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ORIGINAL PAPER

Different effects of a cultivar and nutrition on the response of lettuce (*Lactuca sativa* L.) grown in the Nutrient Film Technique (NFT)*

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Abstract

The aim of this study was to determine the effect of a cultivar and varied nutrient solution composition on the quality and growth of lettuce grown in a recirculating NFT system. Three cultivars were tested: Rouxai (oakleaf type), Corentine RZ (Lollo Rossa type), and Lozano (Lollo Bionda type), under four nutrient solutions varying in the nitrogen (N) content (ranging from 62 to 150 mg dm⁻³) and the ratios of other macronutrients. Chemical composition of the nutrient solution significantly affected plant yielding. Reducing N nutrition significantly decreased nitrate levels in plants, while also significantly reducing crop yields. When the N content was constant, an increase in the proportions of K, Ca, and Mg significantly enhanced yields, demonstrating that nutrient ratios are a key factor. The photosynthetic apparatus of plants was not affected by reduced nitrogen in the nutrient solution, but effectiveness of photosynthesis expressed as dry matter production was reduced. The response of plants varied depending on the cultivar: (1) Lozano took up the most nutrients and achieved the highest fresh weight yield; the measured chlorophyll and fluorescence indices were not the highest. This cultivar accumulated high levels of nitrates; (2) Rouxai had the lowest fresh and dry weight, and stands out with the highest chlorophyll (Relative Chlorophyll Content and NDVI) and fluorescence indices; (3) Corentine shows generally balanced results in nutrient uptake, growth, and physiological parameters. Chlorophyll and fluorescence indicators are valuable diagnostic tools that reveal genotypic differences in response to nitrogen nutrition. The significant variability in plant response across cultivars confirms the difficulty in selecting cultivars suitable for mixed-pot cultivation intended for the consumer market.

Keywords: lettuce, NFT, nutrient solution, nitrates, cultivars

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INTRODUCTION

Lettuce is one of the most popular leafy vegetables in the world (Tabaglio et al. 2020). It contains compounds beneficial for consumers, such as ones with anti-fungal, anti-bacterial and anti-inflammatory capabilities that help to delay the ageing process (Landete 2013). The production of lettuce can be carried out in the field or under cover. Using modern technologies, the greenhouse production of this vegetable can be carried out all year round. It is hard to imagine a grocery shop that does not offer lettuce all year round. It is popular to sell lettuce not only as cut heads of lettuce but also as plants with a root system (in a pot or peat block), and the latter stays fresh longer when brought home (Tabaglio et al. 2020). However, consumers are quickly bored with products in the same category, and so “coloured” lettuce is now being marketed. This “colourful” effect lettuce can be achieved by producing several cultivars of lettuce with different colour and leaf type. In the same pot. Such a product seems interesting to the consumer, but it can be difficult for the producer. Cultivars with different leaf colours or forms have slightly different cultivation requirements and different growth rates. Lettuce cultivars belonging to different types (Romaine and Lollo) showed different responses to decreasing P in the nutrient solution in hydroponic cultivation (Neocleous, Savvas 2019). The Romaine cultivars were more sensitive, showing a reduction in leaf biomass formation associated with lower photosynthetic intensity at lower P doses, while the Lollo cultivars were less sensitive to a reduction in phosphorus in the nutrient solution. Also Sapkota et al. (2019) demonstrated the differential impact of lettuce cultivars and their nutrient requirement.

Hydroponic culture is a cheap and easy option for organic vegetable production (Sapkota et al. 2019). One of the methods of under-cover lettuce production is the nutrient film technique – NFT (Majid et al. 2021). It enables year-round production, allows for increased efficiency in water and fertilizer use, and generates higher yields per unit area. However, given the higher costs of establishing and operating this type of farming, the economic calculation does not always show better results than that of a field cultivation (Meastre-Valero et al. 2018). This type of cultivation appears to be more favourable in terms of the environmental effect (less water and fertiliser consumption) and the use of less land for cultivation (Sambo et al. 2019). Maruo et al. (2002) also stated that from an ecological and management point of view, nutrient concentrations in hydroponic production systems should be reduced. This is also particularly important for the value of the lettuce because by excessive nitrogen supplementation which can negatively impact its quality, particularly its tendency to accumulate nitrates (Escobar-Gutierrez et al. 2002, Manzocco et al. 2011, Kmecl et al. 2017, Zandvakili et al. 2019). This indicates the need for intensive research into the optimisation of nutrition of lettuce crops grown intensively in soilless cultivation.

The aim of the study was to evaluate the effect of using nutrient solutions with different chemical compositions on the multifaceted response of three lettuce cultivars grown hydroponically in an NFT system.

MATERIAL AND METHODS

Plant material and cultivation

The experiment was conducted from March to April 2021 in a commercial greenhouse (Smart Vegetables Innovations, Poland) with an area of 15 000 m², located in Zdunowo near Warsaw, Poland (52°30'14.2"N 20°30'21.4"E). The greenhouse was well equipped with a modern climate control computer system (PRIVA): high pressure fogging system, temperature and humidity regulation and also a shading system. Plants were grown hydroponically in cultivation channels (NFT; Nutrient Film Technique). During the lettuce cultivation, LED lamps were used especially on cloudy days. Lamps switched on when natural light decreased below 130W.

A two-factor experiment was set up in a randomised block design with four replicates on an area about 40 m². Each pot contained three cultivars of lettuce. The first factor of the experiment was the four types of nutrient solutions (I, II, III, and IV), which varied in their overall chemical composition and EC, including four distinct levels (N, P, K, Ca, Mg, S, and Cl), micronutrient content was the same in each of the combinations tested, as detailed in Table 1. The second factor was cultivar of lettuce: Rouxai, Corentine RZ and, Lozano; all cultivars from Rijk Zwaan, (the Netherlands). The cv. Rouxai is an oak-leaved, compact, lettuce with leaves turning intense red in the upper half. Corentine is a cultivar with curly, red-coloured leaves, resistant to bolting and tip-burn. Lozano is a cultivar with green curly leaves, forming compact heads and showing good resistance to tip-burn or bolting (<https://www.rijkszwaan.dk/crop/lettuce>). All three cultivars are recommended by the seed producer for hydroponic cultivation. Seeds of each cultivar were sown in one pot. The end product being a pot with 3 different cultivars of lettuce, colourful and attractive to the consumer (Photo 1).

Cultivation was carried out on mobile channels. The plant density was 16 plants m⁻² and they were 150 pots per each combination. During the beginning of cultivation, the channels were spaced close together, and at the time when lettuce heads developed, the channels were spaced wider, further away from one another. The plant material was harvested on 16.04.2021, 44 days after sowing, and the cultivation in channels lasted 20 days.

