

BIOCHEMICAL AND PHYSIOLOGICAL RESPONSES OF *Eruca sativa* MILL. TO SELECTED NUTRIENT CONDITIONS

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Abstract. The present study deals with biochemical and physiological methods for assessment of the optimal nutrient supply for the growth and development of garden rocket (*Eruca sativa* Mill.), edible vegetable. Two nitrate (0.3; 0.6 g N dm⁻³ of medium) and three potassium doses (0.3; 0.6; 0.9 g K dm⁻³ of medium) in the form of sulphate or chloride were examined. At the higher nitrate dose and the sulphate form of potassium the intense green colour of leaves, higher content of nitrates, flavonoids, L-ascorbic acid and lower carbohydrates content correlated with elevated growth parameters, e.g. the number of leaves and partially plant fresh weight. The proline and anthocyanin contents weakly diversified the nutrient supply. Despite the lack of modification in the photosynthetic pigment concentration, the chlorophyll fluorescence parameters were significantly improved when the higher nitrate dose accompanied the sulphate form of potassium (higher values of fluorescence decrease, maximum quantum efficiency of PSII photochemistry in the dark-adapted state, photochemical quenching and lower values of the fraction of absorbed light energy not used for photochemistry). The biochemical and photosynthetic parameters corresponding to the morphological characteristics (leaf colour, number of leaves and plant fresh weight) indicated that better nutrient conditions were provided to plants under the combined fertilization of the higher nitrate dose and the sulphate form of potassium.

Key words: anthocyanins, carbohydrates, chlorophyll fluorescence, flavonoids, proline

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Abbreviations: Car – carotenoids; Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*; F_0 – minimal fluorescence levels in the dark-adapted state; F_0' – minimal fluorescence levels in the light-adapted state; F_v/F_m – maximum quantum efficiency of PSII photochemistry in the dark-adapted state; HPLC – high performance liquid chromatography; LNU – a fraction of absorbed light energy not used for photochemistry; qP – photochemical quenching coefficient; qN – non-photochemical quenching coefficient; R_{fd} – fluorescence decrease

INTRODUCTION

From an agricultural point of view, it is highly important to avoid nutrient imbalances to achieve appropriate plant growth, development, biomass, and yield. Nutrient imbalances, which cause abiotic stress to plant, can be estimated using diverse parameters derived from primary (e.g. carbohydrates, proline and chlorophyll) and secondary metabolism (e.g. flavonoids, anthocyanins, carotenoids and ascorbic acid) [De Tullio and Arrigoni 2004, Sicher et al. 2012, Arbona et al. 2013, Müller et al. 2013, Filippou et al. 2014].

Soluble carbohydrates function mainly as osmoprotectants, reduce damage to the vital growing regions of the plant [Sicher et al. 2012], and are directly associated with photosynthesis [Arbona et al. 2013]. Moreover, the concentration of glucose and fructose increases in roots and leaves under stress conditions [Sicher et al. 2012]. It is known that high amounts of other saccharides [Arbona et al. 2013], as well as sugar alcohols, such as sorbitol, are accumulated in plant tissues in response to adverse conditions. Another well-known osmoprotectant is proline, the amino acid, which can be involved in not only redox balance and carbohydrate metabolism, but also photosynthesis process [Arbona et al. 2013]. A higher content of proline generally appears in stressful environments [Filippou et al. 2014].

Among the broad range of secondary metabolites, phenolic compounds such as flavonoids and anthocyanins are of great importance for plant defence and survival. Earlier report has shown that the elevated level of flavonoids is highly correlated with the accumulation of carbohydrates or with proteins [Müller et al. 2013]. High flavonoid and anthocyanin concentrations were detected in the leaves of plants lacking N, P, or K in the nutrient solution [Müller et al. 2013]. Ascorbic acid serves as an electron donor and acceptor in electron transport and a cofactor for enzymes involved in flavonoid biosynthesis [De Tullio and Arrigoni 2004]. Interaction of chlorophylls with carotenoids can regulate and dissipate excess energy protecting plants from damage [Arbona et al. 2013].

In addition to the above-mentioned biochemical components, chlorophyll fluorescence also be useful as a nondestructive technique for determination of the effects of nutrient imbalances on the photosynthetic apparatus, yielding selection criteria for verifying plant tolerance or sensitivity to adverse environmental conditions [Maxwell and Johnson 2000].

