Journal of Soil Science and Plant Nutrition (2023) 23:4115–4126
https://doi.org/10.1007/s42729-023-01328-x

ORIGINAL PAPER

Changes in Antioxidant Metabolism and Plant Growth of Wild Rocket *Diplotaxis tenuifolia* (L.) DC cv Dallas Leaves as Affected by Different Nutrient Supply Levels and Growing Systems

Alessandra Villani[1,2] · Martina Loi[2] · Francesco Serio[2] · Francesco Fabiano Montesano[3] · Massimiliano D'Imperio[2] · Silvana De Leonardis[1] · Giuseppina Mulè[2] · Costantino Paciolla[1]

Received: 3 November 2022 / Accepted: 29 May 2023 / Published online: 9 June 2023
© The Author(s) 2023

Abstract

It has been widely assessed that the quality and quantity of nutrient supply and the growing system can strongly affect the growth and development of plants, the nutritional quality, and the levels of minerals, phytochemicals, and vitamins. The study was conducted on leaves of wild rockets grown in soil and soilless to examine the effect of growing conditions and mineral fertilization doses on the quality, mineral content, enzymatic and non-enzymatic antioxidants, and oxidative stress. The experiment was conducted in a plastic greenhouse into two independent sectors, one for soil-bound cultivation and another equipped for soilless cultivation. The crop was subjected to a high compared to a low-input fertilization program. Ion exchange chromatography, spectrophotometrically, and microbiological techniques were utilized. The soil-bound treatments increased the fresh weight, leaves number, chloride content, and microbial load. Exogenous application of higher nitrogen levels significantly boosted the ascorbate and hydrogen peroxide levels. Wild rocket growth in soilless showed a higher content of sulphates and polyphenols, and enhanced activity of the antioxidant enzymes dehydroascorbate reductase and monodehydroascorbate reductase. The fertilization rate and the cultivation system did not modify the content of nitrates, except in the soilless system treated with high fertilization program, where nitrate levels slightly exceeded regulatory limits. The yield and overall quality of wild rockets can be improved by combining the proper fertilizer dose with the growth system (soilless or soil) and suggested fertilization management is provided.

Keywords Ascorbic acid · Anions content · Fertilization strategies · Polyphenols · Soil and soilless cultures

Abbreviations

ASC	Ascorbic acid
CAT	Catalase
Cl^-	Chloride
DHA	Dehydroascorbic acid
DHAR	Dehydroascorbate reductase
DW	Dry weight
FW	Fresh weight
H_2O_2	Hydrogen peroxide
HF	High–input fertilization program
LF	Low–input fertilization program
MDA	Malondialdehyde
MDHAR	Monodehydroascorbate reductase
N	Nitrogen
NO_3^-	Nitrate
NS	Nutrient solution
POD	Peroxidase
SB	Soil-bound
SL	Soilless
SO_4^{2-}	Sulphate
TPC	Total phenolic content

Alessandra Villani and Martina Loi contributed equally to this work.

✉ Costantino Paciolla
costantino.paciolla@uniba.it

[1] Department of Bioscience, Biotechnology and Environment, University of Bari Aldo Moro, Via E. Orabona 4, 70125 Bari, Italy

[2] Institute of Sciences of Food Production, National Research Council of Italy, Via G. Amendola, 122/O, 70126 Bari, Italy

[3] Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Via E. Orabona 4, 70125 Bari, Italy

1 Introduction

Fertilization and water availability are key factors in regulating plant growth, yield, and the biosynthesis of secondary metabolites involved in the nutritional quality of products intended for human consumption (Duncan et al. 2018; Krouk and Kiba 2020). As defined by Evans and Fischer (1999), the potential yield of a crop is the maximum yield attainable in environments to which the plant is adapted, where abiotic and biotic stresses are controlled. Although actual yields are usually much lower than the attainable maximums, improved crop management practices and breeding techniques represent an important strategy to alleviate the negative effects of water and nutrient deficit (Ewert et al. 2005). Optimization of the fertilizer application is one of the crucial management practices to enhance the growth and development of plants and improve the nutritional quality and levels of minerals, phytochemicals, and vitamins.

