculture and Life Sciences, Villányi út 29-43, 1118 Budapest, Hungary; boros.ildiko.fruzsina@gmail.com

² Department of Postharvest, Supply Chain, Commerce and Sensory Science, Institute of Food Science and Technology, Hungarian University of Agriculture and Life Sciences, Villányi út 29-43, 1118 Budapest, Hungary; Sipos.Laszlo@uni-mate.hu

³ Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR 72701, USA; fsravelo@email.uark.edu

* Correspondence: Kappel.Noemi@uni-mate.hu; Tel.: +36-30-2158922

Abstract: The goal of this research was to investigate the effect of electrical conductivity (EC) levels of the nutrient solution on the fresh weight, chlorophyll, and nitrate content of hydroponic-system-grown lettuce. The selected cultivars are the most representative commercial varieties grown for European markets. Seven cultivars ('Sintia,' 'Limeira,' 'Corentine,' 'Cencibel,' 'Kiber,' 'Attirai,' and 'Rouxai') of three *Lactuca sativa* L. types' (butterhead, loose leaf, and oak leaf) were grown in a phytotron in rockwool, meanwhile the EC level of the nutrient solutions were different: normal (<1.3 dS/m) and high (10 dS/m). The plants in the saline condition had a lower yield but elevated chlorophyll content and nitrate level, although the 'Limeira' and 'Cencibel' cultivars had reduced nitrate levels. The results and the special characteristic of the lollo-type cultivars showed that the nitrate level could be very different due to salinity ('Limeira' had the lowest (684 µg/g fresh weight (FW)) and 'Cencibel' had the highest (4396 µg/g FW)). There was a moderately strong negative correlation (−0.542) in the reverse ratio among the chlorophyll and nitrate contents in plants treated with a normal EC value, while this relationship was not shown in the saline condition. Under the saline condition, cultivars acted differently, and all examined cultivars stayed under the permitted total nitrate level (5000 µg/g FW).

Keywords: butterhead; loose leaf; oak leaf; lettuce; EC; nitrate



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1. Introduction

Leafy vegetables have importance in healthy, nutritious eating because they have an adequate quantity of fiber, vitamin, and macro compounds [1–3]. A study conducted by Abdullahi et al. [4] showed that leafy vegetables such as lettuces are very good sources of nitrate. In their study, lettuces contained 2.185 ± 0.157 µg/mL of nitrate. Moreover, lettuce, as one of the most commonly consumed vegetables, can have a wider range of food safety risks compared to other foods because it can be infected with microbiological contaminants and heavy metals, or—depending on the production system and growing conditions—it can accumulate elevated nitrate contents [5–9]. Though nitrate by itself is relatively non-toxic, elevated nitrate levels in water and foods are primarily dangerous to infants because infants have a lower level of nitrate reductase enzyme and nitrate obstructs oxygen transport in blood, which could cause methemoglobinemia [10–12]. As food safety is of utmost importance, many regulations have been released, e.g., Good Agriculture Practice (GAP), Good Manufacture Practice (GMP), Hazard Analysis and Critical Control Point (HACCP), and integrated food safety systems (International Food Standard-(IFS) Food Technical Standard and Protocol for food suppliers, stands for British Retail Consortium (BRC), International Organization for Standardization (ISO 22000)), all of which ensure the

food safety for consumers. Moreover, it is crucial to grant the required growth conditions to achieve optimal production [9].

Leaf and root vegetables contain the highest amounts of nitrates [13], especially spinach and lettuce [14,15]. The Commission Regulation (EU) No 1258/2011 amending Regulation (EC) No 1881/2006 regarding maximum levels for nitrates in foodstuffs stated that the allowed maximum level of nitrate (milligrams of NO_3^- per 1 kg of fresh weight (FW)) in leafy vegetables (spinach, lettuce, and rocket salad) had to be raised by 500 mg. European Commission regulation (EU) No 1258/2011 defined the maximum allowable levels of nitrate in spinach at 3500 mg kg^{-1} FW and in summer/winter lettuce grown under cover at $4000/5000 \text{ mg kg}^{-1}$ FW; this was necessary because some member states could not assure the previous maximum levels of nitrate because of the specific climate conditions, particularly the light conditions in the region [16].

