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Morpho-Physiological, Chlorophyll Fluorescence, and Diffuse Reflectance Spectra Characteristics of Lettuce under the Main Macronutrient Deficiency

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Abstract: The aim of the present work was to assess the physiological state of plants and photosynthetic apparatus activity in lettuce (*Lactuca sativa* L.) by non-invasive methods (leaf diffuse reflectance spectroscopy and chlorophyll fluorescence) under the deficiency of one of the macronutrients (nitrogen, phosphorus, or potassium). Our experiments assessed the deficiency of each of the macronutrients relative to plants vegetating under optimal nutrition. The used methods showed that the deficiency of macronutrients causes changes in the optical characteristics of lettuce plants (cvs. 'Vitaminnyi' and 'Kokarda'), including a decrease in the chlorophyll content (57% and 51%) and a change in metabolism, which leads to a decrease in the efficiency of light energy conversion in photochemical processes of photosynthesis and an increase in the dissipation of excess light energy (19% and 10%). Linear regression equations, describing the relationship between net productivity and spectral characteristics of diffuse leaf reflectance with high accuracy, have been obtained. Changes in all studied indicators of the physiological state of plants under the influence of macronutrient deficiency are more pronounced at the early stages of development than in later periods, when the first symptoms of aging appear (decrease in ChlRI). The observed differences between lettuce cultivars and their response to nitrogen, phosphorus, or potassium deficiency are non-specific and mainly represent quantitative variation. The method for assessing the spectral characteristics of diffuse reflection of leaves seems to be the most promising for monitoring the physiological status of plants and early detection of nitrogen, phosphorus, or potassium deficiency.

Keywords: *Lactuca sativa* L.; macronutrient deficiency; diffuse reflectance indices; chlorophyll fluorescence; photosynthetic apparatus

1. Introduction

Fertilizers are one of the costliest resources of modern agricultural production, the non-application of which currently makes it impossible to obtain high-yielding plants. The need in increasing food reserves implies an increase in the amounts of fertilizers used, of costs of plant products production, but also an increase in the likelihood of environmental pollution. At present, the doses of fertilizers applied in a number of regions often exceed the amounts required for obtaining maximum yields [1,2]. The nonoptimal use of various types of fertilizers leads to aquatic ecosystems pollution, an increase in greenhouse gas emission, and other adverse consequences [3–5]. Strict correction of the fertilizer application norms is necessary, taking into account the actual needs of plants.

The three main macronutrients, namely nitrogen (N), phosphorus (P), and potassium (K), are required by plants in large quantities and play an especially important role in their life [6]. Nitrogen is the main component of chlorophyll and amino acids, all proteins and enzymes, and is part of nucleic acids and cell membranes. Nitrogen deficiency causes a decrease in transpiration, stomatal conductance, soluble sugars concentration, and in

the pool of electron acceptors in PSII. Phosphorus is a component of nucleic acids, which combine with proteins to form chromatin of ribosomes and other nucleoproteins in the nucleus and cytoplasm. Various phosphorus-containing compounds are involved in the reactions of photosynthesis and respiration, ensure the osmotic potential of cell sap, and play an important role in the cell energy metabolism. Although potassium is not part of the structural components of the cell, unlike nitrogen and phosphorus, it plays a key role in cellular osmoregulation. This essential nutrient is necessary to maintain the pH gradient between the inner and outer side of the thylakoid membrane, and it is also involved in the activation of more than 80 enzymes of plant metabolism [6]. Nitrogen, phosphorus, and potassium should be available to plants in optimal amounts, and their deficiency is usually replenished by the application of fertilizers, the excessive use of which entails environmental pollution.

Different species and varieties of cultivated plants differ in the optimal doses of fertilizers necessary for their growth and development, therefore, the determination of nutrients content in the soil alone will not allow optimizing cultivation technologies and the production process. To determine the optimal doses of fertilizers for the growth of a particular crop variety during the vegetation period, the physiological state of plants has to undergo either visual, or chemical, or functional diagnostics. The former is focused on external signs that appear on the leaves of plants in the case of nutrition disturbance. However, the appearance of such signs usually indicates irreversible and uncorrectable processes, which lead to a decrease in the yield and quality of crop products. More reliable is chemical diagnostics based on the laboratory assessment of the plant tissues chemical composition. Chemical methods for assessing the state of plants require considerable time and, due to the significant variability of soil and climatic conditions, do not make it possible to identify deficiency or excess of nutrients and assess the need of plants in a fertilizer on a real-time scale [1,7]. In this regard, the development of non-invasive contact and remote methods for rapid assessment of the state of vegetating plants is a modern tool for monitoring crops and the physiological state of plants in order to adjust agricultural practices depending on the current situation, as well as for managing qualitative and quantitative indicators of the yield.

The use of non-invasive methods, including remote probing, is becoming increasingly popular in crop production, in part due to the growing concerns about surface and groundwater pollution from excessive fertilization of agricultural land, and the necessity to compensate for the spatial heterogeneity of the field. In recent decades, a new trend in agricultural production management has been developing on the basis of the use of information technologies and modern equipment that provides precision monitoring of crops, identification of spatially differentiated heterogeneity in the state of plants within the field, and their need in additional fertilization [1,8,9]. According to this approach, fertilizers are applied only to those areas of the field where it is necessary, which makes it possible to significantly reduce the amounts of applied fertilizers without losses of or even with an increase in the yield, and improve the quality of agricultural products [10–13]. The use of this resource-saving and high-intensity technology ensures an increase in the profitability of agricultural production while reducing the risk of environmental pollution.

The non-invasive methods for crop condition assessment used for improving agricultural technologies, primarily the spatially differentiated application of fertilizers and other agrochemicals, find an increasing application for optimizing plant cultivation in protected ground and determining the response of accessions to environmental conditions, for standardizing experiments on phenotyping and selecting economically valuable genotypes for breeding. Non-invasive methods of monitoring and assessing the condition of plants have great prospects in modern breeding programs aimed at breeding cultivars that are responsive to fertilizers and use them efficiently, and are characterized by high potential yield, tolerance, and adaptability to adverse environmental conditions. An analysis of the spectral characteristics of the diffuse leaf reflectance in spring common wheat (*Triticum aestivum* L.) cultivars differing in drought resistance under optimal conditions and under

soil drought showed that they can be used in genotypes phenotyping and serve as criteria for predicting their potential productivity and resistance to water deficiency [14].

In comparison with visual and chemical methods, the non-invasive methods are less laborious, and require less material and time inputs to monitor the condition of plants or crops [15]. Measurements made by such methods do not require the destruction or damage of valuable genotypes and make it possible to evaluate traits in the same plant during its development or after the influence of stress factors.

Previously, we developed theoretical and methodological grounds for the identification of homogeneous technological zones for differentiated application of nitrogen fertilizers based on the results of a remote assessment of the colorimetric characteristics of the vegetation cover in the experimental field [16]. According to the developed method, the availability of nitrogen for plants and their need in fertilization includes obtaining digital images of the field with specially outlined test sites, in the soil of which strictly defined doses of nitrogen are introduced. Different nitrogen doses, i.e., the maximum one, which is expected to be applied to this field for obtaining the planned yield, as well as the minimum and 2–3 intermediate doses, ensure the formation of crops with different colorimetric characteristics. Quantitative description of the colorimetric characteristics of the vegetation cover in test plots using the 3D CIE LAB color space model [16] makes it possible to construct calibration curves describing the relationship between the colorimetric characteristics and the nitrogen dose. Further on, these curves are used for determining the needs of plants in the fertilizer and the dose of nitrogen fertilizers for each of the elementary areas of the field, the spatial coordinates of which are recorded using a GPS receiver.

Most of the published papers are devoted to the identification of crop field areas that suffer from nitrogen deficiency [17–20], which is mainly judged by the loss of chlorophyll and/or a decrease in the leaf area index. There are extremely few works that consider the optical symptoms of other types of mineral deficiency [21–24].

The aim of the present work was to assess the physiological state of plants by non-invasive methods, to analyze and compare changes in the diffuse reflectance spectra and photochemical processes of PSII in lettuce (*Lactuca sativa* L.) occurring in the case of one of the macronutrients (nitrogen, phosphorus, or potassium) deficiency under the controlled growing conditions.

2. Materials and Methods

2.1. Object of Research

Two early maturing lettuce (*Lactuca sativa* L.) cultivars from the collection of the N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR) where chosen as research objects. According to preliminary tests, they showed a high adaptive ability to growing under intense illumination and differed in morphological characteristics (Table 1).