Nitrogen (N) is one of the most important macronutrients required for plant growth, being involved in the biosynthesis of nucleic acids, proteins, chloroplasts, as well as secondary metabolites, such as alkaloids, non-protein amino acids, glucosinolates (GLS), and cyanogenic glucosides (Aires et al. 2006; Duncan et al. 2018; Wink 2013). It has been reported that N fertilization can have wide-ranging effects on productivity and nutritional quality (Bénard et al. 2009; Stitt 1999). Several studies showed that chlorophyll content, leaf area, and photosynthetic capacity increased with increasing N supply (Hoque et al. 2010; Mahlangu et al. 2016; Zhao et al. 2005). Higher N availability for plant absorption has been also shown to increase lycopene, carotenes, and vitamin B_1 contents in plants (Flores et al. 2004; Mozafar 1993). On the other hand, GLS (Li et al. 2007), total phenols (Fritz et al. 2006; Keller and Hrazdina 1998; Wilkens et al. 1996), and ascorbic acid (ASC) contents (Bénard et al. 2009; Hall et al. 2015; Mozafar 1993; Rajasree and Pillai 2012) were found to be significantly lower when higher N concentrations had been applied. Therefore, adequate N application is essential in order to ensure the optimal nutritional and functional quality of horticultural crops and to avoid the accumulation of nitrates (NO_3) in edible tissues and soil. Although the putative beneficial or harmful effect of nitrate intake on human health is still uncertain and highly debatable, the European Commission regulations No 1881/2006 and 1258/2011 established maximum nitrate limits for fresh mature vegetables, including *Diplotaxis tenuifolia*, since an excessive dietary intake of nitrate could expose some target population groups (vegetarians, infants, and elderly) at an increased risk of developing cancer (EFSA- European Food Safety Authority 2008, Santamaria 2006). Furthermore, it has recently been reported that modulation aimed at increasing or reducing the nitrate content is to be considered a new agronomic approach to obtain tailored products for specific nutritional purposes (e.g. vegetables with low nitrate content for children, vegetables with a high content of nitrates for athletes) (Renna et al. 2022).

N has been seen to play a crucial role in the growth and development of plants. However, there is evidence that the interaction and balance between N and other macronutrients, including phosphorus (P), potassium (K), sulphur (S), and calcium (Ca), can improve crop performance by enhancing resilience to environmental stress, nutrients uptake, and phytochemicals production (Amtmann and Armengaud 2009; Aulakh and Malhi 2004; Duncan et al. 2018). Furthermore, several studies highlight the efficacy of macronutrients interaction in plant growth and secondary metabolites production, and how the concurrent limitation of nutrients can affect plant fitness (de Bang et al. 2021; López-Arredondo et al. 2017), questioning the validity the Liebig's law of the minimum (Gorban et al. 2011), which states that growth is constrained by the scarcest resource (limiting factor).

Considerable effort has been made for understanding the physiological role of each nutrient within the various plant tissues and organelles. However, the impact of combinations of more macronutrients both on crop productivity, nutritional quality (Aulakh and Malhi 2005; Bénard et al. 2009; Duncan et al. 2018), and redox status of plant cells (Mahlangu et al. 2016) has been poorly investigated. In this context, the use of soilless growth systems has been increasing in the greenhouse horticultural sector, and, at present, the hydroponic cultivation of several leafy vegetables used for the fresh-cut industry is the preferred solution as compared to the traditional soil-based cultivation methods, because it may overcome problems related to soil-borne diseases and soil fertility and enhance crop productivity and quality (Di Gioia et al. 2018; Kolega et al. 2020; Mahlangu et al. 2016). The microbiological quality of fresh-cut products is a crucial parameter determining their overall quality because microbial growth may change the nutritive profile of leaves, as well as their physical appearance (Saini et al. 2017).