The hydroponic system is a very popular growing system among horticulture producers [17]. However, the technical issues of hydroponic systems are well known, the production methods have been fully discussed, and the accuracy of the appropriate settings should be emphasized [18]. Publications about how hydroponic methods affect the nutritional composition of fresh vegetables and their bioactive compounds are scarce [19]. Most bioactive compounds have significant health benefits that are influenced by several factors including genotype and environmental conditions (e.g., light, temperature, humidity, atmospheric CO_2 , and nutrients) [20–23]. In a hydroponic system, it is especially important to control the amount of nutrients in order to allow or deny plants the accumulation of beneficial nutrients or undesirables, such as nitrate. For instance, Novaes et al. [24] reported that lettuces cultivated via a hydroponic system (71.5 g/kg dry weight (DW)) presented significantly higher nitrate concentrations than those conventionally cultivated in soil (29.8 g/kg DW).

The salt tolerance levels of vegetables are different, but lettuce is considered to be salt-sensitive crop [22]. There have been several studies examining the tolerance of lettuce in saline conditions and different EC (electrical conductivity) levels. Xu and Mou [25] identified that salt tolerance level differs by genotype after they evaluated 178 cultivars and germplasm accessions of butterhead, iceberg, romaine, leaf, and wild lettuces at $\text{EC} = 5.3 \text{ dS/m}$. Pasternak et al. [26] concluded that there were significant differences between the fresh weight of cultivars of romaine and iceberg lettuces when EC levels were 8.2 or 10.5 dS/m , respectively. Growth was inadequate at 5.6 and 7.6 dS/m EC levels in an NFT (nutrient film technique) system with iceberg lettuces [27]. The decrease in the numbers of leaves, leaf area, and biomass were linear with the elevation of the EC level (0.5 – 4.5 dS/m) [28]. Borghesi et al. [29] investigated the effect of moderate salinity stress (EC level = 2.5 – 6.5 dS/m) on lettuce, but no significant differences were found in either chlorophyll and carotenoids content or in fresh weight. Mota-Cadenas et al. [30] observed that photosynthetic rate and chlorophyll content were significantly lower in those lettuces that were treated with a 6 dS/m EC saline nutrient solution than with a 2 dS/m nutrient solution. In some cases in lettuce, fresh weight decreased while photosynthetic pigments, chlorophylls, and carotenoids increased when sea water was added to the nutrient solution to up to 10 dS/m , but at a higher EC level (12.6 dS/m), sugar and anthocyanin contents were elevated due to the high amount of added NaCl in the nutrient solution [31].

However, there are several practical options for reducing the nitrate contents in leafy vegetables, and growers should be aware of the risk of excessive nitrate levels, mostly in cases of new cultivars.

Since plant variety and (in many cases) the cultivar are major considerations when assessing nitrate levels, the main goal of our experiment was to determine whether, based on previous research, there is indeed a significant difference in the nitrate accumulations of different types of lettuce cultivars under the influence of an exceptionally high nutrient solution EC. Furthermore, we sought evidence that nitrate accumulation in the examined cultivars would not be harmful at such a high EC value. With our results, we want to

provide information primarily for practical cultivation regarding what nitrate content is expected in today's popular lettuce cultivars with such EC growth.

2. Materials and Methods

2.1. Plant Material and Management Practices

Two experiments were conducted in 2017 at the Szent István University, Budapest (47°28'49.8" N 19°2'25.9" E). Plants were cultivated in a Versatile Environmental Test Chamber with Humidity Control Model No. MLR-351H Sanyo™ phytotron with fluorescent lamps (Mitsubishi Osram FL40SS W/37, (4200 K, 37 W, 3000 Lm)). A total of 7 cultivars of 3 lettuce types were used from the assortment of Rijk Zwaan Da Lier (The Netherlands). These cultivars were 'Sintia' (green butterhead), 'Limeira' (green loose leaf), 'Corentine' (red loose leaf), 'Cencibel' (triple-red loose leaf), 'Kiber' (green oak leaf), 'Attirai' (red oak leaf), and 'Rouxai' (quadruole-red oak leaf).

Each treatment had 7 cultivars with 5 biological parallels, and a randomized block design with 5 blocks was applied. Lettuces were treated with the same conditions (temperature, relative humidity, and lighting). The temperature was set to 20/18 °C (day/night), the photoperiod was set to 12/12 h (day/night) with 4000 lux of light intensity, and the relative humidity was set to 80–85%. Water was manually given, and neither pesticides nor phytosanitary product were applied. Seed were sown into Grodan AO rockwool plugs (36 × 40—15 × 15 holes) (Grodan, Milton, Ontario, Canada); after two weeks, they were transplanted into Grodan rockwool cubes (7.5 × 7.5 cm) (Grodan, Milton, Ontario, Canada). During the two experiments, plants were fertigated with nutrient solutions of different concentrations. The first experiment was carried out from 15 May to 10 July 2017, and the second one was carried out from 26 October to 13 December 2017. Nutrient solutions were given after the appearance of the first true leaf. Nutrient solutions of different EC levels were used; the first experiment had a saline condition (EC up to 10 mS/cm), while the second had non-saline condition (EC < 1.3 mS/cm) which was related to the generally recommended range for hydroponic lettuce production [32]. The EC of water and each fertilizer solution was measured with a manual electrical conductivity meter (HI 98311 DiST® 5 EC/TDS-Tester). Each plant was irrigated with the same type and amount of nutrient solution. A total of 3 types of fertilizers were used.