Agronomic practices rely on maximizing the yield of plants, which is associated with plant growth parameters. In previous studies with rocket [Nurzyńska-Wierdak 2009, Nurzyńska-Wierdak et al. 2012], mostly growth and yield were evaluated with a narrowly restricted number of biochemical analysis. In this study, we would like to

explain how the modifications of biochemical and physiological parameters (concentration of nitrate, carbohydrate, proline, non-enzymatic antioxidants, e.g. flavonoids, anthocyanins, ascorbic acid, photosynthetic pigments, and chlorophyll fluorescence) caused by fertilization with two nitrogen (N) and three potassium (K) doses in the sulphate (S) or chloride (Cl) forms affect plant growth (plant height, leaf colour and number, fresh and dry weight). These approaches are important in cultivation of edible vegetables, such as *Eruca sativa* species. The nutrients and their doses selected to the experiment were based on earlier studies and literature data [Nurzyńska-Wierdak 2009, Nurzyńska-Wierdak et al. 2012].

MATERIAL AND METHODS

Plant material and experimental design. The plant material used in this study was obtained from a seed production company PNOS Ożarów Mazowiecki, Poland. The plants were cultivated in the period from March to May 2013 in a greenhouse with the temperatures maintained in the range of 22–25°C during the day and 15–18°C during the night. Seeds of the garden rocket (*Eruca sativa* Mill.) were sowed individually in pots (2 L in volume) filled with a peat substrate. The pH of the substrate was in the range of 5.5–6.5. Before sowing, the seeds were dressed with the fungicide Dithane Neo Tec 75 WG (3 g kg⁻¹ of seeds). Combinations of N and K rates were tested in nutrient solutions. Two N doses (N₁ and N₂ as 0.3 and 0.6 g N dm⁻³ of medium, respectively) were applied in the form of Ca(NO₃)₂ and three K doses (K₁, K₂ and K₃ as 0.3; 0.6 and 0.9 g K dm⁻³ of medium, respectively) in the form of K₂SO₄ (44.9% K; 18.4% S) or KCl (52.4% K; 47.5% Cl). The plants were also supplied with other nutrients (g dm⁻³ of medium): 0.4 P in the form of granulated superphosphate (20% P); 0.2 Mg as MgSO₄ × H₂O; as well as the following micronutrients (mg dm⁻³ of medium): 8.0 Fe (EDTA); 5.1 Mn (MnSO₄ × H₂O); 13.3 Cu (CuSO₄ × 5H₂O); 0.74 Zn (ZnSO₄ × 7H₂O); 1.6 B (H₃BO₃); 3.7 Mo ((NH₄)₆Mo₇O₂₄ × 4H₂O). Fertilizers were divided into 3 equal parts and applied before seeding, 30 days after sowing, and 10 days before harvest. Salts were dissolved in distilled water and supplied to the plants by root application. The plants were watered every day, using 250–300 mL of water each time. Leaf rosettes of the garden rocket were harvested after 54 days.

Growth parameters and nitrate content. Growth parameters such as plant height (the height of the longest rosette leaves), number of leaves, whole plant fresh and dry weight, and the colour of leaves were estimated. The content of N-NO₃ was determined using the Bremner microdistillation method [Bremner and Keeney 1965].

Glucose, fructose, and sorbitol content. The content of glucose, fructose, and sorbitol in fresh rocket leaves was determined by high performance liquid chromatography (HPLC) with near infrared detection [PN EN 12630:2002].

Proline, flavonoid, anthocyanin and L-ascorbic acid content. The concentration of proline and flavonoids was determined spectrophotometrically according to Bates [1973], or Polish Pharmacopoeia VIII [2008], respectively. After extraction [Hawrylak-Nowak 2008] anthocyanins were determined spectrophotometrically [Mancinelli et

al. 1975]. The content of L(+)-ascorbic acid was determined by HPLC with UV detection [PN-EN 14130:2004].

Photosynthetic pigment content and chlorophyll fluorescence. Chlorophyll *a* (Chl *a*), *b* (Chl *b*) and total carotenoids (Car) were extracted using 80% acetone and their concentrations were determined spectrophotometrically [Wellburn 1994].

A PAM 101 Chlorophyll Fluorometer (Waltz, Effeltrich, Germany) with a PDA-100 system and Schott KL 1500 lamps was used to measure Chl *a* fluorescence. The minimal fluorescence levels in the dark- (F_0) and light-adapted state (F_0') were achieved at $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ and after applying far-red light, respectively. The maximal fluorescence levels in the dark- (F_m) and light-adapted state (F_m') were reached at $3800 \mu\text{mol m}^{-2} \text{s}^{-1}$. The following parameters were also calculated: fluorescence decrease (R_{fd} , $R_{fd} = (F_m - F_s) / F_s$), maximum quantum efficiency of PSII photochemistry in the dark-adapted state (F_v/F_m), photochemical (qP) and non-photochemical (qN) quenching coefficients, and a fraction of absorbed light energy not used for photochemistry (LNU, $LNU = 1 - (qP F_v' F_m) / (F_m' F_v)$).

Statistical analysis. All the experiments were conducted with a minimum of 3 replicates from 3 independent experiments (at least 9 measurements). The data was analysed statistically using Statistica 6.0. The significance of differences was carried out using Fisher's LSD test at $P \leq 0.05$. The results were expressed as means \pm SE. In order to reduce the set of parameters obtained in chlorophyll fluorescence (R_{fd} , F_v/F_m , qP, qN, LNU) into a smaller set of uncorrelated components, the principal component analysis (PCA) was carried out on 3 replicates.