Wild rocket salad, with particular reference to *Diplotaxis tenuifolia* (L.) DC species, is gaining popularity, and the cultivation of this plant is in further expansion, both in greenhouse and open field conditions (Schiattone et al. 2017). Different growing techniques are related to the variability of growing cycle duration and specific characteristics of rocket species of agricultural interest (*D. tenuifolia, Eruca sativa* Mill., and *D. erucoides* (L.) DC). However, a few aspects relate to the impact of the cultivation system and nutrient solution composition on the nutritional quality and content of phytochemicals with antioxidant activity in those species have been investigated. Recently, Gioia et al. (2018) compared the GLS profile and content in three rocket salad species belonging to the Brassicaceae (*D. tenuifolia, E. sativa,* and *D. erucoides*) in relation to different growth systems, soil and soilless, and crop management. Another study, conducted by Yang et al. (2021), showed that total GLS, chlorophyll, carotenoids, and nitrate concentrations increased with higher electrical conductivity values in hydroponically grown *E. sativa.* Besides glucosinolates, rocket salad contains a wide range of health-promoting phytonutrients, including ascorbic acid, flavonoids, and polyphenols (Martínez-Sánchez et al. 2006). In the last decade, *D. tenuifolia*, well-known as wild rocket, has become very popular and appreciated by the fresh-cut industry, for its long shelf-life and fast-growing cycle (Hall et al. 2012).

Despite its current importance as a ready-to-eat salad, a proper nutrient management in soilless wild rocket crops is still required, as well as a deeper investigation of physiological and likely oxidative stress responses as affected by the composition of the nutrient solution and culture system. Our working hypotheses were the following: (i) that soilless cultivation could maintain crop productivity and quality compared to soil-bound cultivation (ii) that a low-input fertilization rate could be enough for improving the nutritional quality of *D. tenuifolia* cv Dallas without eliciting nitrate accumulation, to ensure the compliance with the maximum imposed limits (EC Regulation No. 1258/2011).

In this study the effects of different growing techniques (soil-bound and soilless cultivation) and different levels of nutrients supply (low- and high-input fertilization) on growth parameters, anions content, oxidative stress, and microbiological load in leaves of *D. tenuifolia* cv Dallas, which is currently the most widely grown cultivar in the Southern Italian regions, were investigated. In addition, non-enzymatic antioxidants, such as ascorbic acid and polyphenols, and the activity of antioxidant enzymes, such as catalase (CAT), peroxidase (POD), dehydroascorbate reductase (DHAR), and monodehydroascorbate reductase (MDHAR) were assessed.

2 Materials and Methods

2.1 Plant Material and General Growing Conditions

The experiment was carried out at the "La Noria" experimental farm of the Institute of Sciences of Food Production – National Research Council (Mola di Bari, Italy, 41°03' N; 17°04' E; 24 m above sea level). Wild rocket (cv Dallas, Isi Sementi, Fidenza, PR. Italy) plants were cultivated in a plastic greenhouse into two independent sectors, one for soil-bound cultivation and another equipped for soilless cultivation. Crop management practices (e.g., disease and pest control) were the same for both treatments and were based on local practices. In both growing condition cases (soil-bound, SB; soilless, SL), the crop was subjected to a high– compared to a low–input fertilization program (HF and LF, respectively; see Sects. 2.1.1. and 2.1.2 for details). According to the common practice for wild rocket salad, the crop cycle consists of subsequent harvests and re-shootings. For the purposes of this study, plants were harvested during the period occurring between April 28th and May 10th, 2021, when plants of different treatments reached the commercial harvest stage. In particular, the length of the growing period was 17 days for the soilless treatments and 21 days for the soil-bound treatments. During the plant re-shooting period prior to harvest, the average temperature was 18.3 °C, with a minimum temperature of 2.8 °C and a maximum temperature of 41.5 °C, while the air relative humidity ranged from 15.8 to 94.9%, with an average value of 59.5%.

2.2 Soil–Bound Growing Conditions

In soil–bound sector, soil was a typical Mediterranean "Terra Rossa" clay soil, classified as Alfisols according to the USDA soil taxonomy: 1.08% organic matter; pH 7.8; EC 2.4 dS m^{-1}. Plants were watered by drip irrigation, using collected rainwater. In SB–HF treatment, 60 kg ha^{-1} of nitrogen was applied by fertigation as calcium nitrate (Di Gioia et al. 2018); a reduced N dose of 30 kg ha^{-1} was adopted in the SB–LF treatment.