Lettuce production was carried out as follows: on 3 March, seeds were sown using an automatic line into 130 ml capacity pots filled with peat substrate with following chemical composition (mg dm⁻³): N-NO₃ 58; N-NH₄ 29.1; P 73; K 144; Ca 680; Mg 191; Na 17; Cl 25; S-SO₄ 85; Fe 23.6; Mn 3.1;



Photo. 1. A view of the 3 cultivars of lettuce grown in one pot (April 2021)

Zn 1.2; Cu 1.8; B 1.9; pH 5.19; EC 0.70 mS cm⁻¹. Then, the germination process was carried out in a propagation room for 3 days (temp. 17°C, humidity 95-99%, darkness). Transferring germinated plants to transplanting tables took place on 6 March, and the following climate parameters were maintained: temp. 27/14°C per day/night, lighting with LED lamps (Philips Green, Power LED Toplighting 1.2, DR/W MB) from 4 h to 16 h/day. Transfer of transplants at the stage of 3-4 leaves to the growing channels took place on 27 March. In the greenhouse, the following parameters were set: temperature 27/14°C per day/night, lighting with LED lamps (Philips Green, Power LED Toplighting 1.2 DR/W LB; 100 µmol m⁻² s⁻¹).

Four types of nutrient solution with different compositions of nutrients and EC were used. Throughout the manuscript, these solutions are referred to by their corresponding numbers I; II; III and IV, which correspond to the N levels of 62, 120 (both II and III), and 150 mg dm⁻³, respectively. The details of other nutrients in each treatment are presented in Table 1. The nutrient solutions were applied from drippers for 120 s, with a liquid output of 4 dm³ h⁻¹, and depending on external radiation every 45-90 min during the day, and every 180 min at night.

Measurements and analysis

Evaluation of fresh matter

Ten pots from each treatment were selected from the growing channels. Cultivars within each pot were separated and weighed separately.

Dry matter determination

Dry weight was determined separately for each cultivar and treatment. Cultivars within the pot were separated, then labelled and dried in an air-flow dryer at 105°C to constant weight. The percentage of dry matter was calculated.

Table 1

Chemical composition of the nutrient solution used for fertigation of the plants (mg dm⁻³)

Specification	I	II	III	IV
EC (mS cm ⁻¹)	2.1	2.0	1.7	0.9
N total	150	120	120	62
N-NO ₃	134	115	91	61
N-NH ₄	16	5	29	1
K	200	290	216	137
P	50	58	43.2	27.4
Ca	150	145	108	68.5
Mg	30	46.4	34.6	21.9
S	47	76	72	23
Cl	22	87	65	22
Fe	2.20			
Mn	0.20			
Zn	0.26			
B	0.32			
Cu	0.05			
Mo	0.05			

Measurement of the lettuce height

On the day of analysis, cultivars growing in one pot were split and measured from the base of the shoot (pot surface) to the top of the longest leaf using a ruler.

Determination of nitrates

The content of nitrates was determined by high-performance ion chromatography (HPLC/IC) using a Thermo Scientific (USA) Dionex 3000 instrument equipped with a conductivity detector. Analyses were performed according to the PN-EN 12014-2:2001 standard Foodstuffs. Determination of nitrate and/or nitrite content. Part 2: Determination of nitrate content in vegetables and processed vegetable products by HPLC/IC. Lettuce was separated into cultivars. The lettuce samples (at least 6 plants per treatment) were homogenized.

Sample handling: A homogenous laboratory sample was weighed at 5.00 g ± 0.10 g. Two parallel samples were prepared. Each sample was poured with 50 ml of hot water and heated at 95°C ± 5°C in a water bath for 15 minutes. After cooling, the sample was quantitatively transferred using deionized water to a 500 ml volumetric flask and then filtered through paper filters. The extract was clarified using reversed-phase filters (RP II columns) and 0.45 µm nylon filters. The prepared extract was transferred to 1.5 ml

vials, which were placed in an autosampler. Separation was performed using a Dionex IonPac AS9-HC column (250 mm x 4.6 mm; 5 μ m) with an AG9-HC precolumn (50 mm x 4 mm). Results were expressed in mg kg⁻¹ FW.

Determination of Relative Chlorophyll Content

On the day of analysis, the Relative Chlorophyll Content was measured using a CCM 300 portable chlorophyll meter. The measurement was performed on fully grown leaves, without signs of ageing or damage.

Determination of chemical composition of plants and nutrient solutions

Plant samples (several representative heads of lettuce from each treatment) were placed for 48 h in a forced-air dryer at 72°C. Then samples were analysed after grinding and wet mineralisation in a HNO₃ and HClO₄ acid mixture. The concentrations of macronutrients (P, K, Ca, Mg) and micronutrients (Fe, Mn, Cu, Zn, B) were determined in three replications using an ICP spectrometer. Selected elements in plant tissue were determined at their characteristic wavelengths (Boss and Freden 2004). The N content in plant samples was analysed using the Kjeldahl method on a Vapodest Kjeldahl apparatus (Gerhardt GmbH & Co., KG, Königswinter, Bonn, Germany according to Latimer 2012).

Nutrient components in the nutrient solution, as N-NO₃, were analysed by the potentiometric method; P, K, Ca, Mg, and SO₄ by the spectrophotometric method using a sequential emission spectrometer with inductively coupled plasma (ICP Perkin-Elmer model Optima 2000 DV, Boston, MA, USA). All the nutrients in plant samples were determined in three replications.

Determination of the chlorophyll a fluorescence parameters

On the day of harvest, chlorophyll a fluorescence was measured using PAR-FluorPen FP 110D fluorimeter (PSI company, Czech Republic). Leaves were shaded with a special leaf-clip for 20 minutes. The following parameters were considered (Roháček 2002, Stirbet and Govindjee 2011, Stirbet et al. 2018, Tsimilli-Michael 2020): RC/ABS number of QA (plastoquinone A) reducing RCs (reactive centres) per PSII (photosystem II) antenna chlorophyll, apparent antenna size of an active PSII; TR₀/ABS maximum quantum yield of primary PSII photochemistry; ET₀/ABS quantum yield of electron transport from Q_A⁻ to PQ; RE₀/ABS quantum yield of the electron transport flux until the PSI electron acceptors; DI₀/ABS quantum yield of energy dissipation in PSII antenna; ET₀/TR₀ efficiency with which trapped by PSII electron is transferred from Q_A⁻ to PQ; RE₀/ET₀ efficiency with which an electron from the intersystem electron carriers is transferred to PSI acceptors; PI ABS TOTAL – performance index for energy conservation from photons absorbed by PSII antenna, until the reduction of PSI acceptors.

Determination of leaf spectral parameters

On the day of harvest, a handheld spectro-radiometer system for measurement of spectral reflectance of an internal light source (Xenon incandescent lamp 380-1050 nm) from leaves, as well as an apparatus for measurements of transmittance and absorbance of any external light source were used (The PolyPen, PSI, Czech Republic). PolyPen incorporates formulas of commonly used reflectance indices (e.g. ARI1, CRI2, G, MCARI1, NDVI, NPCI, PRI etc.) into its software and displays values for the selected indices.

Uptake of nutrients

The uptake of nutrients by the aerial parts of the plants was calculated by taking into account the dry matter yield and the determined contents of nutrients in the dry matter.

Statistical methods

The data were statistically analysed. The means and results of the two-factor analysis of variance (cultivar \times nutrient solution) are presented in the tables. The assumptions of variance analysis were verified: the presence of a normal distribution with the Shapiro-Wilk test and homogeneity of variance with the Levene test. The letters in the tables indicate homogeneous groups determined using the Tuckey test. The assumed significance level was 0.05. Statistica13 software was used for the analyses (TIBCO Software Inc. (2017). Statistica (data analysis software system), version 13. <http://statistica.io>).