RESULTS

Growth characteristics. The nutrient supply changed the number of leaves and plant fresh weight, but had no effect on plant height and dry weight (tab. 1). The higher N dose with S supplementation stimulated the number of leaves (except for N_2K_2S), but only partially elevated the fresh weight of plants (in N_2K_3S). The lower N dose with Cl supplementation stimulated plant fresh weight (except for N_1K_3Cl), but had no effect on the number of leaves. Compared with Cl, supplementation with S elevated the leaf number only in plants from the N_2K_1S and N_1K_2S combinations and the fresh weight in plants from the N_2K_1S and N_2K_3S with reduction thereof in N_1K_2S (tab. 1). In combinations containing the lower N dose and S the K dose differentiated the leaf number (the highest in N_1K_2S) and the fresh weight (the greatest in the K_3 dose). However, after incorporation of the lower N dose and Cl, the highest fresh weight was shown in the K_2 -treated plants (tab. 1). Moreover, the most intensive dark green colour was observed in plants treated with the higher dose of N (data not shown).

Biochemical characteristics. The higher N dose caused accumulation of nitrates after S supplementation (except for N_2K_2S), but was ineffective after Cl supplementation (except for N_2K_2) (tab. 1). Lower amounts of nitrates were accumulated by plants fed with S than Cl only in the N_1K_1 combination. The K_1 dose significantly elevated the concentration of nitrates only in the Cl-treated plants.

The only differences in sorbitol concentration appeared in the leaves of plants Cl-supplemented (tab. 1). The plant leaves from the N₁K₂Cl combination accumulated more sorbitol than N₁K₁Cl, N₂K₂Cl and N₂K₃Cl, which showed that the N and K ratio was partially significant in diversifying the sorbitol concentration (tab. 1).

Table 1. Growth parameters (plant height, number of leaves, plant fresh and dry weight), N-NO₃ and sorbitol content in 54-day-old *Eruca sativa* plants treated with two various N doses (N₁ and N₂ as 0.3 and 0.6 g N dm⁻³ of medium, respectively), three K doses (K₁, K₂ and K₃ as 0.3, 0.6 and 0.9 g K dm⁻³ of medium, respectively) and S or Cl. Values (±SE) are the means of at least 9 measurements. Different letters mean significant difference according to Fisher's LSD test at P ≤ 0.05

Experimental combination	Plant height (cm)	Number of leaves (plant ⁻¹)	Plant fresh weight (g)	Plant dry weight (%)	N-NO ₃ (% DW)	Sorbitol (mg g ⁻¹ FW)
N ₁ K ₁ S	22.8 ± 1.95 a	11.3 ± 1.77 d	28.6 ± 2.43 cd	9.4 ± 0.41 a	0.73 ± 0.19 c	90.6 ± 0.14 ab
N ₂ K ₁ S	26.7 ± 3.26 a	33.0 ± 2.31 a	30.4 ± 1.10 cd	9.7 ± 0.59 a	1.17 ± 0.15 ab	90.3 ± 0.08 ab
N ₁ K ₂ S	28.0 ± 2.62 a	22.0 ± 1.73 b	27.5 ± 1.55 d	9.4 ± 0.34 a	0.73 ± 0.13 c	90.6 ± 0.31 ab
N ₂ K ₂ S	23.0 ± 2.00 a	18.0 ± 1.53 bc	31.1 ± 1.08 cd	9.7 ± 0.52 a	1.17 ± 0.15 ab	90.4 ± 0.14 ab
N ₁ K ₃ S	26.1 ± 1.62 a	12.3 ± 1.20 d	28.3 ± 1.25 cd	9.9 ± 0.17 a	0.67 ± 0.17 c	90.7 ± 0.28 ab
N ₂ K ₃ S	27.4 ± 0.38 a	18.0 ± 2.08 bc	42.1 ± 1.30 a	9.6 ± 0.83 a	0.93 ± 0.19 bc	90.4 ± 0.12 ab
N ₁ K ₁ Cl	25.6 ± 3.40 a	13.0 ± 1.53 d	33.9 ± 2.03 bc	10.1 ± 0.55 a	1.20 ± 0.12 ab	89.9 ± 0.76 b
N ₂ K ₁ Cl	23.2 ± 2.14 a	11.0 ± 0.58 d	19.9 ± 1.25 e	9.5 ± 0.36 a	1.37 ± 0.09 a	90.5 ± 0.30 ab
N ₁ K ₂ Cl	28.2 ± 1.90 a	14.3 ± 1.20 cd	37.7 ± 4.57 ab	8.9 ± 0.64 a	0.93 ± 0.19 bc	91.1 ± 0.22 a
N ₂ K ₂ Cl	24.3 ± 0.64 a	13.3 ± 2.33 cd	28.7 ± 1.19 cd	9.9 ± 0.17 a	1.17 ± 0.09 ab	90.1 ± 0.26 b
N ₁ K ₃ Cl	26.9 ± 2.43 a	12.3 ± 1.20 d	28.8 ± 2.22 cd	9.2 ± 0.42 a	0.73 ± 0.15 c	90.8 ± 0.47 ab
N ₂ K ₃ Cl	22.1 ± 2.73 a	13.7 ± 0.88 cd	27.5 ± 1.32 d	9.9 ± 1.07 a	0.90 ± 0.10 bc	90.1 ± 0.16 b