Table 1. Morphological characteristics of lettuce accessions *.

VIR Catalogue No.	Name	Origin	Type	Rosette Type	Leaf Type	Leaf Blade Color
k-2886	Kokarda	Russia	Oakleaf	Appressed	Odd, pinnately sected	Dark green with anthocyanin pigmenting
k-2867	Vitaminnyi	Russia	Batavia	Suberect	Entire, sessile	Bright-green

* Accessions described according to Ref. [25].

2.2. Cultivation Conditions

Lettuce was sown as dry seeds in $50 \times 50 \times 5$ cm containers filled with a substrate based on the Agrobalt high-moor peat with a low degree of decomposition ("Pindstrup" LLC, Moscow Region, Russia). After the emergence of seedlings, the containers were placed in the original plant growing light equipment (PGLE) created at the Agrophysical Research Institute (AFI) [26] and located at the agroecobiological testing ground of AFI [27]. The seedlings were grown at a 14-h photoperiod and irradiance of 80–90 PAR/m^2; they were

watered and maintained at relative air humidity of 65–70% and temperatures of 20–22 °C during the day and 18–20 °C at night. Seven days after sowing, individual lettuce plants at the 2nd true leaf stage were transplanted into plastic pots for hydroponic lettuce lines (5 cm height and diameter, 0.12 L volume). After transplanting, the evenly developed seedlings at the three-to-four leaf stage (day 14 after sowing) were maintained in PGLE. Further on, the plants were grown by the Nutrient Film Technique (NFT) using nutrient solutions differing in nitrogen, phosphorus, or potassium content (Table 2, Figure S1, Supplementary Materials). The choice of experimental design was determined by the design features of the plant growing light equipment and the need to evaluate three types of macronutrient deficiency in sufficient replication. In each variant with a deficiency of one of the macroelements, 10 plants were analyzed (5 plants grown in each of 2 trays with a deficiency of N, P, or K). In the control, 15 plants were analyzed (5 plants grown in each of 3 trays). Light and temperature conditions were equalized by placing lamps, using mirror screens around the perimeter of the light equipment and air conditioning. Three times during the vegetation period (every 5 days), the hydroponic trays were removed within the light equipment.

Table 2. Contents of nutrient solutions (mg/L).

Option	Solution Characteristic Feature	N	P	Ca	K	Mg	S	Cl
1	Complete Knop nutrient solution(Control—C)	154	56	119	135	24	33	57
2	Nitrogen deficiency (ND)	86	56	119	135	24	33	57
3	Phosphorus deficiency (PD)	153	27	119	136	24	33	57
4	Potassium deficiency (KD)	153	56	119	78	24	33	57

A solution was supplied 4–6 times a day, depending on the stage of plant growth. The complete Knop nutrient solution with trace elements was used as the control (option 1).

The composition of trace elements in nutrient solution options 1–4 used in the work (g/L) was the same: $Fe(NH_4)_3(C_6H_5O_7)_2$—0.0087; H_3BO_3—0.0029; $MnSO_4(5H_2O)$—0.0019; $ZnSO_4(7H_2O)$—0.0002; and $CuSO_4(5H_2O)$—0.0002.

The main nutrients deficiency was modeled by reducing their content as percentage of the control: the dose of nitrogen, potassium, and phosphorus in options 2, 3, and 4 was 55.6%, 57.8%, and 48.2%, respectively, of these macronutrients content in the control (Table 2).

The vegetation period from sowing to harvesting was 30 days with a photoperiod of 14 h, 65–70% relative air humidity, 20–22 °C air temperature during the day, and 18–20 °C at night. For illumination, DNaZ-400 sodium lamps (Reflax LLC, Moscow, Russia) were used. Irradiation at the upper leaves level was maintained at 80–90 W/m^2 PAR. Morpho-physiological characteristics, spectral characteristics of diffuse leaf reflectance and chlorophyll fluorescence were recorded on days 21 and 28 after sowing.

2.3. Morpho-Physiological Characteristics

At the end of the vegetative experiment performed in 10 replications, biometric parameters of plant growth, including wet (Bw, g) and dry (Bd, g) leaf mass, were measured. The lettuce leaf area was determined from its linear dimensions using 0.67 as the calculated coefficients for different shapes of a brassica crop leaf blade [28]. The leaf area of a plant (LA, dm^2) was calculated by the formula $S_2 = 0.67 \times (S_1 \times n)$, where S_1 is the average area of one leaf, and n is the number of leaves. When determining the morphophysiologcal characteristics of lettuce, the average values were calculated for 10 plants of each experimental variant. The assessment of the structural and functional characteristics of leaves used two criteria, which characterize plant efficiency in the formation of dry matter and of the leaf assimilating surface area: LMA (leaf mass per area) and its reciprocal value SLA (specific leaf area). LMA is the mass of leaves per unit of their leaf surface (g/dm^2), and SLA is a part of the leaf area per unit of its mass, or the degree of the plant leafiness (dm^2/g) [29].

2.4. Pigment Content and Diffuse Leaf Reflectance Spectroscopy

Diffuse reflectance spectra in the 360–1000 nm range were recorded from the upper layer leaves, which had completed growth, using a fiberoptic spectroradiometer system (Ocean Optics, Largo FL, USA). The system includes a fiberoptic spectrometer HR2000, special software SpectraSuite, a reference tungsten-halogen light source LS-1, a diffuse reflectance standard (reference) WS-1, and a standard reflectance/backscatter probe R-200-7-UV-VIS. At least 15 spectra were recorded for each experimental option and used for calculating reflection indices characterizing the photosynthetic apparatus capacity (ChlRI) and the efficiency of its work (SIPI, R_{800}, PRI, and ARI) (Table 3).

Table 3. Reflectance indices characterizing the physiological state of plants, the capacity, and efficiency of their photosynthetic apparatus.

Reflectance Index of	Calculation Formula	Reference
Chlorophyll (ChlRI)	$(R_{750} - R_{705})/(R_{750} + R_{705} - 2R_{445})$	[30]
Total carotenoids to total chlorophylls ratio (SIPI)	$(R_{800} - R_{445})/(R_{800} - R_{680})$	[31]
Light scattering inside leaf tissues (R_{800})	R_{800}	[30]
Photochemical activity (PRI)	$(R_{570} - R_{531})/(R_{570} + R_{531})$	[31]
Anthocyanins (ARI)	$[(1/R_{550}) - (1/R_{700})] \times R_{750}$	[32]

Besides assessing the photosynthetic apparatus activity by the diffuse reflectance spectra of leaves and values of the corresponding reflectance indices, the content of pigments and other physiologically active compounds was also determined by traditional methods by extracting them into 100% acetone [33]. Spectrophotometric analyses of the obtained extracts were performed on a Cary 100 spectrophotometer (Varian Australia Pty Ltd., Mulgrave, Australia) at 645 and 662 wavelengths for chlorophylls '*a*' and '*b*' (Chl *a*, Chl *b*), and at 440 for carotenoids (Car). Anthocyanins were extracted with cooled ethanol containing 1% hydrochloric acid (*v/v*), followed by spectrophotometry [34]. The content of chlorophylls, carotenoids, and anthocyanins was calculated in mg per 100 g of the weighted wet sample [33]. To calculate the proportion of chlorophylls in the light-harvesting complex (Chl Light Harvesting Complex, Chl LHC), the following formula was used [33,35]:

$$\text{Chl LHC} = [(1.2 \times \text{Chl } b) + \text{Chl } b]/(\text{Chl } a + \text{Chl } b)$$

Since all Chl *b* is in the LHC, and the Chl *a/b* ratio in the LHC is about 1.2, the ratio of Chl LHC to reaction centers Chl, i.e., RC (Chl LHC/Chl RC), can be calculated as:

$$\text{Chl LHC/Chl RC} = (2.2 \times \text{Chl } b)/[\text{Chl } a - (1.2 \times \text{Chl } b]$$

This ratio makes it possible to estimate how much chlorophyll in LHC transfers energy to the RC chlorophyll and, like Chl *a/b*, allows the indirect characterization of the photosynthetic apparatus structural organization.