The used fertilizers and EC values during the first experiment were:

- EC Yara Ferticare Starter N:P:K (N₂ 15%, P₂O₅ 30%, K₂O 15%, MgO 2.5%, SO₃ 5%, B 0.02%, Cu 0.01%, Fe 0.1%, Mn 0.1%, Mo 0.002%, and Zn 0.01%) at a concentration of 0.1% = 1.71 dS/m (T = 26.2 °C).
- EC Yara Ferticare I. N:P:K (content: N₂ 14%, P₂O₅ 11.6%, K₂O 25.3%, MgO 2.4%, S 13.75%, B 0.02%, Cu 0.01%, Fe 0.1%, Mn 0.1%, Mo 0.002% and Zn 0.01%) at a concentration of 1% = 10.12 dS/m (T = 23.3 °C)
- EC Calcinit (content: N 15.5%, CaO 26.2%) at a concentration of 0.02% = 0.73 dS/m (T = 20.6 °C)

The used fertilizers and EC values during the second experiment were:

- EC Yara Ferticare Starter N:P:K ((N₂ 15%, P₂O₅ 30%, K₂O 15%, MgO 2.5%, SO₃ 5%, B 0.02%, Cu 0.01%, Fe 0.1%, Mn 0.1%, Mo 0.002%, Zn 0.01%) at a concentration of 0.1% = 1.06 dS/m (T = 25.2 °C)
- EC Yara Ferticare I. N:P:K (content: N₂ 14%, P₂O₅ 11.6%, K₂O 25.3%, MgO 2.4%, S 13.75%, B 0.02%, Cu 0.01%, Fe 0.1%, Mn 0.1%, Mo 0.002% and Zn 0.01%) at a concentration of 0.1% = 1.09 dS/m (T = 25.7 °C)
- EC Calcinit (content: N 15.5%, CaO 26.2%) at a concentration of 0.02% = 0.75 dS/m (T = 27.6 °C)

2.2. Total Fresh Weight Measurement and Leaf Relative Chlorophyll Content Measurement

FW was uniformly recorded 35 days after sowing using a precision balance EMS made by KERN & SOHN, Balingen, Germany. Only shoots were measured without the rockwool cubes and roots. Results are expressed in g/plant for each sample. The relative chlorophyll

contents of the lettuces were measured with a portable Chl meter (Soil Plant Analysis Development (SPAD) chlorophyll meter (SPAD 502; Minolta Camera, Osaka, Japan). After calibration, 5 measurements were taken on a randomly chosen, fully expanded leaf.

2.3. Nitrate Determination

The determination of nitrate content was done with the method of Cataldo et al. [33] following a modified sample preparation (hot extraction and clarification with a Carrez solution). Photometric measurements were done with a UV/VIS spectrophotometer (Thermo Scientific, Waltham, MA, USA) at 410 nm. For data evaluation, the VISIONpro V2.02 (Thermo Scientific, Waltham, MA, USA) software was used.

2.4. Statistical Procedures

Data were subjected to a two-way ANOVA in IBM SPSS 25.0, with two fixed factors: 7 cultivar levels ('Sintia,' 'Cencibel,' 'Corentine,' 'Limeira,' 'Attirai,' 'Kiber,' and 'Rouxai') and treatment with 2 levels (normal and high EC nutrition solution conditions) ($\alpha = 0.05$). Normality was checked by Kolmogorov–Smirnov and Shapiro–Wilk tests. Homogeneity of variances was measured with Levene's test. Factors varieties were separated by Tukey's post hoc analysis or Games–Howell's post hoc analysis. Pearson correlation was performed ($\alpha = 0.05$).

3. Results

3.1. Fresh Weight (FW)

There was a highly significant treatment effect (normal and high EC nutrient solution value on the fresh weight ($F(13, 56) = 73.86; p < 0.001$) (Figure 1). The results showed that when the lettuces were exposed to high EC conditions, there was a significant decrease of the fresh weight by, on average, 42%. In addition, there was a significant variety effect on the fresh weight ($F(13, 56) = 4.47; p < 0.01$). However, there was no significant interaction between cultivars and EC levels ($F(13, 56) = 0.94; p = 0.47$).