The glucose concentration was higher for leaves from the combinations containing N₁ than N₂ (except for K₂Cl and K₃Cl) (Fig. 1A). Moreover, for glucose, the N₁S-treated plants did not differentiate between the K doses, but a decline was observed in the N₁Cl combinations, depending on the increasing K dose. For fructose, in both N₁S- and N₁Cl-treated plants, the elevated K dose reduced its concentration (fig. 1B). In plants from the N₂ combinations, the level of glucose (fig. 1A), but not fructose (fig. 1B), partially differentiated between the K doses (it was higher for K₃S than K₂S, and K₁Cl and K₂Cl than K₃Cl), and between its form, S or Cl (a lower value for K₂S than K₂Cl and a higher value for K₃S than K₃Cl). The highest concentration of the monosaccharides was detected for leaves from the N₁K₁Cl combination (fig. 1A, B).

The analysis of the proline content in the rocket leaves revealed the only significant difference between the two N doses for N₁K₁Cl (the highest value, followed by N₁ and N₂K₃Cl), and N₂K₁Cl (one of the lowest values) (fig. 2A). In comparison with Cl, the S form of K reduced proline accumulation in the leaves from the N₁K₁ and N₂K₃ combinations. The K dose did not change the proline concentration in plants from the combinations with S and both N doses.

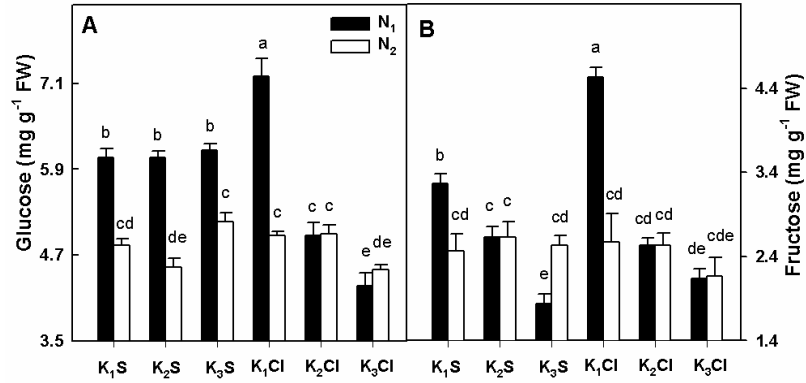


Fig. 1. Glucose and fructose content in 54-day-old *Eruca sativa* plants treated with two N doses (N₁ and N₂ as 0.3 and 0.6 g N dm⁻³ of medium, respectively), three K doses (K₁, K₂ and K₃ as 0.3, 0.6 and 0.9 g K dm⁻³ of medium, respectively) and S or Cl. Values (±SE) are the means; different letters mean significant difference according to Fisher's LSD test at P ≤ 0.05