2.5. The Assessment of the Physiological Status of Plants by the Modulated Chlorophyll Fluorescence Method

The assessment of the physiological status of plants by the modulated chlorophyll fluorescence method under optimal nutrition and deficiency of one of the main macronutrients (nitrogen, phosphorus, or potassium) was based on the activity of the photosynthetic apparatus and used a MINI-PAM-II fluorimeter (MINI-PAM-II/R version) equipped with a source of red measuring and actinic light (maximum emission at 655 nm) and a source of far-red light with the maximum emission at 735 nm (Waltz, Effeltrich, Germany). In dark-adapted lettuce leaves, the minimum Fo and maximum Fm yields of Chl *a* fluorescence were determined. The same parameters, Fo′ and Fm′, were measured after the adaptation of the leaves to light. The determined main fluorescence values were used to calculate

the maximum (Fv/Fm) [36] and effective (Y(II)) [37] photochemical quantum yield of photosystem II (PS II), the coefficients of photochemical fluorescence quenching in the light (qP) [38], of photochemical fluorescence quenching in the case of interconnected PS II antennas (qL) [39], of non-photochemical fluorescence quenching in the light (qN) [38], of zeaxanthin-dependent non-photochemical quenching (NPQ) [40], and of the quantum yield of the light-induced (ΔpH- and zeaxanthin-dependent) non-photochemical fluorescence quenching (Y(NPQ)) [37], as well as of the quantum yield of uncontrolled heat dissipation and fluorescence emission, which do not include transthylakoid ΔpH and zeaxanthin Y(NO) [37]. The measurements of fluorescence parameters were made in five replications for each cultivar, test option, and case of macronutrient deficiency. The fluorescence intensity (μmol m^{-2} s^{-1} PAR) in lettuce leaves was recorded on days 21 and 28 of vegetation under the main macronutrients deficiency. Prior to measurements, the dark adaptation of plants was carried out for 30 min; during the measurements, and a metal clamp was used as a waveguide holder for excluding a possibility of ambient light penetration.

2.6. Statistical Analysis

Statistical processing of the results was performed using Excel 2013 and Statistica 12 (Stat-Soft, Inc., Tulsa, OK, USA). The mean values of the studied parameters and standard errors of the mean ($\pm$SEM) were determined. The significance of differences was also assessed by pairwise comparison of options using the nonparametric Wilcoxon test. For multivariate experiments, the analysis of variance was performed together with its non-parametric alternative, i.e., the assessment of differences between mineral nutrition options according to the Kruskal–Wallis criterion. The effect size (η^2) of a factor (nitrogen, phosphorus, or potassium deficiency) was determined in percent as the ratio of the corresponding sum of squared deviations of optical and morpho-physiological parameters under conditions of deficiency of one of the macronutrients from their average values to the total sum of squares according to the results of the analysis of variance performed in Statistica 12. Differences between options were considered significant at $p \leq 0.05$.

3. Results

3.1. Morpho-Physiological Indicators of Lettuce Plants under Optimal Mineral Nutrition and Main Macronutrients Deficiency

Under the conditions of optimal nutrition (control), the biomass of 'Vitaminnyi' lettuce plants that had formed by the harvesting time was 1.2 times greater less than that of the 'Kokarda' cultivar (Table 4).

The leaf assimilating surface formed by cv. 'Kokarda', which is 1.7 times larger compared with cv. 'Vitaminnyi', is determined by a large number of leaves formed by the time of harvesting, and a larger area of each of them. The number of leaves and their average area in cv. 'Kokarda' are 1.3 times bigger than in cv. 'Vitaminnyi'. Under the conditions of this experiment, lettuce cv. 'Vitaminnyi' had a stronger response to macronutrients deficiency. Under nitrogen deficiency, the mass of plants formed by the harvesting time was 21% less in cv. 'Vitaminnyi' ($p = 0.017$) and 17% in cv. 'Kokarda' ($p = 0.005$). Potassium deficiency reduced the mass of the studied lettuce cultivars by 17% ($p = 0.007$) and 13% ($p = 0.017$), respectively. The leaf assimilating surface area affected by nitrogen deficiency became smaller by 35% smaller in cv. 'Vitaminnyi' and by 24% in cv. 'Kokarda'. No significant changes in the studied morpho-physiological parameters were observed for the option with a phosphorus deficient nutrient solution (Table 4). Dry biomass (Bd) of lettuce cv. 'Vitaminnyi' under nitrogen deficiency was 14.4% less than that in control plants. A decrease in Bd under nitrogen deficiency was also observed in cv. 'Kokarda' (13%). The studied cultivars differed in their response to phosphorus deficiency. The amount of dry matter accumulated by plants of cv. 'Vitaminnyi' by the end of vegetation under conditions of phosphorus deficiency did not differ from the control, while in cv. 'Kokarda' Bd decreased by 8%.

Table 4. Morpho-physiological indicators of lettuce plants under optimal mineral nutrition and main macronutrients deficiency.

Option, Value		NL/P (pcs)	LA/P (dm^2)	Bw (g)	Bd (g)	LA/1l (dm^2)	SLA (dm^2/g)	LMA (g/dm^2)
				'Vitaminnyi'				
C	M *	18.6 ± 0.5	14.6 ± 0.7	72.3 ± 2.3	4.51 ± 0.20	0.78 ± 0.02	3.24	0.31
	%	100.0	100.0	100.0	100.0	100.0	100.0	100.0
ND	M	15.9 ± 0.3 *	9.5 ± 0.6 *	57.3 ± 2.4 *	3.86 ± 0.17 *	0.59 ± 0.03 *	2.46	0.41
	%	85.5	65.2	79.2	85.6	76.2	76.0	132.3
PD	M	18.2 ± 0.4	14.2 ± 0.3	70.4 ± 4.7	4.57 ± 0.28	0.77 ± 0.01	3.11	0.32
	%	97.8	96.2	97.4	101.4	98.9	95.9	103.2
KD	M	15.6 ± 0.3 *	9.4 ± 0.7 *	59.9 ± 3.3 *	4.60 ± 0.21	0.60 ± 0.04 *	2.04	0.49
	%	83.9	64.7	82.8	102.0	77.0	63.1	158.1
				'Kokarda'				
C	M	23.9 ± 1.0	24.3 ± 1.7	90.8 ± 2.8	5.80 ± 0.18	1.00 ± 0.05	4.19	0.24
	%	100.0	100.0	100.0	100.0	100.0	100.0	100.0
ND	M	21.5 ± 0.8 *	18.3 ± 1.0 *	75.2 ± 3.1 *	5.04 ± 0.23 *	0.85 ± 0.03 *	3.63	0.28
	%	89.9	75.6	82.8	86.9	84.7	86.6	116.7
PD	M	23.4 ± 0.5	22.3 ± 1.6	89.1 ± 5.3	5.34 ± 0.15 *	0.94 ± 0.06	4.18	0.23
	%	97.8	91.7	98.1	92.1	98.5	99.7	95.8
KD	M	22.5 ± 0.5	18.7 ± 0.7 *	79.4 ± 2.1 *	6.15 ± 0.37	0.83 ± 0.03 *	3.04	0.27
	%	94.1	77.2	82.8	105.9	77.0	72.6	112.5

Note. C—control, complete Knop nutrient solution, ND—Nitrogen deficiency, PD—Phosphorus deficiency, KD—Potassium deficiency. M denotes the indicator mean value; % is the difference from indicator values in control plants; NL/P is the number of leaves per plant; LA/P is the leaf area of one plant; LA/1l is the one leaf area; Bw is the plant wet biomass; Bd is the dry biomass; * marks the data significantly differing from the control ($p \leq 0.05$). Mean values of the measured indicators and standard errors of means (±SEM) are given; SLA and LMA were determined from LA/P and Bd mean values.

Therefore, the morphometric signs of the studied macronutrients deficiency are the decreased number of the formed leaves and the area of their assimilating surface, and a decrease in Bw and Bd of lettuce plants of both cultivars. Lettuce plants of cvs. 'Vitaminnyi' and 'Kokarda' suffer most of all from nitrogen and potassium deficiency, and the change in morphometric characteristics of cv. 'Vitaminnyi' is more pronounced in comparison with cv. 'Kokarda'.

SLA is mathematically defined as the ratio of the light-capturing leaf surface area to the dry mass spent on its formation. Being the most plastic leaf morphological indicator, SLA is often used as a fundamental one for characterizing photosynthesis, absorption efficiency, and light utilization [41–43], since it has a close linear connection with photosynthetic activity and relative growth rate. SLA has been shown to be closely and positively dependent on the nitrogen content in leaves and is negatively related to leaf lifespan [44].