The normal-EC-treated 'Kiber' cultivar had the highest fresh weight (49.7 g), while 'Rouxai' cultivar had the lowest (31.1 g). The most homogenous (which had the lowest standard deviation compared to its own average fresh weight) cultivar was 'Cencibel' (8.2%), and the most heterogeneous was 'Corentine' (31.9%). Under high EC conditions, the 'Attirai' cultivar (30.2 g) reached the highest fresh weight, while 'Corentine' (17.1 g) had the lowest. The most homogenous cultivar was 'Cencibel' (16.3%), and 'Sintia' was (63.8%) the most heterogeneous. An examination of the pattern of the fresh weight showed that in the case of each cultivar, the average fresh weight decreased when the EC was elevated, but this change was not significant. Overall, 'Cencibel' was the most homogenous cultivar; on average, it only had a 12.3% standard error.

3.2. Leaf Relative Chlorophyll Content

There was a highly significant cultivar effect on the chlorophyll content ($F(13, 56) = 10.40; p < 0.001$) (Figure 2). Additionally, there was a significant treatment effect on the chlorophyll content ($F(13, 56) = 4.97; p < 0.05$). However, there was no significant interaction effect between cultivars and treatments on the chlorophyll content ($F(13, 56) = 0.80; p = 0.57$). In general, lettuces accumulated high chlorophyll when exposed to a high EC compared to the normal conditions. An increasing pattern of chlorophyll by 10% was detected. Under a normal EC, the 'Kiber' cultivar (32.5 SPAD value) had the highest average chlorophyll content and 'Attirai' (22.8 SPAD value) had the lowest value. The most homogenous variety was 'Attirai' (6.6%), and the least homogenous was 'Kiber' (19.0%).

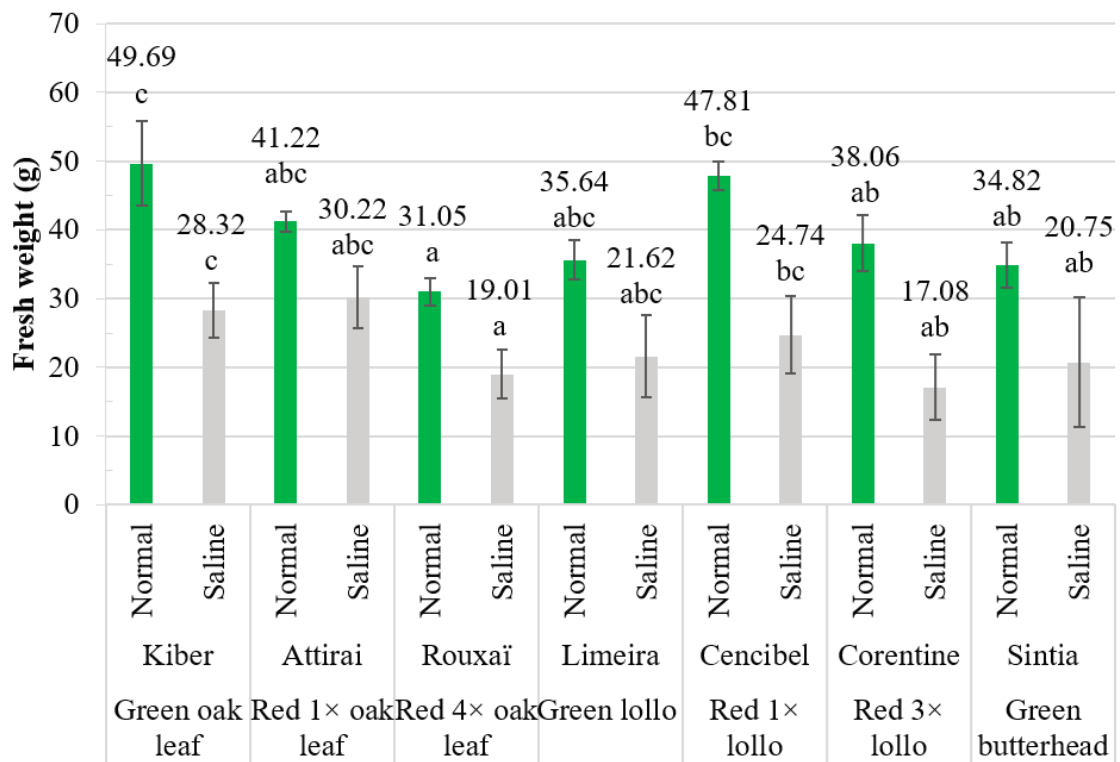


Figure 1. Fresh weight (FW) (g) of different cultivars of lettuce grown in high and normal electrical conductivity (EC) conditions 35 days after sowing. (Different letters indicate significant differences among the treatments at $p < 0.05$ by Tukey-Kramer’s test.