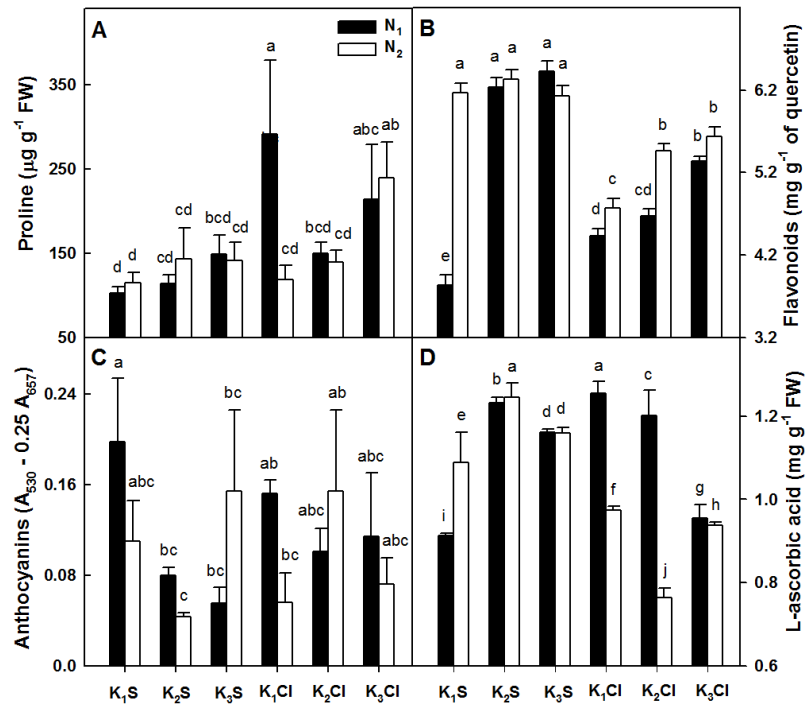


Fig. 2. Proline, flavonoid, anthocyanin, and ascorbic acid content in 54-day-old *Eruca sativa* plants treated with two N doses (N₁ and N₂ as 0.3 and 0.6 g N dm⁻³ of medium, respectively), three K doses (K₁, K₂ and K₃ as 0.3, 0.6 and 0.9 g K dm⁻³ of medium, respectively) and S or Cl. Values (±SE) are the means; different letters mean significant difference according to Fisher's LSD test at P ≤ 0.05

The highest values of flavonoid were found in all the combinations containing S (except for N₁K₁S), therefore the form of K was a differentiating agent in the level of flavonoid synthesis (fig. 2B). After Cl addition, the N₂ more than N₁ dose (except of K₃Cl) elevated the flavonoid concentration.

There were no differences in the anthocyanin content between the N doses and between the S and Cl supplementation (except for N₂K₂S – the lowest value and N₂K₂Cl – high value) (fig. 2C). Moreover, the higher K level in the form of S with N₁ dose lowered anthocyanin content.

Higher values of L-ascorbic acid were obtained in all the combinations with S than in those with Cl (except of N₁K₁) (fig. 2D). The higher N dose elevated (or had no influence in K₃S) the L-ascorbic acid accumulation when S was applied, but lowered its concentration after Cl supplementation. Additionally, the higher K dose in the Cl form with N₁ supplementation lowered L-ascorbic acid content, while in the S form the maximum was recorded with K₂.

There were no differences in the levels of the photosynthetic pigments between the N and K doses, but significantly lower concentrations were found for N₁K₂Cl (Fig. 3A, B, C).

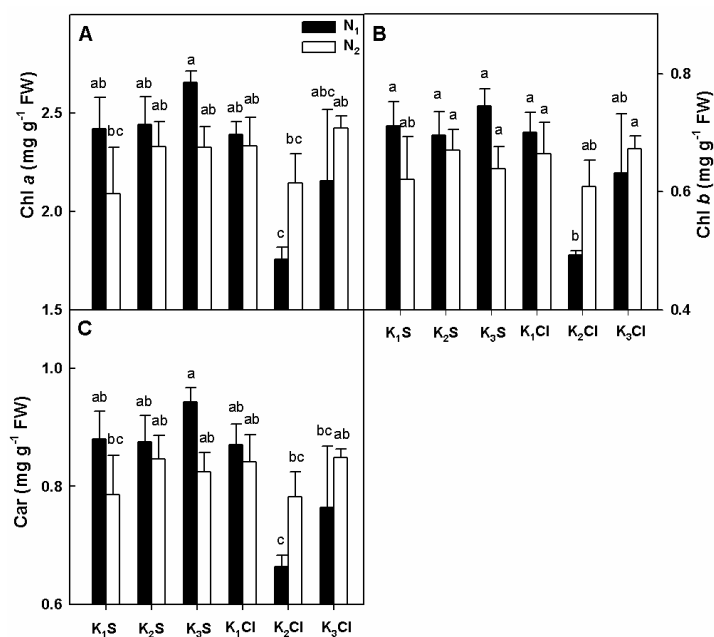


Fig. 3. Chlorophyll *a*, *b*, and carotenoid content in 54-day-old *Eruca sativa* plants treated with two N doses (N₁ and N₂ as 0.3 and 0.6 g N dm⁻³ of medium, respectively), three K doses (K₁, K₂ and K₃ as 0.3, 0.6 and 0.9 g K dm⁻³ of medium, respectively) and S or Cl. Values (±SE) are the means; different letters mean significant difference according to Fisher's LSD test at P ≤ 0.05

Chlorophyll fluorescence characteristics. The lower R_{fd} ratio was obtained at the N_1 and S and whole Cl supplementation than N_2 and S supplementation (except for N_2K_2), then the K form, but not its dose was a diversifying factor (fig. 4A).

The F_v/F_m ratio partially differentiated between the N doses. It decreased for combinations containing N_1 and S (except for K_2) and N_1K_1Cl compared with the N_2 dose (fig. 4B). The form of K was important only in K_3 supplementation; it was lower for N_1S and higher for N_2S combinations than in analogical combinations with Cl.