According to the results of this study, SLA is in close negative with lettuce leaves dry mass Bd (r = −0.97, p = 0.0001) and in positive correlation with leaf area LA/P (r = 0.99, p = 0.00001) and wet biomass (r = 0.98, p = 0.00001). In case of nitrogen deficiency, SLA decreased by 24% in cv. 'Vitaminnyi' and 13% in cv. 'Kokarda'. Significant changes in SLA also occurred in the case of potassium deficiency, but not of phosphorus. Under potassium deficiency, SLA in cvs. 'Vitaminnyi' and 'Kokarda' was 37% and 27.5% less by the time of harvesting than in the control. In plant species with a higher SLA, photosynthesis is efficient because its products are spent less on the formation of leaves and because of the decreased loss of light energy when it passes through a thinner leaf. Cv. 'Kokarda' is characterized by higher SLA values than cv. 'Vitaminnyi', and it can be assumed that high productivity of cv. 'Kokarda' is largely determined by a greater efficiency of photosynthesis processes.

The LMA value is usually associated with the leaf thickness and density [45,46]. The deficiency of nitrogen, phosphorus, and potassium in our experiments led to the formation of a smaller leaf area (LA/P) by plants due to a decrease both in the number of leaves formed per plant (NL/P) and in the average area of each leaf (1LA). These changes are most pronounced in cv. 'Vitaminnyi' under the nitrogen and potassium deficiency, as well as in cv. 'Kokarda' under nitrogen deficiency. As was previously demonstrated in Ref. [47] for maize, wheat, soybean, and cotton, a decrease in both the number of leaves and their area under the deficiency of available nutrients leads to a reduction in the rate of photosynthesis per unit leaf area and, thus, becomes the cause of an overall reduction in the amount of photosynthetic assimilates available for growth. It was suggested by the authors of this work that the increase in the amount of dry matter and LMA under nutrient deficiency is associated with the formation of smaller cells and an increase in the apoplastic contribution to LMA [47]. In addition, under the lack of minerals, LMA can increase at the expense of starch, the accumulation of which is due to the reduced need of the growing organs in photosynthates [48].

Under nitrogen deficiency, LMA of cv. 'Vitaminnyi' increases by 32.3%. Under the same conditions, this indicator in cv. 'Kokarda' was 16.7% larger than in the control. Significant changes also occurred in the case of potassium deficiency in cvs. 'Vitaminnyi' and 'Kokarda' (58% and 12.5%, respectively). The reduced content of phosphorus contributed to the LMA decrease in cv. 'Kokarda' by 4.2% and its increase by 3% in cv. 'Vitaminnyi'. LMA is strongly and negatively correlated with LA/P ($r = -0.95$, $p = 0.0003$) and Bw ($r = -0.95$ $p = 0.0003$), and is positively correlated with Bd ($r = 0.95$, $p = 0.0003$).

The studied cvs. 'Vitaminnyi' and 'Kokarda' significantly differed from each other in the content of chlorophylls, carotenoids, and anthocyanins (Table 5).

Table 5. The content of chlorophylls, carotenoids, and anthocyanins in leaves of lettuce cvs. 'Vitaminnyi', and 'Kokarda' depending on the content of macronutrients in the nutrient solution.

Option, Value		Chl *a* (mg/100 g)	Chl *b* (mg/100 g)	Chl (*a+b*) (mg/100 g)	Car (mg/100 g)	Ant (mg/100 g)
		'Vitaminnyi'				
C	M	28.4 ± 1.9	8.4 ± 0.5	36.8 ± 2.3	10.0 ± 0.5	0.44 ± 0.02
	%	100	100	100	100	100
ND	M	18.2 ± 1.2 *	4.9 ± 0.3 *	23.1 ± 1.7 *	7.4 ± 0.4 *	0.73 ± 0.03 *
	%	64	58	62	74	165
PD	M	25.0 ± 1.5 *	7.2 ± 0.5 *	32.2 ± 4.7	9.6 ± 0.6	0.61 ± 0.03 *
	%	88	86	87	96	138
KD	M	25.9 ± 1.7	7.2 ± 0.5 *	33.2 ± 2.1	9.7 ± 0.6	0.62 ± 0.04 *
	%	91	86	90	97	140
		'Kokarda'				
C	M	48.1 ± 3.0	16.3 ± 1.2	64.4 ± 2.9	15.7 ± 1.2	3.9 ± 0.2
	%	100	100	100	100	100
ND	M	32.2 ± 2.5 *	10.6 ± 0.8 *	42.8 ± 3.5 *	12.0 ± 0.7 *	4.7 ± 0.3 *
	%	67	65	66	76	121
PD	M	49.3 ± 2.9	15.6 ± 0.9	64.9 ± 3.8	17.2 ± 1.1	4.4 ± 0.2 *
	%	102	96	100	110	113
KD	M	43.3 ± 2.5	13.7 ± 0.8 *	57.0 ± 3.5 *	15.8 ± 1.0	4.1 ± 0.2
	%	90	84	88	100	105

Note. Highlighted in bold are the control values (optimal nutrition); * marks the data significantly ($p \leq 0.05$) differing from the control (complete nutrient solution). M denotes the trait mean value. Mean trait values and standard errors of means (±SEM) are given.

In conditions of optimal mineral nutrition, the contents of Chl *a* and *b* in lettuce cv. 'Kokarda' were higher by 70 and 90%, respectively, than those in cv. 'Vitaminnyi'. Relative

to the control, these cultivars differed most significantly in the content of carotenoids (1.6 times) and anthocyanins (8.9 times). Differences in the chlorophyll content between the studied cultivars persisted in the options with a low content of nitrogen, phosphorus, or potassium. Nitrogen deficiency caused a decrease in the content of Chl *a* (by 36%) and Chl *b* (by 42%) in lettuce cv. 'Vitaminnyi'. Cv. 'Kokarda' also responded to the reduced nitrogen content in the nutrient solution by reducing chlorophylls concentration in leaf tissues (by 33% and 35%, respectively). Phosphorus deficiency reduced the concentration of Chl *a* and *b* by 12–14% in cv. 'Vitaminnyi', while it did not have a significant effect on the content of these pigments in leaves of lettuce cv. 'Kokarda'. Potassium deficiency contributed to a small but statistically significant decrease in the content of Chl *b*. In the case of nitrogen deficiency, the content of carotenoids in the leaves of lettuce cvs. 'Vitaminnyi' and 'Kokarda' was 26 and 24% less and did not change under phosphorus and potassium deficiency (Table 5).

The Chl *a* to Chl *b* ratio (Chl *a*/*b*) is an indicator of functional pigment equipment and light adaptation of the photosynthetic apparatus [32]. Chl *b* is located exclusively in the antennas of the light harvesting complex (LHC), while Chl *a* is present in the reaction centers of photosystems I and II (PSI and PSII) and in light harvesting antennas. The level of LHC-FS II varies and characterizes the adaptation of plants to light. The shaded plants form more LHC-FSII than the sun-exposed ones, and hence Chl *a*/*b* ratios in the former are lower than those of the sun-exposed plants [30,32]. Therefore, the decrease in the Chl *a*/*b* ratio can be interpreted as an expansion of the FS II antenna system. An increase in the Chl *a*/*b* ratio in lettuce plants in nutrient solution options with nitrogen, phosphorus, or potassium deficiency seems to be an indirect evidence of a decrease in the LHC antenna system and, accordingly, of a lower efficiency of light absorption by plants (Figure 1).
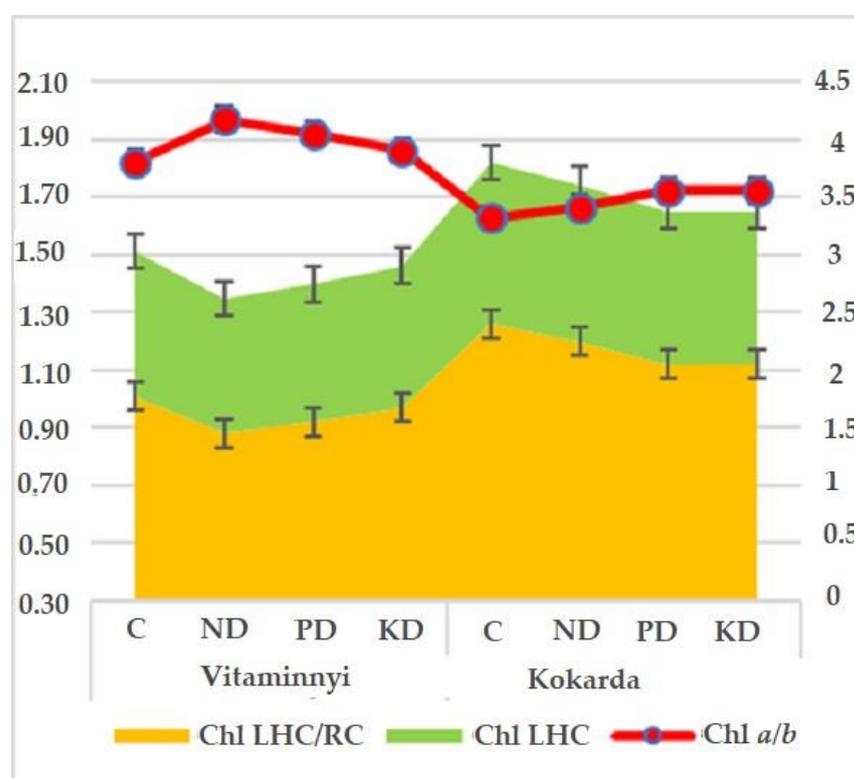