2.3 Soilless Growing Conditions

Rocket plants were grown in a peat:perlite (3:1) mixture in 4.5 L plastic pots. Nutrient solution (NS), prepared by mixing soluble fertilizer salts with pre-collected rainwater, was provided to plants via drip irrigation according to a prefixed irrigation schedule controlled by a timer (the schedule was periodically adjusted according to plant water need variations). In the SL–LF treatment, plants were fertigated with a NS containing N (11.0 mM), phosphorus (1.0 mM), potassium (4.4 mM), magnesium (1.7 mM), calcium (3.2 mM), sulphur (2.1 mM) (Di Gioia et al. 2018). In the SL–HF treatment a NS containing N (15.0 mM), phosphorus (1.0 mM), potassium (6.0 mM), magnesium (2.0 mM), calcium (5.0 mM), sulphur (2.9 mM) was used. In both treatments, iron (20 μM), manganese (5 μM), zinc (2 μM), boron (25 μM), copper (0.5 μM), and molybdenum (0.1 μM) were added in NS as micronutrients (Hoagland and Arnon 1950).

2.4 Measurements

All chemicals used in this study were of the highest grade available (Sigma-Aldrich). Ultrapure water was produced by a Milli-Q system 84 (Millipore, Bedford, MA, USA). Spectrophotometric analyses were performed using a Beckman Coulter DU800 (Beckman Coulter, Fullerton, CA, USA).

2.5 Plant Growth, Nitrate, Chloride, and Sulphate Contents

All treatments were harvested at commercial stage when leaves reached 10–12 cm in length. After determining the number of leaves, fresh weight, and leaf area (Li–3100, Licor, NE, USA), a sub-sample for each experimental unit of approximately 100 g was dried in a forced–air oven at 65 °C until constant weight and the dry matter was determined. Dried samples were finely ground and used to determine chloride (Cl^-), nitrate (NO_3^-), and sulphate (SO_4^{2-}) contents. Those were determined by ion exchange chromatography technique (IC–Dionex DX120, Dionex Corporation, Sunnyvale, CA, USA) with a conductivity detector, according to D'Imperio et al. (2021). Briefly, dried vegetable material was treated with solution of Na_2CO_3 (3.5 mM) and $NaHCO_3$ (1 mM), for 30 min, at room temperature. Then, the extracts were diluted and filtered by using 0.45 µm (RC) followed by a Dionex OnGuard IIP (Thermo Scientific) in order to remove organic compounds. The solutions obtained were analysed by ion chromatography (IC–Dionex DX120) with a conductivity detector, by using an IonPac AG14 precolumn and an IonPac AS14 separation column (Thermo Scientific) at 35 °C, flow 1 mL min^{-1}. In addition, the accuracy and precision of the chemical analysis of NO_3 were evaluated by using certified reference materials (CRM) SPIN–1_spinach. The recovery of NO_3 ions was 102 ± 6%.

2.6 Levels of Reduced, Oxidized, and Total Ascorbic Acid

The ascorbate pool contents including ASC, dehydroascorbic acid (DHA), and total ascorbic acid (ASC + DHA) were quantified according to the methods of Law et al. (1983). Briefly, one gram of fresh leaf tissue was homogenized with four volumes of cold 5% metaphosphoric acid in a porcelain mortar with quartz sand. After centrifugation (20,000 × *g*, 4 °C, 15 min), the supernatant was collected and immediately assayed for ASC and total ascorbic acid. The sample extract (0.1 mL) was mixed with 0.2 mL 0.15 M phosphate buffer solution (pH 7.4) containing 5 mM ethylenediaminetetraacetic acid (EDTA) and 0.05 mL 10 mM dithiothreitol (DTT) and then incubated for 10 min at room temperature to reduce all DHA to ASC. After incubation, 0.05 mL of 0.5% N–ethylmaleimide (NEM), 0.15 mL 10% (w/v) trichloroacetic acid (TCA) containing 3% (w/v) $FeCl_3$, 0.2 mL 44% H_3PO_4, and 0.2 mL 4% (w/v) 2,2–bipyridyl in 70% ethanol were added to the mixture and mixed. For the ASC content, DTT and NEM were substituted with deionized H_2O. The reaction mixtures were incubated at 40 °C for 40 min and then the absorbance was read at 525 nm. DHA was calculated as the difference between the total ascorbic acid and reduced ASC.