RESULTS

Quality parameters

The compared cultivars differed in the weight they reached in the pot (Table 2). Taking into account the impact of a single factor, it was noted that the largest heads were formed by the green-leaved cv. Lozano, while the smallest were formed by the oak-leaved cv. Rouxai. The smallest plants were determined after treatment with the nutrient solution with the lowest nitrogen content. However, it appears that it was not nitrogen that was the most important parameter in the formation of heads, as at the same N-120 larger heads were formed at a higher potassium, magnesium and calcium dose.

Analysing the influence of both factors simultaneously, it can be seen that each cultivar produced similar-sized plants at the weakest nutrient solution (IV). Lozano plants were larger than the other two cultivars in nutrient solutions I and III, while in nutrient solution II, Corentine plants were of similar size to Lozano - both larger than Rouxai. The percentage dry

Table 2

Some quality parameters of three lettuce cultivars cultivated under four different nutrient solution treatment

Cultivar	Nutrient solution				Mean
	I	II	III	IV	
Fresh weight (g plant ⁻¹)					
Corentine RZ	56.52 <i>bc</i>	67.97 <i>cd</i>	47.37 <i>ab</i>	36.46 <i>a</i>	52.08 <i>B</i>
Lozano	75.30 <i>d</i>	74.72 <i>d</i>	70.33 <i>cd</i>	46.80 <i>ab</i>	66.79 <i>C</i>
Rouxai	41.00 <i>ab</i>	50.60 <i>ab</i>	50.68 <i>ab</i>	39.83 <i>ab</i>	45.53 <i>A</i>
Mean	57.61 <i>BC</i>	64.43 <i>C</i>	56.13 <i>B</i>	41.03 <i>A</i>	
Dry weight (%)					
Corentine RZ	3.23	2.98	3.38	3.82	3.354
Lozano	3.21	3.00	3.17	3.34	3.181
Rouxai	3.31	2.98	3.13	4.14	3.389
Mean	3.25 <i>A</i>	2.98 <i>A</i>	3.23 <i>A</i>	3.77 <i>B</i>	
Dry weight (g plant ⁻¹)					
Corentine RZ	1.83 <i>abc</i>	2.02 <i>bcd</i>	1.60 <i>ab</i>	1.39 <i>a</i>	1.71 <i>A</i>
Lozano	2.42 <i>d</i>	2.24 <i>cd</i>	2.23 <i>cd</i>	1.56 <i>ab</i>	2.11 <i>B</i>
Rouxai	1.36 <i>a</i>	1.51 <i>ab</i>	1.58 <i>ab</i>	1.65 <i>ab</i>	1.52 <i>A</i>
Mean	1.87 <i>B</i>	1.92 <i>B</i>	1.81 <i>B</i>	1.53 <i>A</i>	
Height (cm)					
Corentine RZ	15.13	16.88	15.50	14.13	15.4 <i>B</i>
Lozano	14.50	16.25	14.38	12.88	14.5 <i>A</i>
Rouxai	17.75	18.00	18.13	16.13	17.5 <i>C</i>
Mean	15.79 <i>B</i>	17.04 <i>C</i>	16.00 <i>B</i>	14.38 <i>A</i>	
Nitrates (mg N-NO ₃ ⁻ kg ⁻¹ F.W.)					
Corentine RZ	1877 <i>d</i>	1978 <i>e</i>	1909 <i>d</i>	1017 <i>b</i>	1695 <i>A</i>
Lozano	2412 <i>i</i>	2300 <i>h</i>	2101 <i>f</i>	1597 <i>c</i>	2103 <i>C</i>
Rouxai	2081 <i>f</i>	2009 <i>e</i>	2225 <i>g</i>	965 <i>a</i>	1820 <i>B</i>
Mean	2124 <i>C</i>	2096 <i>B</i>	2078 <i>B</i>	1193 <i>A</i>	
Relative Chlorophyll Content					
Corentine RZ	2.96 <i>bcd</i>	3.19 <i>cd</i>	3.40 <i>d</i>	3.10 <i>cd</i>	3.16 <i>B</i>
Lozano	2.16 <i>abc</i>	1.96 <i>ab</i>	1.54 <i>a</i>	1.85 <i>a</i>	1.88 <i>A</i>
Rouxai	6.98 <i>ef</i>	6.50 <i>e</i>	7.75 <i>f</i>	6.81 <i>ef</i>	7.01 <i>C</i>
Mean	4.03	3.88	4.23	3.92	

Key for Tables 2-6: Data were statistically analysed with two-way analysis of variance and assumed significance level of 0.05. Small letters indicate homogenous groups according to two factors, capital letters indicate homogenous groups according to a single factor, lack of letters indicates no significant differences.

matter content was similar in all plants under the influence of the two factors tested. It is only noticeable that the plants accumulated a higher dry matter content under the influence of the nutrient solution with the lowest mineral content. The results for dry matter accumulation in the plant are slightly different. The highest dry mass expressed per plant was determined in plants of cv. Lozano and the lowest dry mass accumulation was observed under the nutrient solution IV - describing the influence of the individual factors separately. However, in nutrient solution IV, plants of each cultivar accumulated similar dry matter. Lozano plants in nutrient solutions I and III also had the highest dry weight, whereas in nutrient solution II, the dry weight of Corentine and Lozano plants was similar.

The plants of cv. Lozano were the lowest and those of cv. Rouxai were the highest, with the lowest plants obtained on the nutrient solution with the lowest content of macronutrients, while the highest ones grew in nutrient solution II, with the highest K:N ratio. The highest amount of nitrate was determined in the leaves of cv. Lozano and the lowest was in the leaves of cv. Corentine. Lowering the nitrogen content of the nutrient solution resulted in a lower nitrate content in plant leaves. Whereby a reduction of nitrogen from 150 to 62 mg dm⁻³ resulted in an almost 2-fold reduction in leaf nitrate content. The leaves of the Lozano plants showed the highest levels of nitrate under the influence of nutrient solutions I, II and IV; interestingly, when nutrient solution III was used, cv. Rouxai accumulated the most nitrate. In the case of Corentine, no reduction in leaf nitrate content was observed following a reduction in the nitrogen content of the nutrient solution from 150 to 120 mg dm⁻³. Only a reduction to N-62 resulted in a significant reduction in leaf nitrate content. Similar relationships can be observed for cv. Rouxai. It can only be emphasised that the use of the weakest nutrient solution reduces nitrate accumulation, but cv. Rouxai had a more than twofold reduction in nitrate content and cv. Lozano exhibited only a 40% reduction compared with the highest values. Generally, the most effective reduction in nitrates was found with N-62, but this reduction was accompanied by a significant decrease in fresh and dry plant mass yield and an increase in %DM, which may indicate some stress on the plants.

The cultivars differed significantly in the Relative Chlorophyll Content – Rouxai had the highest levels, while Corentine RZ had the lowest RCC. Interestingly, despite the fact that Lozano is a green cultivar, it had the lowest Relative Chlorophyll Content. This phenomenon was observed regardless of the level of plant nutrition used. This may indicate, for example, less effective assimilation of nitrogen into chlorophyll, which points to possible problems reducing nitrate content. In turn, the Rouxai may potentially be better at converting the nitrogen it takes up into organic forms (including chlorophyll) and stores less of it as nitrates. Importantly, however, despite the use of different levels of nutrition, the Relative Chlorophyll Content does not indicate significant morphological changes in the leaves or severe stress related to nitrogen supply.