Figure 1. Elements of the photosynthetic apparatus of lettuce cvs. 'Vitaminnyi' and 'Kokarda' depending on the content of macronutrients in the nutrient solution. Control is the complete Knop's nutrient solution with trace elements. ND, PD, and KD are the Knop's nutrient solution with a reduced content of nitrogen, phosphorus, and potassium. Chl LHC/RC and Chl LHC are located on the main axis, Chl *a*/*b* on the auxiliary one. The standard errors of mean trait values (±SEM) are given.

Chlorophyll of light-harvesting complexes (Chl LHC), as well as the Chl *a/b* and the chlorophylls to carotenoids (Chl (*a+b*)/Car) ratios change under stress conditions [49,50]. This adaptation of the photosynthetic apparatus occurs due to changes in the composition and dimensions of the PSII antenna, and to the adjustment of the PSII/PSI ratio [43].

An increased Chl *a/b* ratio, a decreased content of chlorophyll in LHC and Chl LHC/RC were observed to be most pronounced under the nitrogen deficiency in cv. 'Vitaminnyi'. In contrast to the latter, cv. 'Kokarda' displayed a stronger change in these indicators under the phosphorus and potassium deficiency (Figure 1).

The considered changes in the content of chlorophylls, carotenoids, anthocyanins, and the values of Chl *a/b*, Chl LHC, and Chl LHC/RC under the influence of nitrogen, phosphorus, and potassium deficiency were determined at the end of vegetation period when the plants had finished active growth.

3.2. Diffuse Leaf Reflectance Indices of Lettuce under the Main Macronutrients Deficiency

According to the results of our previous works [14,51,52], the photosynthetic apparatus operation efficiency can be assessed with a high degree of reliability using the parameters characterizing the dissipation of energy as heat (PRI), the accumulation of carotenoids and, as a consequence, the change in the total carotenoids to total chlorophylls ratio (SIPI), anthocyanins and flavonoids accumulation (ARI and FRI), as well as a change in reflectance in the near infrared region (R_{800}), which is a consequence of the structural changes associated with the epidermal cell size, the presence of cuticle, and pubescence.

The indices characterizing the capacity and efficiency of the photosynthetic apparatus of lettuce cvs. 'Vitaminnyi' and 'Kokarda', measured on day 21 after sowing and placing the seedlings in PGLE, are presented in Table 6. The obtained results evidence a significant difference in the spectral characteristics of leaves of lettuce cvs. 'Vitaminnyi' and 'Kokarda'. The latter is characterized by higher values of chlorophyll and anthocyanin indices both under optimal conditions of mineral nutrition and in the case of a reduced content of main macronutrients in the nutrient solution (Table S1, Supplementary Materials). A deficiency in mineral nutrition had a significant impact on the optical characteristics of leaves of cv. 'Vitaminnyi' and changed almost all reflection indices. The only exception was in the absence of significant changes in the SIPI and R_{800} indices in the case of potassium deficiency. Nitrogen deficiency had the strongest effect on ChlRI (52.7%), SIPI (40.2%), and ARI (57.2%). The effect size of nitrogen deficiency on the reflection of radiation in the near infrared region (R_{800}) equaled 10.1%, and 18.8% on the value of the photochemical reflection index (PRI). The effect size of phosphorus deficiency on the reflectance indices of cv. 'Vitaminnyi' was also significant and reached 37.7, 26.9, and 47.3% for ChlRI, PRI, and ARI, respectively. Phosphorus deficiency had a lesser effect on SIPI than nitrogen deficiency (11.7% vs. 37.7%).

The effect size (η^2) of a factor and the level of significance of the effect of nitrogen, phosphorus, and potassium deficiency on plants of lettuce cvs. 'Vitaminnyi' and 'Kokarda' are shown in Table 6.

Potassium deficiency did not cause a significant change in SIPI and R_{800}, but its effect on the values of ChlRI, PRI, and ARI indices was statistically significant. The effect size of the factor on ChlRI, PRI, and ARI reached 20.4, 18.8, and 17.9%, respectively. It can be concluded that phosphorus and nitrogen deficiencies had a similar effect on the spectral characteristics of the diffuse leaf reflectance in lettuce cv. 'Vitaminnyi', and the differences between the deficiencies of these macronutrients were only in the size of the effect on each index. Potassium deficiency had a lesser effect on the spectral characteristics of leaves in the radiation reflectance ranges included in the calculation formulas of ChlRI, PRI, and ARI, but, unlike nitrogen and phosphorus deficiencies, it did not lead to a significant change in the indices characterizing the total carotenoids to total chlorophylls ratio (SIPI) and R_{800} indicator related to structural features of leaf tissues (Table 6).

Table 6. The effect size (η^2) of a factor and the level of significance of the effect of nitrogen, phosphorus, and potassium deficiency (day 21 of vegetation of lettuce plants).

Reflectance Index	ND η^2 *	ND p	PD η^2	PD p	KD η^2	KD p
			'Vitaminnyi'			
ChlRI	52.7 **	<0.0001	37.7	<0.0001	20.4	<0.0001
SIPI	40.2	<0.0001	11.7	0.0026	3.6	0.101
R$_{800}$	10.1	0.0056	10.0	0.0057	0.01	0.93
PRI	18.8	0.0001	26.9	<0.0001	12.0	0.0023
ARI	57.2	<0.0001	47.3	<0.0001	17.9	0.0002
			'Kokarda'			
ChlRI	50.9	<0.001	32.1	<0.0001	30.7	<0.0001
SIPI	0.02	0.893	0.6	0.473	0.6	0.829
R$_{800}$	3.5	0.091	12.8	0.0012	1.1	0.362
PRI	9.7	0.005	12.0	0.0018	5.2	0.042
ARI	0.3	0.865	2.1	0.202	2.6	0.157

* η^2 is the effect size of a factor in %, p is the level of significance of the factor effect; ** highlighted in italics are significant differences from the control values (optimal nutrition), $p \leq 0.05$. Mean values of reflectance indices and standard errors of their determination ($\pm$SEM) are presented in Table S1, Supplementary Materials.

An analysis of changes in reflectance indices characterizing the photosynthetic apparatus activity yields a conclusion that under conditions of unbalanced mineral nutrition both lettuce cvs. 'Kokarda' and 'Vitaminnyi' are sensitive to the deficiency of nitrogen, phosphorus, and potassium. The size of effect of these macronutrient deficiencies on the content of chlorophyll in the leaves of cv. 'Kokarda' was the highest and equaled 50.9, 32.1, and 30.7%, respectively. No effect of macronutrients on SIPI and ARI was found. The PRI value became larger at a reduced content of phosphorus and potassium (12 and 5%, respectively). A significant increase in R$_{800}$ was recorded only under the phosphorus deficiency (η^2 = 12.8%).