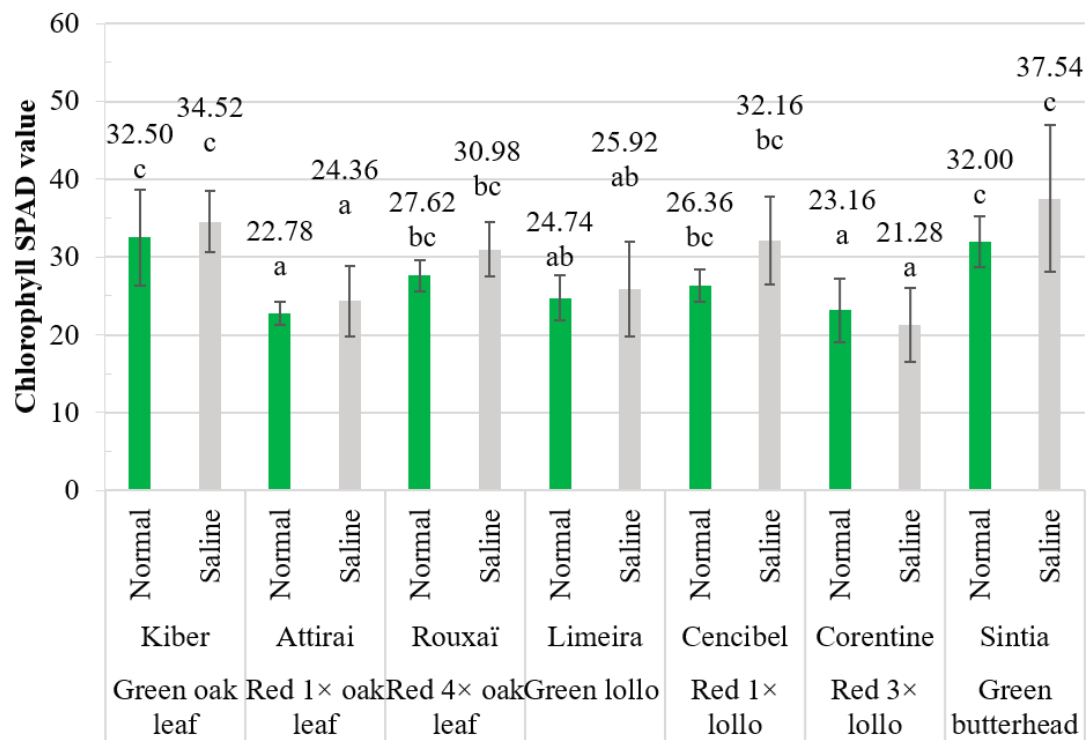


Figure 2. Chlorophyll content (SPAD value) of different cultivars of lettuce grown in saline and normal conditions (Games-Howell’s; $p < 0.05$). Different letters indicate significant differences among the treatments at $p < 0.05$ by Games-Howell’s test.

Under high EC conditions, the ‘Sintia’ cultivar had the highest chlorophyll content (37.5 SPAD value) and ‘Corentine’ had the lowest (21.3 SPAD value). The ‘Rouxai’ cultivar (11.3%) was the most homogeneous, and ‘Sintia’ (25.0%) was the most heterogeneous. The pattern of the chlorophyll content showed that this compound was typically increased in saline conditions except for in the ‘Corentine’ cultivar, where chlorophyll was reduced, though this change was not significant.

3.3. Nitrate Content

There was a highly significant cultivar effect on the nitrate content ($F(13, 56) = 25.66$; $p < 0.001$). Furthermore, there was a significant treatment effect on nitrate ($F(13, 56) = 6.80$; $p < 0.05$). Additionally, there was a highly significant interaction effect between cultivars and treatments ($F(13, 56) = 25.89$; $p < 0.001$) (Figure 3). At first glance, when exposed to high EC values, some cultivars accumulated more nitrate in saline conditions (average increase of 39%) than in normal conditions. However, for the ‘Corentine’ and ‘Limeria’ cultivars, a significant decrease (average of 45%) of nitrate content was noticed in saline conditions.