Chlorophyll a fluorescence parameters

In this paper, only a few selected parameters obtained from the OJIP test are presented, which may generally indicate the condition of the photosynthetic apparatus of the examined lettuce (Table 3). As regards chlorophyll fluorescence results, it can be seen that cv. Rouxai has the highest number of active reaction centres per chlorophyll antenna in PSII. However, plants of each cultivar treated with nutrient solutions I, II and IV showed no differences in the number of reaction centres in PSII. Only under the influence of nutrient solution III had cv. Rouxai significantly more reaction centres than the other two cultivars. In line with this observation, another finding was that more of the absorbed energy in the leaves of the Rouxai cultivar was transferred to QA (trapping) than in other two cultivars. And, as before, only when plants were treated with nutrient solution III were the differences between cultivars in their ability to transfer an electron to QA observed.

The quantum yield of electron transport from QA⁻ (reduced plastoquinone A) to PQ (plastoquinone) (ET_0/RC) was similar in all tested plants and nutrient solutions. The quantum yield of the electron transport to the PSI (photosystem I) electron acceptors was greater in the cv. Rouxai compared to the other cultivars, but only when applied fertilisation was not taken into account. In agreement with these observations is the least dissipation of absorbed energy in cv. Rouxai. However, taking into account the two experimental factors, this relationship is only apparent when treating the plants with nutrient solution III. Cultivar Rouxai also shows a different efficiency of electron transport from QA⁻ to PQ (ET_0/TR_0) as well as from the intersystem electron carriers to PSI acceptors (RE_0/ET_0). Rouxai differs in electron transport efficiency, which is particularly evident for nutrient solution III.

Leaf spectral parameters

In the case of nutrient solutions I-III, the parameters were generally similar, while changing significantly for nutrient solution IV (regarding ARI1, CRI2, NDVI). At the same time, a significant influence of the cultivar was found (Table 4). The cv. Rouxai showed significantly higher levels of anthocyanins (ARI1) than Lozano and Corentine RZ. Regardless of the nutrient solution tested, this cultivar also had the highest carotenoid content (CRI2), which is important for PSII protection. Interestingly, there was no positive correlation between Relative Content of Chlorophyll (CCM measurement) and the G index for this cultivar, which may indicate that this parameter also takes leaf colour into account. MCARI1 (Modified Chlorophyll Absorption Ratio Index 1) indicates higher chlorophyll content in the cv. Lozano and lower content in cvs. Corentine and Rouxai. These results also indicate that the Relative Chlorophyll Content may not be sufficient to assess the chlorophyll content in lettuce leaves. NDVI (Normalized Difference Vegetation Index) is a very popular indicator of overall vitality, biomass, and vegetation density. Higher NDVI values indicate healthier, denser vegeta-

Table 3

Selected chlorophyll a fluorescence parameters obtained in the OJIP test of three lettuce cultivars cultivated under four different nutrient solution

Cultivar	Nutrient solution				Mean
	I	II	III	IV	
RC/ABS					
Corentine RZ	0.275 <i>abc</i>	0.282 <i>abc</i>	0.257 <i>ab</i>	0.246 <i>ab</i>	0.265 <i>A</i>
Lozano	0.255 <i>ab</i>	0.271 <i>abc</i>	0.241 <i>a</i>	0.298 <i>abc</i>	0.266 <i>A</i>
Rouxai	0.322 <i>bc</i>	0.341 <i>c</i>	0.466 <i>d</i>	0.311 <i>abc</i>	0.360 <i>B</i>
Mean	0.284 <i>A</i>	0.298 <i>AB</i>	0.321 <i>B</i>	0.285 <i>A</i>	
TR ₀ /ABS					
Corentine RZ	0.77 <i>ab</i>	0.77 <i>ab</i>	0.77 <i>ab</i>	0.77 <i>ab</i>	0.77 <i>A</i>
Lozano	0.76 <i>ab</i>	0.77 <i>ab</i>	0.75 <i>a</i>	0.79 <i>bc</i>	0.77 <i>A</i>
Rouxai	0.79 <i>bc</i>	0.79 <i>bc</i>	0.82 <i>c</i>	0.79 <i>bc</i>	0.80 <i>B</i>
Mean	0.78	0.78	0.78	0.78	
ET ₀ /ABS					
Corentine RZ	0.454	0.448	0.452	0.428	0.446
Lozano	0.432	0.433	0.407	0.463	0.434
Rouxai	0.402	0.397	0.439	0.447	0.421
Mean	0.429	0.426	0.433	0.446	
RE ₀ /ABS					
Corentine RZ	0.113	0.134	0.113	0.098	0.114 <i>A</i>
Lozano	0.097	0.106	0.106	0.107	0.104 <i>A</i>
Rouxai	0.127	0.138	0.166	0.128	0.140 <i>B</i>
Mean	0.112	0.126	0.128	0.111	
DI ₀ /ABS					
Corentine RZ	0.227 <i>bc</i>	0.226 <i>bc</i>	0.230 <i>bc</i>	0.235 <i>bc</i>	0.229 <i>B</i>
Lozano	0.238 <i>bc</i>	0.229 <i>bc</i>	0.250 <i>c</i>	0.210 <i>ab</i>	0.232 <i>B</i>
Rouxai	0.210 <i>ab</i>	0.207 <i>ab</i>	0.178 <i>a</i>	0.210 <i>ab</i>	0.201 <i>A</i>
Mean	0.225	0.221	0.219	0.218	
ET ₀ /TR ₀					
Corentine RZ	0.588	0.580	0.587	0.559	0.578 <i>B</i>
Lozano	0.566	0.562	0.544	0.587	0.565 <i>B</i>
Rouxai	0.508	0.501	0.534	0.566	0.527 <i>A</i>
Mean	0.554	0.547	0.555	0.571	
RE ₀ /ET ₀					
Corentine RZ	0.247	0.298	0.250	0.230	0.256 <i>A</i>
Lozano	0.225	0.243	0.259	0.231	0.240 <i>A</i>
Rouxai	0.319	0.352	0.379	0.285	0.334 <i>B</i>
Mean	0.264	0.298	0.296	0.249	
PI _{-Abs}					
Corentine RZ	1.36 <i>a</i>	1.35 <i>a</i>	1.23 <i>a</i>	1.03 <i>a</i>	1.24 <i>A</i>
Lozano	1.08 <i>a</i>	1.20 <i>a</i>	0.87 <i>a</i>	1.62 <i>ab</i>	1.19 <i>A</i>
Rouxai	1.35 <i>a</i>	1.32 <i>a</i>	2.61 <i>b</i>	1.58 <i>ab</i>	1.71 <i>B</i>
Mean	2.16	1.29	1.57	1.41	

Table 4

Selected leaf spectral parameters of three lettuce cultivars cultivated under four different nutrient solution