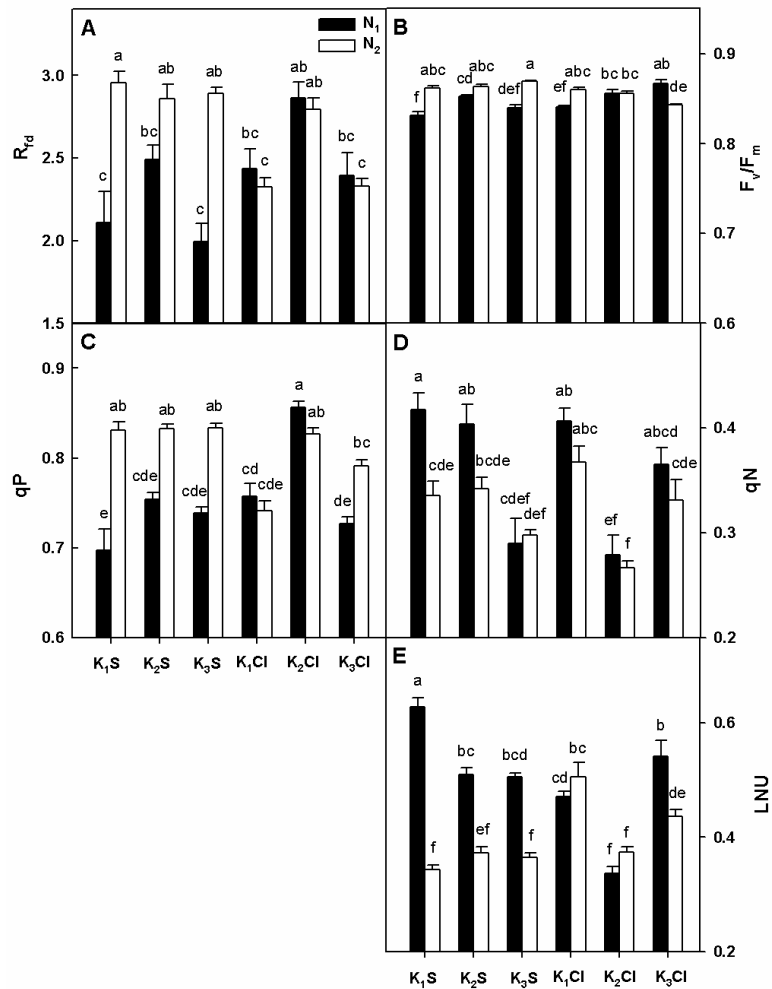


Fig. 4. Fluorescence parameters: R_{fd} , F_v/F_m , qp, qN and NPQ in 54-day-old *Eruca sativa* plants treated with two N doses (N_1 and N_2 as 0.3 and 0.6 g N dm^{-3} of medium, respectively), three K doses (K_1 , K_2 and K_3 as 0.3, 0.6 and 0.9 g K dm^{-3} of medium, respectively) and S or Cl. Values (\pm SE) are the means; different letters mean significant difference according to Fisher's LSD test at $P \leq 0.05$

The value of qP decreased in all the combinations containing N₁ and S, compared with N₂ and S and only in N₁K₃Cl compared with N₂K₃Cl, so the rate of N and partially the form of K were the differentiating agents (fig. 4C). Furthermore, S rather than Cl caused a significant decrease in the combinations containing N₁K₁ and N₁K₂, but an increase in N₂K₁.

The N dose was not a differentiating agent in the level of qN (except for increased value of N₁K₁S) (fig. 4D). The form of K and its dose were partially the differentiating factors because qN increased in N₁K₂S, N₂K₂S, and N₁K₃Cl compared with the N₁K₂Cl, N₂K₂Cl, and N₁K₃S combinations.

The LNU value increased in all the combinations containing N₁ and S compared with N₂ and S and in N₁K₃ compared to N₂K₃, therefore, the rate of N was a diversifying agent (fig. 4E). The S form of K elevated the LNU value at the K₁ and K₂ doses compared with Cl. In the N₁S-treated plants, the lower the K dose, the higher the LNU value. In the Cl-treated plants after N₁ addition, LNU value was the biggest at K₃ and the smallest at K₂, but after N₂ supplementation the opposite tendency was observed.

The first two factors of the PCA explained 90.41% of the variation. F_v/F_m, qP and R_{fd} registered a strong negative association and LNU strong positive relation with PC 1 (73.30%), but qN registered a strong negative association with PC 2 (17.11%). Generally, the PCA demonstrated that the data obtained from the combinations containing N₂ were quite well grouped and separated from the data containing N₁ (except for N₁K₂Cl and N₂K₁Cl). Secondly, when comparing the same experimental combination differing only the form of K, the data concerning S were separately grouped from those for Cl.