The optical characteristics of leaves of lettuce cvs. 'Vitaminnyi' and 'Kokarda' after 28 days of cultivation under conditions of basic nutrients deficiency differed from the control to a lesser extent than in the earlier part of the vegetation period (Table 7; Table 2, Supplementary Materials). The data in Table 7 evidence that the effect size of nitrogen deficiency on ChlRI of cv. 'Vitaminnyi' at the end of the vegetation period (day 28) decreases (η^2 = 23.2%), while in the cases of phosphorus and potassium deficiency no significant changes in this indicator were found (η^2 = 3.8% and η^2 = 1.4%, respectively). The effect of nitrogen deficiency on ChlRI was significantly higher in cv. 'Kokarda' than in cv. 'Vitaminnyi' (η^2 = 46.8%). Phosphorus and potassium deficiency had no significant effect on the studied leaf reflectance indices of cv. 'Kokarda', with the exception for a significant increase in PRI under the phosphorus deficiency (η^2 = 9.8%).

It has been established that there is a close correlation between the chlorophyll reflectance index ChlRI and the total chlorophyll content (determined in acetone extracts by the traditional method), which can be described by the linear regression equation:

$$\text{ChlRI} = 0.0863 + ((0.0069 \times \text{Chl } (a+b)); \text{r} = 0.954, p = 0.0002, \text{r}^2 = 0.910,$$

where Chl ($a+b$) is the total chlorophyll determined by the traditional method [33].

A close correlation relationship also exists between the anthocyanin index (ARI) and the anthocyanins content (Ant) in leaf tissues, between the index of the total carotenoids to total chlorophylls ratio (SIPI) and the same ratio determined by the traditional method (Car/Chl):

$$\text{SIPI} = 0.950 + (0.208 \times \text{Car/Chl}); \text{r} = 0.85, p = 0.0077, \text{r}^2 = 0.72,$$

$$\text{ARI} = 0.548 + (0.044 \times \text{Ant}); \text{r} = 0.76, p = 0.029, \text{r}^2 = 0.57,$$

Table 7. The effect size of the factor and the level of significance of the influence of nitrogen, phosphorus, and potassium deficiency (day 28 of vegetation of lettuce plants).

Reflectance Index	ND		PD		KD	
	η^2 *	p	η^2	p	η^2	p
			'Vitaminnyi'			
ChlRI	*23.2* **	*<0.0002*	3.8	0.111	1.4	0.313
SIPI	*19.0*	*0.0002*	1.2	0.371	1.0	0.701
R_{800}	*6.2*	*0.041*	23.4	<0.0001	3.1	0.149
PRI	0.9	0.90	*10.9*	*0.006*	*10.0*	*0.009*
ARI	*22.3*	*<0.0001*	1.0	0.407	0.9	0.756
			'Kokarda'			
ChlRI	*46.8*	*<0.0001*	0.2	0.907	0.7	0.948
SIPI	1.2	0.426	3.6	0.162	0.007	0.846
R_{800}	0.04	0.989	6.9	0.541	9.7	0.471
PRI	6.5	0.058	*9.8*	*0.018*	0.005	0.989
ARI	1.3	0.790	1.1	0.809	6.80	0.052

* η^2 is the effect size of nitrogen, phosphorus, or potassium deficiency in %, p is the level of significance of the factor effect; ** highlighted in italics are significant differences from the control values (optimal nutrition), $p \leq 0.05$. Mean values of reflectance indices and standard errors of their determination ($\pm$ SEM) are presented in Table S2, Supplementary Materials.

The spectral characteristics of diffuse reflectance make it possible to assess not only the content of photosynthetic and non-photosynthetic compounds in leaf tissues, but can also be used to predict plants yielding ability. The closest relationship between the lettuce plants yield (Bw) and the spectral characteristics of the diffuse leaf reflectance in the first half of the vegetation period (day 21) was found for ChlRI, SIPI, and R_{800}:

$$Bw = 32.1 + (111.9 \times ChlRI); r = 0.93, p = 0.0007, r^2 = 0.87$$

$$Bw = 679.3 - (597.4 \times SIPI); r = -0.76, p = 0.027, r^2 = 0.58$$

$$Bw = -37.3 + (3.19 \times R_{800}); r = 0.78; p = 0.022, r^2 = 0.61$$

ChlRI, one of the indices closely correlated with Bw, characterizes the photosynthetic apparatus capacity and the potential ability of a plant to absorb light, while the other two, SIPI and R_{800}, characterize the efficiency of its work. There is a reverse relation between the SIPI index and net productivity, and an increase in the value of this indicator is a sign of plant suppression and inhibition of their growth. Since the Chl (*a+b*) content is included in the denominator of the calculation formula for determining SIPI, it can be assumed that the close negative relationship of this index with the Bw value is more likely determined by a decrease in the chlorophyll content than by an increase in the carotenoid content.

The light scattering indicator R_{800} is related to the internal structure of the leaf and depends on the size of the cells and organelles. This relationship between the reflectance of leaves in the near infrared range at 800 nm (R_{800}) and characteristics of their structure was previously studied in 48 species of Alpine angiosperms [53]. The wavelength of 800 nm was chosen by the authors of Ref. [53] to distinguish the influence of the structure from that of the chemical composition of the leaf or the water content in it. The multiple regression analysis showed that R_{800} correlated with the proportion of mesophyll occupied by intercellular air spaces and with the ratio of the mesophyll cells surface area with intercellular air spaces to a leaf surface area unit [53]. In our experiment, a close positive linear relationship between R800 and the SLA leaf specific area was found:

$$R_{800} = 30.7 + (2.08 \times SLA); r = 0.79; p = 0.019; r^2 = 0.63$$

Ordoñez et al. [54] used 150 plant species to demonstrate that, depending on the soil fertility gradient, the specific leaf area (SLA), leaf nitrogen concentration (LNC), and leaf phosphorus concentration (LPC) show the same type of response to the compromise

between fast growth and leaf longevity. The authors suggested that SLA and LPC are regulated according to changes in plant size, while LNC is apparently regulated at the leaf level only by factors associated with leaf longevity [54].

3.3. Changes in Fluorescence Parameters of Lettuce Plants Influenced by Nitrogen, Phosphorus or Potassium Deficiency

The results of evaluating the efficiency of the lettuce photosynthetic apparatus depending on the regime of mineral nutrition were obtained using a MINI-PAM-II fluorimeter after 21 days of vegetation, and the determined significance levels of differences in the fluorescence parameters of control plants and those experiencing a deficiency of nitrogen, phosphorus, or potassium, are shown in Tables 3 and 4, Supplementary Materials, and the corresponding data after 28 days are presented in Tables 8 and 9. A comparison of the values of the maximum yield of leaves fluorescence after dark adaptation (Fm) and the light-adapted ones (F′m) revealed their significant decrease after 21 days of cv. 'Vitaminnyi' cultivation in conditions of phosphorus and potassium deficiency. Despite the trend of an increase in Fm and F′m, no changes in these indicators in cv. 'Kokarda' were observed. A lower Fm value indicates that not all PSII electron acceptors in cv. 'Vitaminnyi' can be fully restored and that plants are under stress. Due to the non-photochemical quenching in both studied cultivars, the F′m values in all mineral nutrition options are slightly less than those of Fm.

Table 8. Significance of differences between fluorescence indices in lettuce plants under optimal nutrition and under nitrogen, phosphorus, or potassium deficiency (day 21 of vegetation).

FL	'Vitaminnyi'			'Kokarda'		
	ND	PD	KD	ND	PD	KD
F	0.110	0.463	0.115	*0.027*	0.115	*0.027*
Fm′	0.500	*0.046*	*0.046*	0.173	0.463	0.074
Y(II)	*0.046*	0.173	0.463	*0.028*	*0.027*	*0.027*
Fo′	*0.027*	0.074	*0.027*	0.600	0.463	0.685
qP	*0.046*	0.249	0.345	*0.027*	*0.028*	*0.027*
qN	*0.027*	0.916	0.753	*0.027*	*0.046*	*0.046*
qL	*0.043*	0.248	0.225	*0.028*	*0.027*	*0.027*
NPQ	*0.027*	0.916	0.753	*0.028*	*0.046*	*0.046*
Y(NO)	*0.027*	0.172	0.600	*0.028*	*0.027*	*0.027*
Y(NPQ)	*0.027*	0.753	0.753	0.074	*0.046*	0.074
Fo	*0.027*	0.115	*0.027*	0.600	0.345	0.916
Fm	0.910	*0.046*	*0.043*	0.248	0.345	0.115
Fv/Fm	0.110	0.753	0.115	0.463	0.892	0.074

Note. Highlighted in italics are significant differences from the control values (optimal nutrition), $p \leq 0.05$. Mean values of reflectance indices and standard errors of their determination (±SEM) are presented in Table S3, Supplementary Materials.