Cultivar	Nutrient solution				Mean	
	I	II	III	IV		
ARI1						
Corentine RZ	0.984 <i>a</i>	2.739 <i>b</i>	0.922 <i>a</i>	0.571 <i>a</i>	1.304 <i>B</i>	
Lozano	-0.094 <i>a</i>	-0.073 <i>a</i>	-0.090 <i>a</i>	-0.103 <i>a</i>	-0.090 <i>A</i>	
Rouxai	6.840 <i>d</i>	5.241 <i>cd</i>	6.434 <i>d</i>	3.732 <i>bc</i>	5.562 <i>C</i>	
Mean	2.577 <i>B</i>	2.636 <i>B</i>	2.418 <i>B</i>	1.400 <i>A</i>		
CRI2						
Corentine RZ	3.606 <i>bc</i>	4.132 <i>cd</i>	3.376 <i>abc</i>	2.204 <i>ab</i>	3.330 <i>B</i>	
Lozano	1.747 <i>ab</i>	1.887 <i>ab</i>	1.591 <i>a</i>	1.710 <i>a</i>	1.733 <i>A</i>	
Rouxai	8.749 <i>g</i>	6.852 <i>ef</i>	7.558 <i>fg</i>	5.492 <i>de</i>	7.163 <i>C</i>	
Mean	4.700 <i>B</i>	4.290 <i>B</i>	4.215 <i>B</i>	3.135 <i>A</i>		
G						
Corentine RZ	1.975	2.013	1.912	2.164	2.016 <i>B</i>	
Lozano	2.301	2.400	2.237	2.362	2.325 <i>C</i>	
Rouxai	1.134	1.052	1.160	1.316	1.165 <i>A</i>	
Mean	1.803 <i>A</i>	1.822 <i>AB</i>	1.811 <i>A</i>	1.947 <i>B</i>		
MCARI1						
Corentine RZ	0.645 <i>abc</i>	0.696 <i>bcd</i>	0.608 <i>ab</i>	0.759 <i>de</i>	0.677 <i>B</i>	
Lozano	0.817 <i>e</i>	0.704 <i>bcd</i>	0.754 <i>cde</i>	0.754 <i>cde</i>	0.720 <i>C</i>	
Rouxai	0.621 <i>ab</i>	0.544 <i>a</i>	0.621 <i>ab</i>	0.538 <i>a</i>	0.581 <i>A</i>	
Mean	0.694	0.648	0.661	0.684		
NDVI						
Corentine RZ	0.462 <i>bc</i>	0.459 <i>bc</i>	0.449 <i>bc</i>	0.359 <i>a</i>	0.432 <i>B</i>	
Lozano	0.400 <i>ab</i>	0.409 <i>ab</i>	0.408 <i>ab</i>	0.408 <i>ab</i>	0.406 <i>A</i>	
Rouxai	0.548 <i>de</i>	0.517 <i>cd</i>	0.616 <i>e</i>	0.488 <i>cd</i>	0.542 <i>C</i>	
Mean	0.470 <i>B</i>	0.462 <i>B</i>	0.490 <i>B</i>	0.418 <i>A</i>		
NPC1						
Corentine RZ	0.110 <i>def</i>	0.087 <i>bcde</i>	0.130 <i>f</i>	0.103 <i>def</i>	0.108 <i>B</i>	
Lozano	0.095 <i>cde</i>	0.092 <i>cde</i>	0.064 <i>abc</i>	0.064 <i>abc</i>	0.078 <i>A</i>	
Rouxai	0.083 <i>bcd</i>	0.116 <i>ef</i>	0.049 <i>a</i>	0.057 <i>ab</i>	0.076 <i>A</i>	
Mean	0.096 <i>B</i>	0.099 <i>B</i>	0.081 <i>A</i>	0.074 <i>A</i>		
PRI						
Corentine RZ	-0.005 <i>cd</i>	-0.016 <i>bc</i>	-0.019 <i>bc</i>	0.008 <i>de</i>	-0.008 <i>B</i>	
Lozano	0.010 <i>de</i>	0.008 <i>de</i>	0.013 <i>e</i>	0.013 <i>e</i>	0.011 <i>C</i>	
Rouxai	-0.030 <i>ab</i>	-0.043 <i>a</i>	-0.044 <i>a</i>	-0.027 <i>ab</i>	-0.036 <i>A</i>	
Mean	-0.008 <i>B</i>	-0.017 <i>A</i>	-0.017 <i>A</i>	-0.002 <i>B</i>		

tion. The cultivars studied differed significantly in this parameter, with the lowest values found in cv. Lozano (for mean) and the highest in cv. Rouxai confirming the good vitality of this cultivar despite its lower weight. The reduced NDVI for nutrient solution IV (especially for Corentine) is consistent with the observed reduction in growth. NPCI (Normalized Pigment Chlorophyll Index) is an indicator sensitive to stress and chlorophyll content. Low values may indicate a higher chlorophyll content in relation to carotenoids – in the case of cvs Rouxai and Lozano, they had lower values than in cv. Corentine.

PRI (Photochemical Reflectance Index) is an indicator of photosynthetic efficiency, related to changes in the xanthophyll cycle and the rate of heat energy dissipation. Cultivar Lozano has the highest PRI, which would suggest high photosynthetic efficiency, while Rouxai has the lowest PRI value. However, this is not consistent with other fluorescence indices, which indicated that cv. Rouxai was the most efficient cultivar. The marked differences are likely due to differences in energy regulation between the cultivars.

Nutrient uptake

The determined nutrient uptake by the plants is presented in Table 5. Chemical composition of plants is shown in supplementary materials (S1,2). A reduction in the nitrogen content of the nutrient solution led to a decrease in its uptake by plants. The uptake of most macro- and microelements (N, P, K, Ca, Fe, Cu, Zn) was the lowest when using solution IV (the lowest yield), which is consistent with the observed decrease in biomass. Corentine showed a significant decrease in N uptake in nutrient solution IV, similar to Lozano. Interestingly, the decrease in nutrient solution IV was not as pronounced for cv. Rouxai, which may suggest that N uptake was also low in nutrient solutions I-III. While the lowest N uptake was observed in the lowest N content solution (IV), the highest uptake was unexpectedly found in solution II rather than I. The highest N uptake was recorded in solution II, and the lowest in IV. The cultivars differed in terms of an average P uptake, as was the case with N. Similar relationships (regarding the means) also applied to Mg and Ca. Significantly, the highest uptake of these nutrients was found in cv. Lozano, and the lowest in cv. Rouxai. Cultivars Lozano and Corentine absorbed more of both N and P. The reduction of nitrogen in nutrient solution IV probably results in lower phosphorus uptake, as the plant is unable to grow as intensively. At the same time, the average uptake of P, K, and Ca was lower for nutrient solution IV than for the others. The determined uptake of Mg positively related with the obtained plant yield and the uptake of other nutrients – cv. Lozano had the highest uptake, while cv. Rouxai had the lowest. In the case of nutrient solution II, where plant yield was highest, Ca uptake was also highest, while it was lowest in nutrient solution IV, which corresponds positively with the plant yield obtained. At the same time, cv. Lozano absorbed the most of the all nutrient, meanwhile cv. Rouxai showed the lowest absorption.