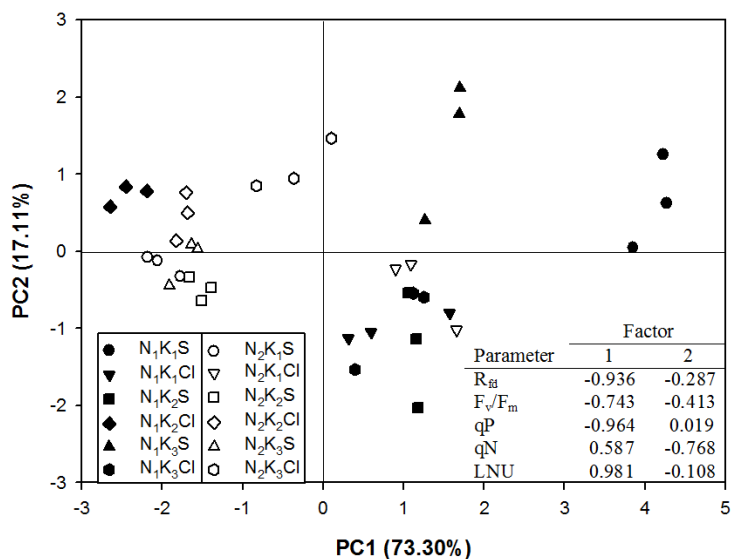


Fig. 5. Principal component analysis (PCA) of chlorophyll fluorescence parameters of the *Eruca sativa* plants. Factor 1 is strongly positively correlated with LNU, and negatively with F_v/F_m, qP and R_{fd}; factor 2 is negatively correlated with qN

DISCUSSION

The growth of the plants was dependent on the balanced concentration of nutrients. The environmental stresses can cause nutritional imbalances, which constitute an additional source of adverse conditions to plants [Sicher et al. 2012].

The nutritional value of leaf vegetables is associated with the amount of accumulated nitrates. The N content in rocket leaves increased (mostly significantly in the combinations with S) by increasing the amount of N in the nutrient medium, which is in accordance with other reports [Ceylan et al. 2002]. Our research showed that the application of potassium chloride could not be used as a strategy to decrease the N content in vegetables.

The pronounced promotion of glucose and fructose, as a response to stress conditions, was stated for the lower N dose with the weak fluorescence parameters. Moreover, growth inhibition could decrease carbohydrate export from leaves causing accumulation of sucrose and feedback inhibition of photosynthesis [Vinit-Dunand et al. 2002], which was confirmed in our experiment mostly for glucose, partially for fructose, and for fluorescence parameters. The reduced translocation of photoassimilates was proposed in rice [Moya et al. 1993], and this statement was generally true also for the examined garden rocket.

It has also been found that proline accumulates in response to stresses such as nutrients imbalances [Ashraf and Foolad 2007], which was detected in our experiment in a few combinations with Cl. However, the concentration of proline in the leaves from the combinations with N and S was not significantly different, which is in agreement with the results obtained by others [de Bona et al. 2013].

The amount of flavonoids depends on the balanced NPK dose [Oloyede et al. 2014]. It was evident in the conducted experiment that the flavonoid concentration was mainly higher for the supplementations with S and was partially dependent on N and K dose. Moreover, we found a mostly similar correlation between the anthocyanin (belonging to flavonoids) and flavonoid content, which was in agreement to other results [Müller et al. 2013].

Vitamin C is a sugar acid; therefore prevention of the decrease in vitamin C content was accompanied by an increase in the content of glucose, and fructose [Noichinda et al. 2007], which was clearly observed for the N₁K₁Cl combination in our research. Similar to the alterations in the flavonoid content, the vitamin C content was generally higher for the supplementations with S, which is in accordance with the statement that the S content in vegetables correlates with the L-ascorbic acid content [Lee and Kader 2000]. The specific influence of Cl on the quality of vegetables is not clear. Both the soluble sugars and the vitamin C content in lettuce decreased significantly when Cl alleviated [Xu et al. 2000], which was mostly confirmed by our results. The level of vitamin C largely depends on the developmental stage; at maturity, the vitamin C level in plant tissues declines [Noichinda et al. 2007]. A high L-ascorbic acid content can also be associated with high radical scavenging activity inevitable in stress conditions [Anjum et al. 2014].

Photosynthetic pigments were not severely affected by the nutritional changes in *Eruca sativa* plants, which showed that the plants possess ability to protect the photo-

synthetic apparatus against damage. Some articles reported the lowest chlorophyll content in the youngest wheat leaves under low N nutrition with higher distribution of N to the photosynthetic apparatus [Li et al. 2013], which stays in accordance with N₁K₃Cl combination from our experiment.

Nitrogen is known to depress F_v/F_m in *Fagus sylvatica* [Percival et al. 2008], which is partially in accordance with our findings. The F_v/F_m ratio decreased mainly due to inhibition of electron transport at the acceptor side of the PSII reaction centre [Filippou et al. 2014]. However, some experiments showed that F_v/F_m ratios in S starved barley plants were marginally affected despite the development of strong deficiency symptoms [Schmidt et al. 2013], similarly to our results – changing S into Cl depicted mainly a weak effect, which reflects the protective mechanism working to avoid photodamage to the photosynthetic apparatus.