2.7 Total Phenolic Content (TPC)

Total phenols were determined using the Folin–Ciocalteu method, as previously described by Loi et al. (2019). Briefly, around 0.6 g were homogenized with 5 mL of ethanol and centrifuged at 6000 × *g* for 10 min at 4 °C. The supernatant (50 µL) was mixed with 950 µL of distilled water and 50 µL of a 1:1 water-diluted Folin-Ciocalteu reagent (Sigma-Aldrich, Milan, Italy). After 3 min, 100 µL of a 0.1 M NaOH solution containing 20% (W/V) Na_2CO_3 was added, and the resulting solution was incubated at 25 °C for 1 h. The total phenolic content (TPC) was determined spectrophotometrically at 760 nm and gallic acid (GA) was used as standard and the results were expressed as gallic acid equivalents (GAE) in mg g^{-1} dry weight (DW).

2.8 Levels of Hydrogen Peroxide and Lipid Peroxidation

Hydrogen peroxide (H_2O_2) content was determined spectrophotometrically according to Velikova et al. (2000), homogenizing one gram of fresh leaf tissue with 0.1% TCA, and using a calibration curve obtained with H_2O_2 standard solutions prepared in 0.1% TCA for quantification. Lipid peroxidation was measured in terms of malondialdehyde (MDA) content, following the method reported by Villani et al. (2021). Leaves were ground with four volumes of 0.1% (w/v) TCA. The homogenate was centrifuged at 12,000 × *g*, for 10 min, at 4 °C. One milliliter of the supernatant was mixed with 4 mL of 20% TCA containing 0.5% (w/v) thiobarbituric acid (TBA). Absorbance was measured at 532 and 600 nm and MDA content was calculated and expressed as nmol g^{-1} DW.

2.9 Enzymatic Activities

One gram of leaf tissue was homogenized in 50 mM Tris–HCl, pH 7.8 containing 0.3 mM mannitol, 1 mM EDTA, and 0.05% (w/v) cysteine, at 4 °C. After centrifugation for 20 min at 12,000 × *g*, at 4 °C, the supernatant was used for the determination of soluble protein content according to Bradford (1976) with serum albumin as a standard. Activities of total peroxidase (POD; EC 1.11.1.7), catalase (CAT; EC 1.11.1.6), dehydroascorbate reductase (DHAR; EC 1.8.5.1), and monodehydroascorbate reductase (MDHAR; EC 1.6.5.4) were determined according to Paciolla et al. (2008) and Loi et al. (2020).

2.10 Microbial Analysis

A total of 10 g samples was transferred to sterile stomacher bags, added with 90 mL sterile saline solution, and homogenized for 1.5 min in a stomacher (Seward, London, UK). After 20 min of incubation at room temperature, the suspension was decimally diluted. Aliquots of 100 µL were spread–plated onto count plates (Merck, Darmstad, Germany) of selective media following the manufacturers' instructions. Total Viable Count (TVC) was determined on Plate Count Agar (PCA, Difco, 30 °C, 48 h), Pseudomonas spp. on Pseudomonas Agar Base (PAB, Oxoid Ltd; 25 °C, 48 h), yeasts and fungi on Potato Dextrose Agar (PDA, Oxoid Ltd; 25 °C, 48–96 h). Microbiological counts were performed on three replicates and expressed as log cfu g^{-1} of fresh weight.

2.11 Statistical Analysis

All statistical analyses and graphical representations were performed on GraphPad Prism software version 9.0.0 for Windows, GraphPad Software, San Diego, CA, USA (www.graphpad.com). The data were examined for normality of distribution using the D'Agostino-Pearson test to determine whether they required log transformation before performing a one-way analysis of variance (ANOVA) followed by Tukey's *post–hoc* test ($p < 0.05$). Reported data are mean values out of at least three replicates of three independent experiments. The error bars in all figures represent the standard deviation of the means.

3 Results

3.1 Plant Growth

Rocket shoot fresh weight, leaf number, and area were higher in SB compared to SL growing conditions (13.9 *vs* 7.4 g/plant, 22.6 *vs* 15.6 number of leaves/plant, and 253.7 *vs* 148.9 cm^2/plant, respectively, on average) (Fig. 1). In SL conditions, the plant fresh weight increased (28%) with increasing the fertilizer dose (8.3 vs. 6.5 g/plant) (Fig. 1a). The shoot dry matter was higher in SB system (9.1 mg 100 g^{-1} of fresh weight – FW) than in SL (7.2 mg 100 g^{-1} of FW), with no differences between fertilization dose (data not shown).