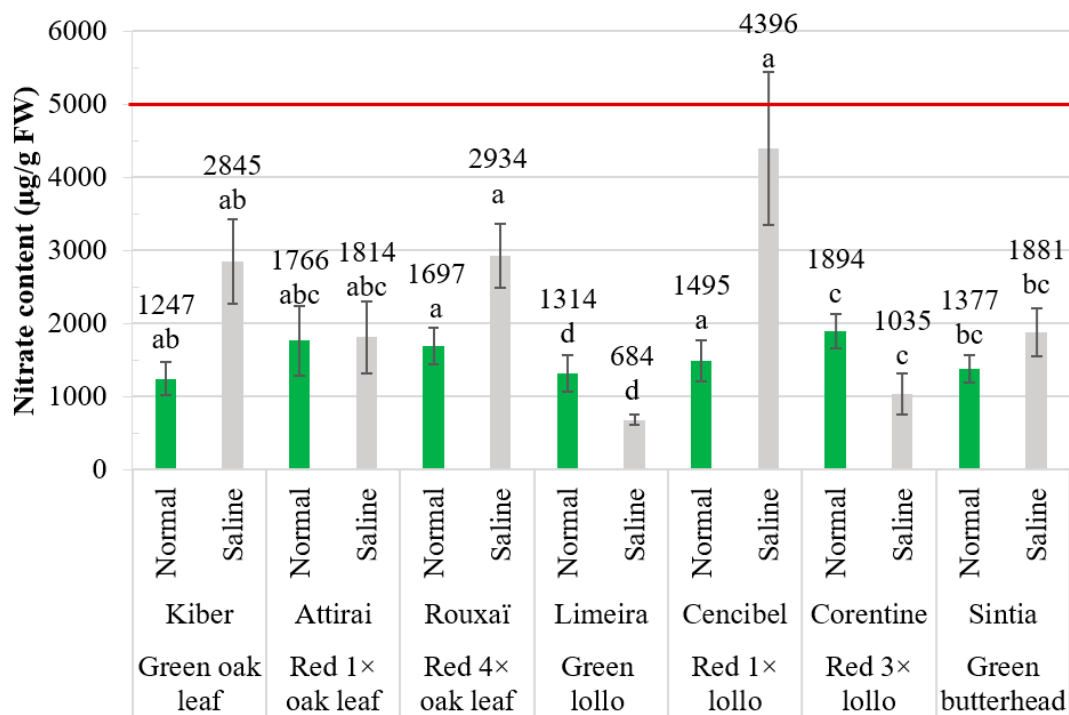


Figure 3. Nitrate content ($\mu\text{g/g FW}$) of different cultivars of lettuce grown in saline and normal conditions. Different letters indicate significant differences among the treatments at $p < 0.05$ by Tukey-Kramer’s test.

In normal conditions, the nitrate contents were the highest in the ‘Corentine’ (1894 $\mu\text{g/g FW}$) cultivar and ‘Kiber’ (1247 $\mu\text{g/g FW}$) had the lowest value. The most homogenous variety was ‘Corentine’ (12.1%), and ‘Attirai’ (26.9%) was the most heterogeneous.

Based on the results of the salted plants, the ‘Cencibel’ cultivar (4396 $\mu\text{g/g FW}$) had the highest nitrate content and ‘Limeira’ (684 $\mu\text{g/g FW}$) had the lowest. In this case, ‘Limeira’ (11.1%) was the most homogenous and ‘Attirai’ (26.9%) was the most heterogeneous.

During the examination of the nitrate content pattern, it was found that cultivars responded differently to salinity. The ‘Cencibel,’ ‘Kiber,’ and ‘Rouxai’ cultivars’ nitrate contents were significantly elevated, while that of ‘Corentine’ was significantly decreased. There was no significant difference between normal and saline plants’ nitrate content of the ‘Attirai,’ ‘Limeira,’ and ‘Sintia’ cultivars.

The correlation analysis of normally treated samples showed that the correlation was -0.542 among the chlorophyll and nitrate contents, which signified a moderately strong

interaction (Table 1). A reversed proportionality was observed, as nitrate content was elevated when chlorophyll content was decreased. In saline treatments, there were no significant interactions.

Table 1. Correlation matrix (Pearson) between variables in normal treatment.

Variables	Nitrate Content	Fresh Weight	Chlorophyll Content
Nitrate content	–	–0.156	–0.542
Fresh weight	–0.156	–	–0.015
Chlorophyll content	–0.542	–0.015	–

Values in bold are different from 0 with a significance level $\alpha = 0.05$.

4. Discussion

Zapata et al. [34] and Mola et al. [35] stated that lettuce is a salt-sensitive crop. A reduction in plant growth under saline conditions is a common phenomenon; a high concentration of nutrients (EC values of 6 and 10 dS m^{-1}) in the root zone reduces the yield of lettuce through a combination of decreased stomatal conductance and leaf area [36].