The qP coefficient represents the reduced state of the primary quinone electron acceptor of PSII (Q_A). The significantly higher qP coefficient for the N₂ dose in all the combinations with S implied higher PSII electron transfer activity; therefore, the results were in accordance with those presented for sunflower [Ciompi et al. 1996]. Moreover, our data showed a relationship between F_v/F_m and qP under S and lower N supplementation. Not only F_v/F_m, but also qP decreased suggesting that the nutrient stress conditions induced inhibition of PSII electron transport and reduction of the photochemical activity of PSII, which is in accordance with other findings connected with low N-supply [Lin 2013].

The qN coefficient indicated the level of photoprotective capacity of the plant against damage caused by excess energy. Generally, our results depicted mainly lack of differentiation between the N doses. However, the stress was potentiated and qN increased at low N supply in the N₁K₁S combination compared with N₂ supplementation, which is in accordance with the results obtained by other scientists [Ciompi et al. 1996, Li et al. 2013].

A higher value of LNU was found for all combinations with the lower N content and S as well as the N₁K₃Cl combination, which in turn was accompanied by lower efficiency in utilization of absorbed energy in the photochemical processes of photosynthesis. The R_{fd} ratio is an indicator of CO₂ fixation. Increased R_{fd} values obtained in our experiment in combinations with the higher N dose and application of S indicate elevation in photosynthetic activity. According to PCA results, to obtain better parameters of photosynthesis, it is desirable to apply higher N doses and use S, not Cl. Moreover, the chlorophyll content did not explain the variability of the data acquired for fluorescence parameters.

In conclusion, the current study revealed that modifications in biochemical and physiological parameters caused by fertilizer application (N dose and K dose and its form, S and Cl) affected *Eruca sativa* growth. We showed that N shortage affected the whole plant metabolism and higher level of N enabled plant growth and development. The proline and anthocyanin contents weakly diversified the nutrient supply. Generally, the best condition for garden rocket growth was provided by cultivation on higher N dose with S form of K. It was determined by more intense green colour of leaves, higher number of leaves and partially also plant fresh weight, elevated content of nitrates, flavonoids, L-ascorbic acid and lower content of carbohydrates (especially glucose).

Moreover, besides analysed morphological and biochemical parameters, also the chlorophyll fluorescence parameters were good indicators of nutritional requirements. The photosynthetic apparatus worked most efficiently under higher N dose with S form of K, which was manifested by high F_v/F_m , qP , R_{fd} and low LNU values. However, strong changes in chlorophyll parameters did not influence photosynthetic pigment levels.

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**BIOCHEMICZNE I FIZJOLOGICZNE ODPOWIEDZI *Eruca sativa* Mill.
NA WYBRANE WARUNKI ŻYWIENIOWE**

Streszczenie. W badaniu zastosowano metody biochemiczne i fizjologiczne w celu optymalizacji zapotrzebowania na składniki odżywcze sprzyjające wzrostowi i rozwojowi rockety siewnej (*Eruca sativa* Mill.). Zastosowano dwie dawki azotu (0,3 i 0,6 g N dm⁻³) i trzy dawki potasu (0,3; 0,6 i 0,9 g K dm⁻³) w formie siarczanu i chlorku. Po zastosowaniu wyższej dawki azotu i siarczanowej formy potasu intensywny zielony kolor liści, wyższa zawartość azotanów, flawonoidów, kwasu askorbinowego i niższa zawartość węglowodanów odpowiadały podwyższonym wartościom parametrów wzrostu, tj. większej liczbie liści i częściowo wyższemu plonowi świeżej masy liści. Zawartości prolina i antocyjanów słabo różnicowały zapotrzebowanie rośliny na składniki odżywcze. Po zastosowaniu wyższej dawki azotu i siarczanowej formy potasu parametry fluorescencji chlorofilu poprawiły się (wyższe wartości wskaźnika witalności PSII, maksymalnej fotochemicznej wydajności PSII, wygaszania fotochemicznego i niższe wartości wskaźnika energii świetlnej pochłoniętej, a nie zużytej w procesach fotochemicznych) przy jednoczesnym braku wpływu na zawartość barwników fotosyntetycznych. Zmierzone parametry biochemiczne i fotosyntetyczne odpowiadały charakterystyce morfologicznej (kolor liści, liczba liści i świeża masa liści) wskazując, że lepsze warunki żywieniowe były dostarczalne roślinom w warunkach nawożenia wyższą dawką azotu i siarczanową formą potasu.

Słowa kluczowe: antocyjany, węglowodany, fluorescencja chlorofilu, flawonoidy, prolina

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