3.2 Nitrate, Sulphate, and Chloride Contents

The effects of the adopted production system and the fertilization rate applied on anions content (NO_3^-, SO_4^{2-} and Cl^-) were reported in Fig. 2. The highest nitrate content was observed in SL–HF (8,478 mg kg^{-1} of FW), which was 1.4-fold higher than the maximum imposed limit of 6,000 mg kg^{-1} permitted by EC Regulation No. 1258/2011 in the harvest time between 1 April to 30 September (Fig. 2a). The other treatments showed values below the limit (5702 mg kg^{-1} of FW, on average). The fertilization rate did not modify the content of SO_4^{2-} and Cl^-, while differences were related to the cultivation system (Fig. 2b,c). The highest SO_4^{2-} value was found in the SL system (34,538 mg kg^{-1}, on average, compared to 27,849 mg kg^{-1}, on average, in SB). On average, the SO_4^{2-} concentration of rocket produced by using SL was about 24% higher than the SB system, without differences related to fertilization level. On the contrary, the SL showed the lowest Cl^- content (5785 mg kg^{-1}, on average) while the highest was found in the SB system (16,619 mg kg^{-1}, on average). In our experimental conditions, the SL–HF cultivation system led to the highest N accumulation, while comparable levels were detected for SL–LF and SB–LF. NO_3^- levels slightly exceeded regulatory limits only in SL–HF (8478 mg kg^{-1} *vs* 6000 mg kg^{-1}), while they were compliant for all other treatments.

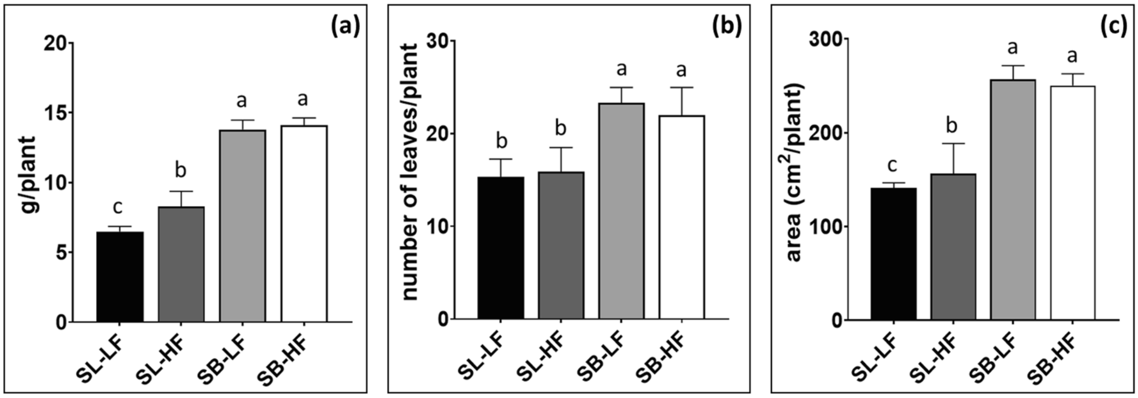

Fig. 1 Growth parameters of wild rocket leaves grown in soil (SB) and soilless (SL) treated with high– (HF) and low–input (LF) fertilization program. **a** Yield expressed as gram of fresh weight (FW)/plant; **b** Number of leaves for plant; **c** Leaf area. Vertical bars indicate ± standard errors of means of 10 measurements. Means with different lowercase letters indicate significant differences at $P < 0.05$ between different treatments, according to the Tukey's test