Table 5

Macroelement uptake (mg plant⁻¹) by three lettuce cultivars cultivated in four different nutrient solution

Cultivar	Nutrient solution				Mean
	I	II	III	IV	
N					
Corentine RZ	76.95 <i>e</i>	88.20 <i>f</i>	66.80 <i>c</i>	52.10 <i>a</i>	71.01 <i>B</i>
Lozano	110.77 <i>i</i>	100.00 <i>h</i>	97.30 <i>g</i>	63.05 <i>b</i>	92.78 <i>C</i>
Rouxai	62.97 <i>b</i>	71.60 <i>d</i>	70.78 <i>d</i>	65.10 <i>bc</i>	67.36 <i>A</i>
Mean	83.56 <i>C</i>	86.27 <i>D</i>	78.29 <i>B</i>	60.08 <i>A</i>	
P					
Corentine RZ	10.47 <i>d</i>	12.00 <i>e</i>	10.13 <i>cd</i>	7.53 <i>a</i>	10.03 <i>B</i>
Lozano	15.48 <i>g</i>	13.14 <i>f</i>	15.57 <i>g</i>	9.80 <i>cd</i>	13.50 <i>C</i>
Rouxai	8.63 <i>b</i>	9.45 <i>c</i>	10.37 <i>d</i>	8.53 <i>b</i>	9.24 <i>A</i>
Mean	11.53 <i>B</i>	11.53 <i>B</i>	12.03 <i>C</i>	8.62 <i>A</i>	
K					
Corentine RZ	146.70 <i>c</i>	166.35 <i>d</i>	140.95 <i>c</i>	84.99 <i>a</i>	134.71 <i>A</i>
Lozano	166.05 <i>d</i>	163.72 <i>d</i>	186.08 <i>e</i>	93.50 <i>a</i>	152.33 <i>B</i>
Rouxai	117.63 <i>b</i>	140.33 <i>c</i>	146.12 <i>c</i>	111.75 <i>b</i>	128.96 <i>A</i>
Mean	143.46 <i>B</i>	156.80 <i>C</i>	157.71 <i>C</i>	96.71 <i>A</i>	
Mg					
Corentine RZ	6.53 <i>cd</i>	7.18 <i>ef</i>	5.20 <i>a</i>	6.20 <i>bc</i>	6.27 <i>B</i>
Lozano	9.87 <i>ch</i>	8.83 <i>g</i>	8.60 <i>g</i>	7.73 <i>f</i>	8.76 <i>C</i>
Rouxai	5.03 <i>a</i>	5.60 <i>ab</i>	5.93 <i>bc</i>	6.83 <i>de</i>	5.84 <i>A</i>
Mean	7.14 <i>B</i>	7.20 <i>B</i>	6.58 <i>A</i>	6.92 <i>B</i>	
Ca					
Corentine RZ	18.60 <i>d</i>	19.93 <i>d</i>	15.70 <i>c</i>	15.17 <i>bc</i>	17.35 <i>B</i>
Lozano	33.70 <i>h</i>	31.23 <i>g</i>	28.15 <i>f</i>	22.83 <i>e</i>	28.97 <i>C</i>
Rouxai	12.40 <i>a</i>	13.23 <i>ab</i>	12.75 <i>a</i>	14.90 <i>bc</i>	13.32 <i>A</i>
Mean	21.57 <i>C</i>	21.45 <i>C</i>	18.86 <i>B</i>	17.63 <i>A</i>	

The trend of Fe uptake is generally consistent with the uptake of macro-nutrients: cv. Lozano showed the highest Fe uptake, while cv. Rouxai – the lowest (Table 6). Also, cultivation in nutrient solution IV, where yields were lowest, significantly reduced Fe uptake, which is consistent with the overall reduction in nutrient uptake. Again, Mn uptake was also lower by lettuce plants grown in nutrient solution IV, with cv. Lozano had the highest and cv. Rouxai the lowest uptake of this element. Similar uptake relationships were found for zinc and copper. Unlike the uptake of micronutrients, B uptake was similar in plants grown in nutrient solutions I and IV. As with the other micronutrients, cv. Lozano had the highest uptake, but the differences between Corentine and Rouxai were less pronounced.

Table 6

Micronutrient uptake ($\mu\text{g plant}^{-1}$) by three lettuce cultivars cultivated in four different nutrient solution

Cultivar	Nutrient solution				Mean
	I	II	III	IV	
Fe					
Corentine RZ	2558.7 <i>d</i>	2495.3 <i>d</i>	2297.4 <i>cd</i>	1487.1 <i>a</i>	2209.6 <i>A</i>
Lozano	3303.5 <i>e</i>	3886.7 <i>f</i>	3273.4 <i>e</i>	1615.2 <i>a</i>	3019.4 <i>B</i>
Rouxai	2126.0 <i>bcd</i>	1907.8 <i>abc</i>	2276.0 <i>cd</i>	1812.4 <i>ab</i>	2030.6 <i>A</i>
Mean	2662.8 <i>B</i>	2763.9 <i>B</i>	2615.6 <i>B</i>	1638.2 <i>A</i>	
Mn					
Corentine RZ	913.8 <i>bc</i>	1025.1 <i>c</i>	1020.5 <i>c</i>	1013.1 <i>c</i>	993.1 <i>B</i>
Lozano	1313.4 <i>d</i>	1404.8 <i>d</i>	1681.4 <i>e</i>	1313.0 <i>d</i>	1428.1 <i>C</i>
Rouxai	642.4 <i>a</i>	813.4 <i>b</i>	895.5 <i>bc</i>	999.6 <i>c</i>	837.4 <i>A</i>
Mean	956.5 <i>A</i>	1081.1 <i>B</i>	1198.8 <i>C</i>	1108.6 <i>B</i>	
Cu					
Corentine RZ	67.00 <i>cd</i>	94.53 <i>g</i>	85.48 <i>f</i>	55.80 <i>ab</i>	75.68 <i>B</i>
Lozano	104.75 <i>h</i>	112.10 <i>h</i>	133.90 <i>i</i>	78.20 <i>ef</i>	107.24 <i>C</i>
Rouxai	54.30 <i>ab</i>	60.20 <i>bc</i>	74.10 <i>de</i>	49.40 <i>a</i>	59.50 <i>A</i>
Mean	75.35 <i>B</i>	88.911 <i>C</i>	97.83 <i>D</i>	61.13 <i>A</i>	
Zn					
Corentine RZ	530.00 <i>b</i>	695.65 <i>cd</i>	742.65 <i>d</i>	413.58 <i>a</i>	595.22 <i>B</i>
Lozano	846.03 <i>e</i>	963.93 <i>f</i>	1205.20 <i>g</i>	547.10 <i>b</i>	890.56 <i>C</i>
Rouxai	416.18 <i>a</i>	492.03 <i>b</i>	640.48 <i>c</i>	384.45 <i>a</i>	483.28 <i>A</i>
Mean	597.40 <i>B</i>	716.87 <i>C</i>	862.78 <i>D</i>	448.38 <i>A</i>	
B					
Corentine RZ	316.80 <i>ab</i>	364.18 <i>b</i>	384.70 <i>b</i>	329.92 <i>ab</i>	348.90 <i>A</i>
Lozano	515.70 <i>c</i>	500.68 <i>c</i>	505.93 <i>c</i>	369.92 <i>b</i>	473.06 <i>B</i>
Rouxai	277.13 <i>a</i>	345.20 <i>ab</i>	381.13 <i>b</i>	384.43 <i>b</i>	346.97 <i>A</i>
Mean	369.88 <i>A</i>	403.35 <i>AB</i>	423.91 <i>B</i>	361.42 <i>A</i>	

DISCUSSION

The study aimed to evaluate the multifaceted response of three lettuce cultivars to four nutrient solutions with different chemical compositions, specifically varying nitrogen (N) content (ranging from 62 to 150 mg N dm⁻³).