Nevertheless, there have been many publications about the optimal EC value for hydroponic lettuce cultivation in consideration with several factors. For instance, Samarakoon et al. [37] found a general trend of lowering EC to improve lettuce growth in a high-temperature environment because high transpiration rates can lead to a buildup of EC. A reduction in EC levels from 1.8 to 1.0 mS/cm and from 3.5 to 1.5 mS/cm increased the fresh weight of lettuce in soilless culture [38]. According to this, the EC values applied in our experiments were chosen by considering the characteristics of the used cultivars and what fertilizers allowed for the easiest way to prepare the nutrient solution in cultivation practice. Research conducted by Shannon and Grieve [39] demonstrated a wide variation in salt tolerance among lettuce cultivars. For instance, romaine cultivars were found to be far more tolerant to salinity than iceberg cultivars [28] after they measured the response of romaine and iceberg varieties to saline (EC = 1.2, 3.5, 8.2, and 10.5 ds/m) irrigation water during growth. They concluded that significant differences between the fresh weights of cultivars can be seen above 8.2 ds/m . In our study, when the saline condition was induced (EC = 10 dS/m), the cultivar' responses were distinct. Our findings were similar to those reported by Miceli et al. [40]. In lettuce grown in a hydroponic system, they found that nitrate content decreased from 2218 to 1634 mg/kg of FW in leaves and the level of salinity of the nutrient solution increased from 1.6 to 4.6 dS/m .

Andriolo et al. [41] also found that when exposed to a solution with an EC of 2.8 dS/m , yield linearly decreased by 16.5% per unit of EC. This significant decrease in fresh weight with increasing EC levels was also confirmed by the results published by Ouhibi et al. [42] and Schrader [43]. Soares et al. [44] observed that lettuces had smaller leaf areas and decreases in fresh and dry shoot weight when they were grown in briny water (5.5 dS/m). Only oak leaf lettuces showed a pattern in fresh weight loss, as the green variety had the highest fresh weight, red had a lower fresh weight, and quadruole-red had the lowest fresh weight, but lollo varieties did not have any linearity. The range of fresh weight of normal plants was between 49.7 and 31.1 g, and that of saline lettuces was between 30.2 and 17.1 g—the fresh weight was always decreased, and differences between the treatments (among same variety) were 26.7–55.12%. This was similar to Xu and Mou's finding [25] that the fresh weight also decreased in saline conditions (EC = 5.3 dS/m) when fresh weight loss among control and saline conditions were between 15 and 56% and between 2 and 52%, respectively, in romaine; between 5 and 62% in leaf varieties; and between 18 and 50% in wild lettuce. Additionally, one iceberg lettuce gained fresh weight by 4.5%.

In our study, salinity induced an increase of chlorophyll (SPAD value) in general. Salinity increased SPAD values in most genotypes, indicating higher chlorophyll contents. Salt stress usually caused an uptrend (4.7–22%) in chlorophyll content, except for in the 'Corentine' cultivar, where it declined by 8.1%, which Mota-Cadenas et al. [30] also observed but with iceberg lettuce. However, Borghesi et al. [29] reported that chlorophyll content was

not affected for lollo-type lettuce, even in a high salt concentration. Research conducted by Wang and Nii [45], Pérez-López et al. [46], and Xu and Mou [25] supported that there was an increasing pattern in chlorophyll contents with an increase of salinity levels, and this change enhances salt tolerance. Nonetheless, many researchers have found that salinity reduced the chlorophyll content in the cases of other crops [47–49].

The present experiment revealed that higher levels of salinity showed a tendency to increase leaf nitrate content in general. This result was supported by the findings of Chung et al. [50], Eraslan et al. [51], and Quy et al. [52]. Furthermore, Jin et al. [53] stated that nitrate accumulation in lettuce was increased by 18.6% in saline conditions, which was in agreement with our findings. However, this increase was smaller than our results. We found an enormous uplift, in that nitrate content was more than twice as high in the ‘Kiber’ cultivar (228%) and almost three times higher in the ‘Cencibel’ cultivar (294%) than in normal plants. Typically, differences were between 2.7 and 72.9% when looking at all cultivars. The highest nitrate contents in normal and saline conditions were 1894.24 mg/kg FW (‘Corentine’ lollo) and 4395.80 mg/kg FW (‘Cencibel’ lollo). Though the nitrate level did not reach the maximum allowance (5000 mg nitrate/kg FW) of lettuces in the ‘Cencibel’ cultivar, its standard error did exceed that. There were two lollo cultivars (‘Limeira’ and ‘Corentine’) in which we found a decreased nitrate level (47.9% and 45.3% reduction, respectively) in saline plants.