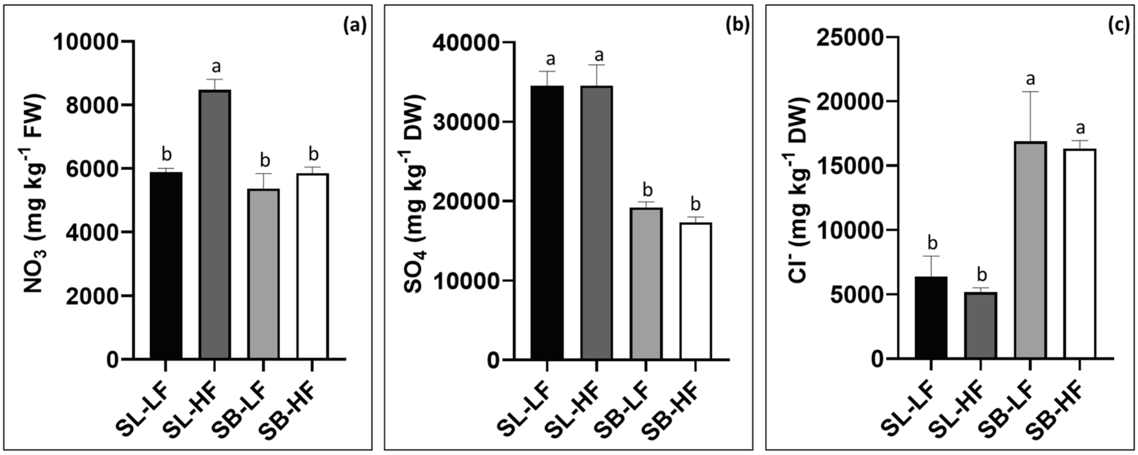

Fig. 2 Concentrations of nitrate (**a**), sulphate (**b**), and chloride (**c**) in wild rocket leaves grown in soil (SB) and soilless (SL), as influenced by high– (HF) and low–input (LF) fertilization program. FW: fresh weight; DW: dry weight. Data represent the mean (± SD) of at least 3 replicates. Means with different lowercase letters indicate significant differences at $P < 0.05$ between different treatments, according to the Tukey's test

3.3 Ascorbic Acid, Dehydroascorbic Acid and Total Phenolic Contents

The level of ASC, DHA, and ASC + DHA was higher ($P < 0.05$) in the higher nutrient–enriched soil treatment (SB–HF) compared to the other treatments (Fig. 3a). Similarly, a higher level of fertilization significantly increased the contents of ASC and ASC + DHA in the leaves grown in the SL system (SL–HF) compared with those treated with a lower concentration in the nutrient solution (SL–LF). However, DHA contents were not altered by the fertilization treatment in the SL plants (SL–HF and SL–LF). In our study, the amount of ASC (mg 100 g^{-1} FW) ranged from 60.3–69.4 (SL–LF and SL–HF, respectively) to 95–122.6 (SB–LF and SB–HF, respectively), while DHA level (mg 100 g^{-1} FW) ranged from 116.8–122.7 (SL–LF and SL–HF, respectively) to 245.5–465 (SB–LF and SB–HF, respectively) (data not shown).

In SB treatments the DHA amounts changed as a function of supplied nitrogen level, while in SL treatments it did not change (Fig. 3a). Overall, a higher concentration of nutrient solution shifted the ASC/ASC + DHA ratio towards the oxidized form compared to the SL–LF, slightly unbalancing the cell redox state (Fig. 3b).

In this study, the SL treatments showed a significantly higher phenolic content compared to SB treatments (Fig. 3c). SB–HF treatment showed the lowest phenolic content, with 39 and 34% lower TPC in comparison with SL–LF and SL–HF, respectively. Moreover, the SL treatments showed a similar TPC, independently from the nutrient solution