We assessed the nutrient solutions' impact on plant growth, development, physiological activity (expressed by chlorophyll fluorescence), leaf reflectance spectrum (vegetation indices), and chemical composition. The analysis focused on macro- and micronutrient content, as well as nitrate levels, which are a key quality parameter in lettuce due to the potential for excessive accumulation (Kmecl et al. 2017; Zandvakili et al. 2019). While nitrates are generally nontoxic, a portion is converted to harmful nitrites in the human body, making it a health concern (Pinto et al. 2015, Fewtrell 2004, Salehzadeh et al. 2020). Therefore, a reduction in dietary nitrate intake from vegetables is strongly recommended (Manzocco et al. 2011).

The use of different nutrient solutions had a significant impact on lettuce quality. In each combination tested, the nitrate content was significantly lower than the current EU standards (UE, 2023). Clear correlations can be observed between the effect of the applied nutrient solution on the nitrate content of lettuce leaves, indicating that the cultivars responded differently to the applied fertilisation. The European Union law sets the maximum limits for nitrates in lettuce at 5000 in lettuce plants grown in winter and 4000 mg kg⁻¹ of fresh produce in the remaining seasons of the year. Growth conditions (greenhouse, indoor, field), light and nutrient content, especially nitrogen in a nutrient solution, can significantly affect the nitrogen content in leafy vegetables (Samouliene et al. 2009, Maynard and Hochmuth 2013, Iammarino et al. 2014, Sabat et al. 2015). Light can play an important role in nitrate accumulation in lettuce leaves, especially when it is cultivated year round in a greenhouses (NFT, the Nutrient Film technique with plants grown in nutrient channels, or DFT – the Deep Flow Technique) (Liu et al. 2016, Pinho et al. 2017, Matysiak et al. 2021).

The effects produced by the use of nutrient solutions II and III did not differ significantly, but the application of nutrient solution IV, with the lowest nitrogen content, significantly reduced the nitrate content in lettuce leaves (by as much as 48% compared to nutrient solution III). This reduction, however, was accompanied by a 26.9% decrease in yield. The low nitrate content (1193 mg N-NO₃⁻ kg⁻¹ F.W.) in solution IV suggests that plants were unable to effectively absorb this key nutrient, leading to a direct inhibition of growth and development. Despite the yield loss, our studies showed no damage to the photosynthetic apparatus in the nutrient solution IV treatment, as indicated by fluorescence (TR₀/ABS), although the increase in dry matter yield may point to a certain level of metabolic stress. Our research is generally consistent with earlier studies, which showed that the nitrate content in leaves is positively correlated with fresh plant weight, but negatively correlated with dry matter yield (Nicola, Fontana 2014, Di Gioia et al. 2017). The average NDVI value for nutrient solution IV (0.418) was the lowest of all variants, while the Relative Chlorophyll Content values remained low, indicating less chlorophyll and an impaired overall physiological condition. Generally, our studies are consistent with the research suggesting that

a temporary nitrogen deficiency (2-4 days before harvesting) – in our case reduction from the start of the vegetative growth - can reduce nitrate accumulation (Sago, Shigemura 2018, Tabaglio et al. 2020), but our results also highlight the negative impact on yield. In soilless cultivation, optimising the nutrient solution concentration is necessary to maximise yields and maintain appropriate lettuce quality (Fallovio et al. 2009, Sago, Shigemura 2015). This is particularly important during light deficiency, when the conversion of nitrogen to proteins is limited, which can lead to the accumulation of nitrates (Escobar-Gutierrez et al. 2002, Gromaz et al. 2017). The key is to finding an optimal nutrition strategy that balances the nitrogen level in the plant with its ability to grow and minimises nitrate accumulation. The research indicates that a simple reduction of nitrogen in the nutrient solution (as in Nutrient IV) is effective in reducing nitrates. Future research should focus on finding an optimal strategy that balances nitrogen levels to minimise nitrate accumulation while maintaining high yields. However, the yield and quality of plants obtained when using nutrients solutions II and III was better.

From the agrotechnical perspective, balancing a nutrient solution and the proportions between its components is crucial for achieving optimal crop yields. In our study, even though nutrient solutions II and III had the same nitrogen (N) content (120 mg N dm^{-3}), the average yield of plants grown in solution II was significantly higher for two cultivars. This highlights the substantial impact of the relationships between nutrients on plant yield, likely due to ionic antagonisms and synergisms. In nutrient solution II, the content of potassium, calcium, and magnesium was significantly higher (+34.2%) than in solution III, which underscores the vital role these nutrients play in yield production. Our findings are consistent with previous research by Sapkota et al. (2019), who also demonstrated that the proportions between nutrients significantly influenced lettuce yield. Vought et al. (2024) further support this by arguing that in hydroponic cultivation, plants should be fed with a nutrient composition tailored to their specific development phase.

It is important to note the variability in research findings. While Petropoulos et al. (2016) found that increasing the N dose from 100 to 200 mg dm^{-3} boosted fresh lettuce weight by 23.5%-113%, our results showed no increase in fresh weight yield when the N concentration was raised to from 120 to 150 mg N dm^{-3} . This suggests that for the specific cultivars we tested, using higher concentrations of this nutrient may be ineffective. As Hameed et al. (2022) stated, optimal N applications improve lettuce quality and nutritional value. For better ecology and management, Maruo et al. (2002) also recommend reducing nutrient concentrations in hydroponic systems. Ultimately, as Sapkota et al. (2019) concluded, different lettuce cultivars respond differently to fertilisation. Developing low-cost, easy-to-use hydroponic systems that account for these cultivar-specific needs can help growers produce high-quality vegetables efficiently. But with 3 varied cultivars in one pot – which is what consumers expect – it will be very difficult to grow them this way.

In our study, nutrient solution composition and cultivar differentiated the plant nutritional status. Despite the significant impact of a reduced nitrogen content on plant yield, none of the combinations tested revealed morphological changes suggestive of nutrient deficiencies. The nitrogen content determined in our study (Table S1) positively corresponds with studies by other authors, who, depending on the cultivation season, determined N levels ranging from 1.3-3.1% (spring) – Djidonou, Leskovar (2019) to 2-5% (Djidonou, Leskovar 2019, Samarakoon et al. 2020). Also in the case of phosphorus, they were within the ranges (0.39-1.05% P) reported by other authors (Pinho et al. 2017, Aini et al. 2019, Samarakoon et al. 2020, Singh et al. 2020). Also in the case of calcium (0.35-1.53%), magnesium (0.17-0.65%), iron (0.0047-0.0141%) and manganese (0.0098-0.483%), their amounts were generally within ranges shown by other authors (Domingues et al. 2012, Pinho et al. 2017, Samarakoon et al. 2020, Singh et al. 2020, de A. Moreira et al. 2022).