The minimum fluorescence yield (Fo) after excitation with a very low intensity light and F′0 in the light-adapted plants significantly changes only in cv. 'Vitaminnyi' after 21 days of vegetation under conditions of nitrogen and phosphorus deficiency. The Fo parameter, which represents fluorescence under conditions when all reaction centers are open and photochemical quenching qP is at its maximum, is an indicator of energy losses during the excitation energy transfer from the antenna to the PSII reaction center.

The maximum photochemical efficiency (or potential quantum efficiency) of PSII (Fv/Fm) is considered in many works as a criterion for the photosynthetic apparatus photochemical activity. For most plants cultivated in favorable growth conditions, the maximum Fv/Fm value is 0.83 [36,55]. In the framework of this experiment, no significant changes in Fv/Fm were found in either cv. 'Vitaminnyi' or cv. 'Kokarda' after 21 and 28 days of vegetation in conditions of a reduced content of nitrogen, phosphorus, or potassium.

Table 9. Significance of differences between fluorescence indices in lettuce plants under optimal nutrition and under nitrogen, phosphorus, or potassium deficiency (day 28 of vegetation).

FL	'Vitaminnyi'			'Kokarda'		
	ND	**PD**	**KD**	**ND**	**PD**	**KD**
F	0,07	0.07	0.89	0.138	0.685	0.685
Fm′	0.07	0.13	0.89	0.138	0.345	0.892
Y(II)	0.07	*0.043*	0.89	0.079	0.500	0.224
Fo′	0.07	0.07	0.68	0.224	0.500	*0.043*
qP	0.07	*0.043*	0.89	0.079	0.345	0.225
qN	0.89	0.89	0.68	0.500	0.345	0.225
qL	0.06	*0.043*	0.22	0.079	0.345	0.224
NPQ	0.89	0.89	0.68	0.715	0.345	0.224
Y(NO)	0.34	0.68	0.22	0.225	0.500	1.000
Y(NPQ)	0.89	0.89	0.34	0.685	0.345	0.224
Fo	0.13	0.07	0.89	0.245	0.500	*0.043*
Fm	0.13	0.22	0.89	0.138	0.685	0.685
Fv/Fm	0.50	0.50	0.50	0.893	0.893	0.079

Note. Highlighted in italics are significant differences from the control values (optimal nutrition), $p \leq 0.05$. Mean values of reflectance indices and standard errors of their determination (±SEM) are presented in Table S4, Supplementary Materials.

When studying correlations between Fv/Fm and morpho-physiological characteristics of leaves and diffuse reflectance indices, the analyzed samples data included the results of the analysis of cvs. 'Vitaminnyi' and 'Kokarda' growing under different conditions of mineral nutrition (nitrogen, phosphorus, and potassium deficiency) for 21 and 28 days. When assessing the results of the expanded sample analysis, a significant negative relationship was found between Fv/Fm and ChlRI (r = −0.57, p = 0.045), Chl LHC (r = −0.57, p = 0.025), and Chl LHC/Chl RC (r = −0.58, p = 0.023), with morpho-physiological parameters such as LA (r = −0.66, p = 0.008), Bw (r = −0.64, p = 0.009), SLA (r = −0.87, p = 0.011), and R_{800} (r = −0.55, p = 0.031), as well as a positive association with LMA (r = 0.87, p = 0.011) and Chl *a/b* (r = 0.57, p = 0.028). The obtained results indicate that the maximum fluorescence quantum yield depends on both the content of chlorophyll, its content in LHC, RC, and on the structural features of leaves.

The Fv/Fm parameter is considered as an indicator of the D1 protein degradation during plant stress, which leads to the inactivation of PS reaction centers [56]. The absence of significant changes in Fv/Fm in lettuce cultivars at different stages of vegetation indicates that the created deficiency of the main macronutrients did not lead to the D1 protein degradation and did not cause damage to the reaction centers.

The effective photochemical quantum yield of PSII (YII) characterizes the ratio of the number of quanta used in photochemical reactions to the total number of absorbed quanta [37]. Previously, a linear relationship between Y(II) values and the carbon dioxide fixation rate during photosynthesis was found under laboratory conditions [37]. A decrease in assimilation efficiency in a stressed plant can lead to the slowing of electron transfer to PSI and PSII and a decrease in Y(II). It has also been shown that under the effect of unfavorable environmental factors this relationship can be disrupted due to changes in the carboxylation efficiency upon activation of photorespiration or the appearance of pseudo-cyclic electron transfer [57]. However, these processes can depend on the genotypic characteristics of the studied crops. The experiments by Ibrahimova et al. [58] have shown differences in the rate of linear electron transport between winter wheat accessions with sensitivity and resistance to a stressor (salt stress). It significantly decreased in sensitive accessions, while it did not change or even increased in resistant ones. A similar picture was observed when studying the proton transport at the thylakoid membrane [58].

In our experiments, Y(II) decreased most significantly in cv. 'Kokarda', in which quantum yield losses in photosynthetic photochemical reactions amounted to 53% under the nitrogen deficiency, and to 56 and 57%, respectively, under the phosphorus and potassium deficiency. In cv. 'Vitaminnyi', Y(II) reduced by 53%, while the sensitivity of this cultivar to

phosphorus and potassium deficiency was significantly less than that in cv. 'Kokarda'. A decrease in the effective quantum yield of PSII in cv. 'Vitaminnyi' under the phosphorus deficiency was 26%, and 10% under the potassium deficiency. However, these values fall within the measurement error due to the significant variation of the measured parameter in plants in the sample.

Determination of fluorescence parameters at different periods of plant vegetation showed that Y(II) was significantly higher in 21-day-old lettuce plants than in 28-day-old ones (Table S3, Supplementary Materials; Table 8 and Table S4, Supplementary Materials; Table 9).

Under optimal nutrition of plants of cvs. 'Vitaminnyi' and 'Kokarda' in the control, the effective quantum yield of Y(II) decreased by 58 and 56% with the increasing age of the plants. On the same dates of measurements under nitrogen deficiency, Y(II) of plants of cv. 'Vitaminnyi' was 47 and 45% relative to the control, and 47 and 56% of those of cv. 'Kokarda', respectively.

The correlation analysis showed the absence of a close relationship between Y(II) and the content of chlorophyll (ChlRI) in lettuce leaves. A decrease in Y(II) under the influence of nitrogen, phosphorus, or potassium deficiency is mainly determined by the intensity of photochemical quenching (qP), which consists of the conversion of light energy into chemical energy, which is then used in photosynthesis:

$$Y(II) = -0.0017 + (1.2377 \times qP); r = 0.99; p \leq 0.0005; r^2 = 0.99$$

The qP parameter represents the fraction of light energy consumed by the open centers in photosynthesis reactions, from the total energy absorbed by PSII. Changes in qP are caused by the closing of reaction centers due to the saturation of photosynthesis with active light. Under the deficiency of nitrogen, phosphorus, or potassium, the qP value reaches only 10, 75, and 87% in cv. 'Vitaminnyi' and 47, 41, and 42% in cv. 'Kokarda' relative to the values of this indicator in plants with optimal mineral nutrition. Changes in qP are caused by the closing of reaction centers due to the saturation of photosynthesis with active light.

The qL index also characterizes the photochemical quenching of fluorescence in the light, provided that many RCs are uniformly spread over a common antenna [39]. According to the results of our studies of chlorophyll fluorescence in lettuce leaves of cvs. 'Vitaminnyi' and 'Kokarda' vegetating under different regimes of mineral nutrition, there is a very close correlation between qP and qL (r = 0.993, $p \leq 0.987$).