In studying the correlations between nitrate concentration and agronomic and physiological parameters, we found that there was a moderately strong negative correlation (-0.542) in the reverse ratio among chlorophyll and nitrate contents of normally treated samples; meanwhile, this relationship was not shown in the saline conditions. Behr and Wiebe [54] even showed a negative correlation between the nitrate concentration and photosynthetic ability of lettuce cultivars. In their study, Hamdi et al. [55] reported that the correlation between nitrate accumulation and chlorophyll fluorescence was dependent on nitrogen level.

A high nitrate accumulation could be further explained by the high nutrient uptake of lettuce. Lettuce needs to keep a high turgor pressure, which results in this leafy vegetable accumulating nitrate content in its leaves [56,57]. The tendency of the increase in nitrate content with higher levels of salinity can also be explained by the osmotic adjustment that allows plants to absorb water under conditions of low total water potential [58]. According to Krohn et al. [59], younger leaves accumulate more nitrate than mature leaves. This could be also justified by the higher concentration of nitrate in the leaves of lettuce because our experiments were carried out for approximately 35 days. Nevertheless, the results of Tesi et al. [60] disagreed with the results from the current study by indicating that rising salinity reduced nitrate accumulation in the leaves of lettuce. This reduction of the nitrate contents of lettuce cultivars under saline stress was also reported by Pérez-López et al. [46]. Those investigations were therefore in partial conformity with the results that showed a decreasing pattern in the nitrate content of lollo-type lettuce when exposed to saline conditions.

The amount of nitrate in plants is mainly determined from their genetically-based metabolism, age, and amount of available nitrate in the root zone (which is also related to the fertilizer management). There are other options for reducing nitrate content in cultivation practices. Previous studies have shown that nitrate accumulation in leafy vegetables is related to the water content in vegetable tissues [61]. Nevertheless, low radiation is the major factor responsible for excessive nitrate accumulation, depending on the season and growing system [62]. Additionally, a suitable temperature regulation strategy is needed to guarantee the production of vegetables with low nitrate concentrations. However, in terms of reducing nitrate concentration and enhancing yield, CO₂ enrichment should be combined with other environmental regulatory activities [63].

Gruda et al. stated that both the quantity of absorbed nitrogen and the way in which it is utilized in plant metabolism, mainly with respect to the nitrate nitrogen content in the edible plant tissues, are better managed in soilless culture [62]. Accordingly, they found

that the replacement of a nutrient solution with rain water three days before harvesting resulted in a one third nitrate reduction in leaves [62].

However, further studies using a wider range of concentrations have to be performed to identify the nutrient concentration thresholds for the different lettuce types, and it might be possible to reconsider the determination of the optimal EC value of a nutrient solution recommended for different hydroponically-grown types and new lettuce cultivars.

5. Conclusions

Nitrate accumulation is a natural phenomenon in plants and can be affected by many factors, e.g., the form and level of nitrogen fertilizer, EC level, light condition, and lettuce type and cultivar. The goal of this investigation was to examine the EC sensitivity of hydroponically-grown, consumer-preferred lettuce types and new cultivars under normal and high EC nutrient solution conditions by comparing their fresh weight, chlorophyll content, and nitrate content. In conclusion, the nitrate content in all examined cultivars was below the safety level established by the European Commission for lettuce, even at a very high EC level. The results showed that there was an overall decrease in the fresh weight of lettuces in saline conditions. Under saline conditions, lettuces accumulated chlorophyll and showed a different change in nitrate contents. The accumulation of nitrate was significant for ‘Kiber’, ‘Rouxai’, ‘Cencibel’ and ‘Sintia’ cultivars and not significant for the ‘Attirai’ cultivar. Furthermore a significant decrease in nitrate content was reported for the ‘Corentine’ and ‘Limeria’ cultivars. Therefore, loose leaf/lollo-type cultivars did not accumulate nitrates under the high EC nutrient solution condition. Considering all of the measured properties, in normal EC conditions, the most advantageous cultivar was ‘Kiber’ because it had the greatest fresh weight, a relatively high chlorophyll content, and the lowest nitrate content. In our experiment, the butterhead type (‘Sintia’ cultivar) was shown to be the most sensitive to salinity.

Author Contributions: All authors contributed to this research. The design of the experiment was done by N.K., I.F.B., and F.S.R. I.F.B. and F.S.R. did the recording and the processing of the data, as well as with L.S. the result of the evaluation. The manuscript was written by I.F.B., L.S., and N.K. assisted in writing the paper. N.K. contributed to designing the research and revised the manuscript. The work presented in the paper was conceived within research projects led by N.K. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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