Measurements of chlorophyll fluorescence are a valuable diagnostic tool for assessing the physiological condition of plants, especially when morphological changes are not yet visible (Suliburska et al. 2024). Also spectrum measurements can be useful, among other things, for determining the harvest time for individual varieties (Ribeiro et al. 2023) or describing differences between varieties (da Silva et al. 2025). The high-performance phenotyping technique can be used to evaluate leaf pigments in breeding programs, as well as in crops for monitoring biofortification levels in lettuce (Clemente et al. 2023). In our studies, the results of spectral analysis of leaves and chlorophyll fluorescence were generally varied by the studied factor. TR_0/ABS , which reflects the maximum photochemical efficiency of the active centre of PSII, is one of the most important fluorescence parameters. Greater TR_0/ABS values result in higher light utilisation efficiency and a better ability to adapt to low-light conditions. Under normal physiological conditions, TR_0/ABS values range from 0.8 to 0.84. In our study, where the stress factor was N nutrition, the determined TR_0/ABS values were similar regardless of the nitrogen nutrition level (0.78), but varied depending on the cultivar (0.77-0.80). Values outside this range may indicate plant stress (Li et al. 2002, Lu et al. 2004, Yang et al. 2004, Chen et al. 2006). Therefore, even despite significant deterioration in lettuce yield in the case of N-62, there was no damage to the PSII system, but rather an adjustment of the plants to the uncomfortable situation of nitrogen deficiency. Another sensitive physiological indicator is PRI, which assesses whether plants are efficiently using energy for photosynthesis. Low, negative values indicate better condition and higher efficiency. In our study, a decrease in the level of N was accompanied by an increase in PRI, which positively corresponds to the opinion of Kohzuma et al. (2021) that the PRI index is significantly correlated with both net CO_2 uptake and radiation use efficiency. Pigment disruption of the photosynthetic complex promotes changes in electron transport, reduces the net assimilation rate of CO_2 , and deprives plants of essential sugars

(Lanquar et al. 2005), resulting in a reduction of dry matter production. The PRI is also sensitive to changes in carotenoid pigments (Wong, Gamon 2015). In our study, we demonstrated a positive effect of nitrogen nutrition on the increase in the content of anthocyanins and carotenoids, with the results for carotenoids corresponding positively to previous data reported by Qadir et al. (2017), who also showed a positive effect of nitrogen nutrition on the level of carotenoids in lettuce leaves.

Chlorophyll indices such as the Relative Chlorophyll Content (measured by CCM) and NDVI (Normalised Difference Vegetation Index) provide information about the chlorophyll content in leaves. Chlorophyll is essential for photosynthesis, hence its amount directly translates into a plant's ability to produce sugars. NDVI is a vegetation index, often used in remote sensing, which shows the overall condition and vigour of a plant. Higher NDVI values indicate healthier and greener biomass, and lower values can suggest some stress of plants (DeTar et al. 2006, Mirik et al. 2007, Liu et al. 2010). The Relative Chlorophyll Content (determined by CCM) measures the chlorophyll content at a given point on a leaf. It is a direct measure of a plant's greenness. In our study, the Relative Chlorophyll Content measurements did not indicate a linear relationship between the nitrogen nutrition level and chlorophyll content. But Hameed et al. (2022) indicates the modifying effect of the proportions between nitrogen forms (N-NO_3 and N-NH_4) on the chlorophyll content. In turn, Sapkota et al. (2019) found that the nitrogen content of the solutions showed a significant positive effect on the chlorophyll content. In our study, however, the cultivars differed in terms of the Relative Chlorophyll Content, which positively corresponds with the opinion of Caldwell and Britz (2006), who showed varietal differences in the chlorophyll content in greenhouse leaf lettuce. Cultivar Rouxai has the highest values for both the Relative Chlorophyll Content and NDVI, indicating its high chlorophyll content and excellent photosynthetic condition. High chlorophyll levels and favourable fluorescence ratios (RC/ABS , TR_0/ABS) suggest that it is very efficient at capturing light energy. Low biomass may be the result of a compromise, where the plant invests resources in the production of the photosynthetic apparatus rather than rapid mass growth, which may be an adaptive strategy to growing conditions. On the other hand, the low NDVI value for nutrient solution IV (0.488) clearly indicates deterioration in the physiological condition of the plants due to nutrient deficiency. Thus, these indicators correlate with yield, as a plant that is unable to photosynthesise efficiently will not produce much biomass. The explanation could be that cv. Rouxai has a growth strategy that focuses on photosynthetic efficiency rather than biomass growth rate alone. In contrast, cv. Lozano has low fluorescence indices, which may indicate that even with high nutrient uptake, its ability to process them is limited. The phenomenon can be explained by the concept of luxury consumption (Pen et al. 2023). The plant absorbs more nutrients than necessary for optimal growth. Instead of using them efficiently to build biomass, some of them are simply stored in the tissues (the high nitrate con-

tent). This may be a genetic trait of this cultivar, which intensively absorbs nutrients, but is less efficient at converting them into leaves and growth, making it susceptible to nitrate accumulation, but at the same time this does not translate into a proportionally higher yield. Its main feature is the intensive uptake of nutrients, not necessarily their effective use.

CONCLUSIONS

Reducing N content in the nutrient solution effectively lowers nitrate levels in plants, but this is accompanied by a significant reduction in crop yields, highlighting a critical trade-off in quality vs. quantity. At the same time, reducing the N level in the nutrient solution to 62 mg dm^{-3} did not damage the photosynthetic apparatus, although due to the growth achieved, it does not ensure proper plant growth. Even with the same N level, an increase in the content of K, Ca, and Mg (nutrient solution II vs. III) had a significant yield-forming effect, demonstrating that nutrient ratios are often more critical than the N concentration alone. The response of plants varied depending on the cultivar: (1) Lozano took up the most nutrients and achieved the highest fresh weight yield. However, the measured chlorophyll and fluorescence indices were not the highest. This cultivar accumulated high levels of nitrates. (2) Rouxai had the lowest fresh and dry weight, and stood out with the highest chlorophyll (Relative Chlorophyll Content, NDVI), pigment (ARI1, CRI2) and fluorescence indices, which means that it is very photosynthetically efficient and effectively uses the available components to build its photosynthetic apparatus. Its low biomass may be a genetic trait. (3) Corentine shows generally balanced results in nutrient uptake, growth, and physiological parameters. It is difficult to select cultivars with relatively balanced growth that are suitable for growing in a single pot. Chlorophyll and fluorescence indicators are valuable diagnostic tools that allow the assessment of plant condition in terms of their response to varying nitrogen nutrition. Their correlation with yield is clear, but not always direct, as different genetic strategies can differentiate plant response. Successful cultivation depends not only on the supply of nutrients, but also on selecting the right cultivar, whose genetics are best suited to the prevailing growing conditions and the expected yield.

Author contributions

T.K., J.T., A.K., W.K. – conceptualisation, data curation, conducting experiments, formal analysis, funding acquisition, investigation, methodology, project administration, validation, writing – original draft preparation, writing – review & editing, K.W., K.A. – chemical analysis, A.K., T.K. – statistical analysis, A.W. project administration, co-conducting vegetation experiments

All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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