Since the chlorophyll content in plant leaves usually allows them to absorb more light than they require for photosynthesis, some part of the energy not used in photosynthesis is dissipated as heat. Under plant stress, the regulatory mechanism preventing the supply of excitation energy to reaction centers is an increase in heat dissipation in the light-harvesting antenna (non-photochemical quenching). The present work considers several parameters of excess energy dissipation, namely the non-photochemical fluorescence quenching in the light (qN) [38]; zeaxanthin-dependent non-photochemical quenching (NPQ) [40], as well as additional PSII fluorescence yield coefficients, i.e., the light-induced (ΔpH- and zeaxanthin-dependent) non-photochemical fluorescence quenching (Y(NPQ)) [37] and quantum yield of unregulated heat dissipation and fluorescence emission, which do not include transthylakoid ΔpH and zeaxanthin (Y(NO)) [37]. According to the results of our study, the last indicator is most closely related to the effective quantum yield Y(II) of photosynthesis:

$$Y(II) = 0.9673 - [0.9682 \times Y(NO)]; r = -0.963; p = 0.00005; r^2 = 0.928$$

The Y(NO) value in cv. 'Vitaminnyi' was 11% higher under nitrogen deficiency and exceeded values of this indicator in the control under phosphorus and potassium deficiency only by 4 and 2%, respectively. Cv. 'Kokarda' responded to macronutrient deficiency by increasing the yield of unregulated heat dissipation and fluorescence emission by 11% (ND) and 13% (PD and KD).

The value of the non-photochemical fluorescence quenching in the light (qN) in cv. 'Vitaminnyi' did not change under phosphorus and potassium deficiency and was 56% less under nitrogen deficiency. A decrease in qN also occurred in cv. 'Kokarda' under nitrogen, phosphorus, and potassium deficiency by 40, 68, and 56%, respectively.

Similar to qN, the NPQ indicator is associated with thylakoid pH- and zeaxanthin-dependent quenching mechanisms. NPQ is an alternative to qN, which undergoes similar changes under nitrogen, phosphorus, or potassium deficiency. This parameter is related to heat losses and can take values from 0 to infinity. For the majority of healthy plants, NPQ values are in the 0.5–3.5 range, although significant differences can be observed in plants belonging to different species or grown under different conditions [58].

NPQ values in lettuce are low and vary after 21 days of vegetation under the main macronutrients deficiency from 0.018 (ND) to 0.04 (C, PD and KD) in cv. 'Vitaminnyi' and from 0.012 (PD) to 0.038 (C) in cv. 'Kokarda', which, apparently, can be considered as an insignificant involvement of pigments of the xanthophyll cycle and a partial decrease in the excitation energy transferred to PSII reaction centers, and their protection from photoinhibition.

The uncontrolled excitation energy losses, including heat dissipation and fluorescence emission Y(NO), and the controlled excitation energy losses due to heat dissipation involving ΔpH- and zeaxanthin-dependent Y(NPQ) mechanisms make it possible to estimate the distributions of light energy absorbed by a plant. For example, the value of Y(NPQ) in the case of exposure to strong light, is much higher than Y(NO). This indicates that photosynthetic energy flows are well regulated and excess excitation energy is dissipated at the antenna level. However, a high Y(NO) value indicates that the excess excitation energy reaches the reaction centers. This leads to a strong decrease in PS II acceptors and photodamage, for example, due to the formation of reactive oxygen species [39].

When comparing the measured fluorescence and diffuse reflectance spectra of leaves, a close correlation between some of them was found. A close negative relationship was found between the photochemical reflectance index PRI and Y(II), the effective quantum yield of PSII:

$$Y(II) = 0.6651 - (0.6313 \times PRI); r = -0.8207; p = 0.0125; r^2 = 0.673$$

The positive correlation between PRI and Y(II) can be explained by an increase in the reflectance coefficient at 570 nm due to the loss of chlorophyll (decrease in the PRI value) under the main macronutrients deficiency, especially nitrogen, and a decrease in its photosynthetic activity (effective quantum yield, Y(II), of PSII in [59]). The PRI value is determined by the intensity of thermal dissipation and is closely related to the fluorescence parameters characterizing the non-photochemical quenching: qN (r = 0.60, p = 0.014), NPQ (r = 0.59, p = 0.017), and Y(NPQ) (r = 0.56, p = 0.023). In addition, a negative correlation was found between PRI and the photochemical quenching indicator qP (r = −0.81, p = 0.016) and qL (r = −0.82, p = 0.013).

It was observed in this study that nitrogen, phosphorus, and potassium deficiencies have a nonspecific effect on PSII photochemistry. The question arises if it is possible to identify their deficiency using non-invasive optical methods, in particular, spectroscopy of diffuse leaf reflectance and fluorescence parameters. This question remains open, although the results of a number of studies suggest a positive answer. For example, Kalaji et al. [60] were able to recognize the deficiency of the main nutrients by analyzing key fluorescence parameters. They studied the effect of deficiency of some macronutrients (Ca, S, Mg, K, N, and P) and micronutrients (Fe) in hydroponic culture of tomato (*Solanum lycopersicum* L.) and maize (*Zea mays* L.). In these experiments, as well as in the present study with a deficiency of macro- and micronutrients, most accessions demonstrated only non-specific changes (a decrease in photochemical efficiency, an increase in non-photochemical dissipation, and a decrease in the number of active PSII reaction centers). A more detailed and in-depth study of the response of tomato and maize plants to nutrient deficiency revealed a specific response of the photosynthetic apparatus [60]. The strongest decrease in electron

donation by the oxygen-releasing complex was noted when Mg and Ca were deficient. Sulfur deficiency caused a limitation of electron transport beyond PSI, most likely due to a decrease in the content of PSI or the activity of PSI electron acceptors, while Ca deficiency had an opposite effect under which the activity of PSII was influenced much more than that of PSI.

Thus, fluorescence parameters provide information about the structure (PSII antenna size, photosystem integrity) and functions of PSII and of the electron transport chain. It should be emphasized once more that the usefulness of Chl *a* fluorescence is not limited to PSII. Fluorescence measurements also provide information about PSI functioning, the cyclic flow of electrons, the transfer of excitation to photosystems, and its distribution between them. Although the technical quality of Chl *a* fluorescence measurements and the efficiency of their performance have improved over the years, there still remain many fundamental questions about the obtained data interpretation. One of the most important problems associated with the use of fluorescence parameters is the inhomogeneity of photosynthetic samples. Different factors, such as aging and many other hard-to-control characteristics of plants and their environment, can cause variation in photosynthesis and fluorescence parameters within the area of the leaf where chlorophyll fluorescence is measured. It is difficult to take this type of photosynthetic inhomogeneity into account when making measurements.

The method for the evaluation of spectral characteristics of diffuse leaf reflectance seems to be more promising for diagnosing the physiological state of plants, studying their response to environmental stress factors, and their ability to adapt to environmental conditions, predicting productivity and selecting economically important accessions when implementing breeding programs. As shown above, the chlorophyll reflectance index is closely and positively related to plant productivity and can be used for yield prediction. Other reflectance indices (SIPI, R_{800}, PRI, ARI), the change in which was studied in the present work under the main macronutrients deficiency, are also associated with plant productivity and are suitable for the express evaluation of accessions in terms of their efficiency of using light energy in photosynthesis. Accurate determination of fluorescence parameters requires much more time, but they allow a more precise and comprehensive characterization of the photosynthetic process.

4. Conclusions

In our experiments, lettuce plants were grown by means of the Nutrient Film Technique (NFT) using nutrient solutions varying in nitrogen (N), phosphorus (P), and potassium (K) content. The effect of deficiency of these macroelements was assessed in relation to plants growing under optimal nutrition. Changes in the optical characteristics of lettuce leaves under conditions of macronutrient deficiency indicate a decrease in the pool of photosynthetic pigments (intensity of the photosynthetic apparatus) and a decrease in the efficiency of light energy conversion in the photochemical processes of photosynthesis. Under the influence of N, P, or K deficiency, changes in indicators characterizing plant growth and the state of their photosynthetic apparatus are more pronounced in the early stages than in the later stages of development, which is most likely associated with plant aging, natural growth inhibition, a decrease in chlorophyll content, and a decrease in the intensity and efficiency of photosynthesis. The observed changes in the optical characteristics of lettuce leaves with a reduced content of N, P, or K in the nutrient solution are non-specific and consist mainly of quantitative variations in the values of diffuse reflectance indicators of leaves and fluorescence parameters. It has been shown that reflectance indices characterizing the content of photosynthetic and non-photosynthetic pigments in leaf tissues are closely related to their content determined by traditional methods using organic solvents. The resulting linear regression equations accurately describe the relationship between net productivity and the spectral characteristics of leaf diffuse reflectance and can be used to predict potential productivity. The method for assessing the spectral characteristics of diffuse reflection of leaves seems more promising for studying the re-

sponse of plants to N, P and K deficiency, their ability to adapt to environmental conditions, forecasting productivity, and selecting economically valuable samples when implementing breeding programs.

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