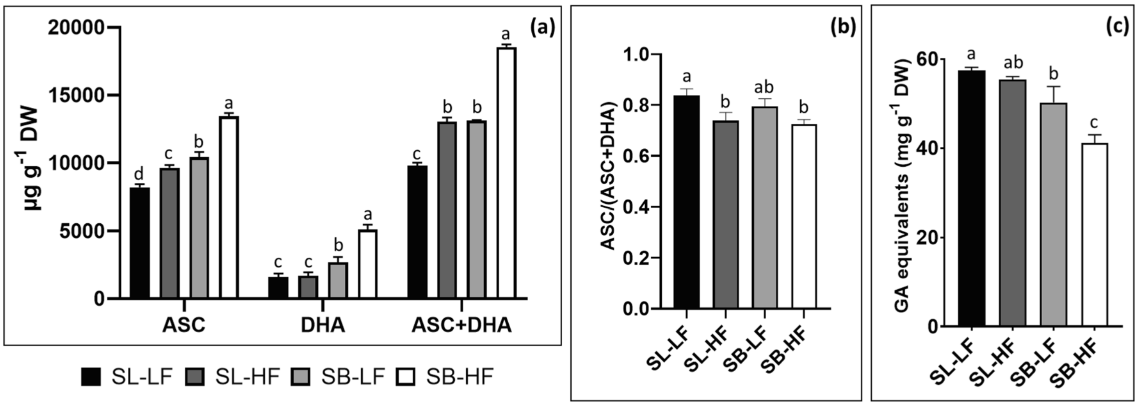

Fig. 3 **a** Ascorbic acid (ASC), dehydroascorbic acid (DHA), and total ascorbate levels (ASC + DHA), **b** ASC/DHA ratio, and **c** polyphenols content in wild rocket leaves grown in soil (SB) and soilless (SL), as influenced by high– (HF) and low–input (LF) fertilization program. DW: dry weight. Data represent the mean (± SD) of at least 3 replicates. Means with different lowercase letters indicate significant differences at $P < 0.05$ between different treatments, according to Tukey's test

applied, while between the soil rocket leaves, SB–LF showed a significant increase of TPC compared to SB–HF.

3.4 *Lipid Peroxidation and H_2O_2 Content*

The lipid peroxidation, as an indicator of oxidative stress, was measured in terms of MDA content (Fig. 4a). In the SL–HF leaves the MDA content was the highest ($P < 0.05$). Both SB treatments (SB–LF and SB–HF) showed higher levels of MDA compared to SL–LF, while no significant differences were observed between them. Similarly, H_2O_2 content increased in the soil-grown treatments compared to SL–LF (Fig. 4b). However, significant differences were observed between SB–LF and SB–HF, showing an enhanced production of H_2O_2 in the latter.

3.5 Enzymatic Activity

The trend of antioxidative activity of the enzymes examined in this study is reported in Fig. 5. The SB–LF showed the lowest CAT activity, while no significant changes were observed between soilless treatments and the SB–HF treatment (Fig. 5a). Nutrient supply dose did not affect POD activity in wild rocket plants grown in the soil (SB–LF and SB–HF), while between the soilless treatments, the increased concentration of nutrients (SL–HF) induced a higher POD activity as compared to SL–LF (Fig. 5b). DHAR and MDHAR enzymes showed a similar pattern (Fig. 5c, d). Both activities significantly increased in soilless treatments (SL–LF and SL–HF) compared to the soil-bound treatments (SB–LF and SB–HF). The different concentrations of nutrient supply only affected MDHAR, which showed a slightly increased activity in the SB–HF treatment compared to SB–LF (Fig. 5c).

3.6 Microbiological Load

Microbial loads of mesophiles, moulds, and yeasts were shown in Fig. 6. The SB treatments showed higher levels of mesophiles (3.47 ± 0.10 log cfu g^{-1} VS 2.9 ± 0.27 log cfu g^{-1}) and moulds and yeasts (3.67 ± 0.10 log cfu g^{-1} *vs* 3.1 ± 0.28 log cfu g^{-1}) count with respect to the SL counterparts. The nutrient supply level affected the microbial counts of mesophiles and moulds and yeasts only in the SL treatments, with SL–LF having lower counts than SL–HF. As regards Pseudomonas counts, no statistically significant difference emerged amongst all samples, and the contamination level was 3.27 ± 0.26 log cfu g^{-1}.

4 Discussion

The effect of the composition of the culture system on the growth parameters measured in this study was concordant with previous results obtained by Di Gioia et al. (2018), in which fresh yield of wild rock was lower in the soilless cultivation system compared to the conventional soil system. Furthermore, the fertilization dose influenced the plant's fresh weight in the SL growing conditions, showing increased yield with a higher fertilization rate applied. Similar results were obtained for basil, where increasing nitrogen fertilization was followed by a likewise increase in yield and dry biomass (Corrado et al. 2020). Based on these results, a clear effect on growth parameters can be observed for the cultivation system (SB *vs* SL), but not for

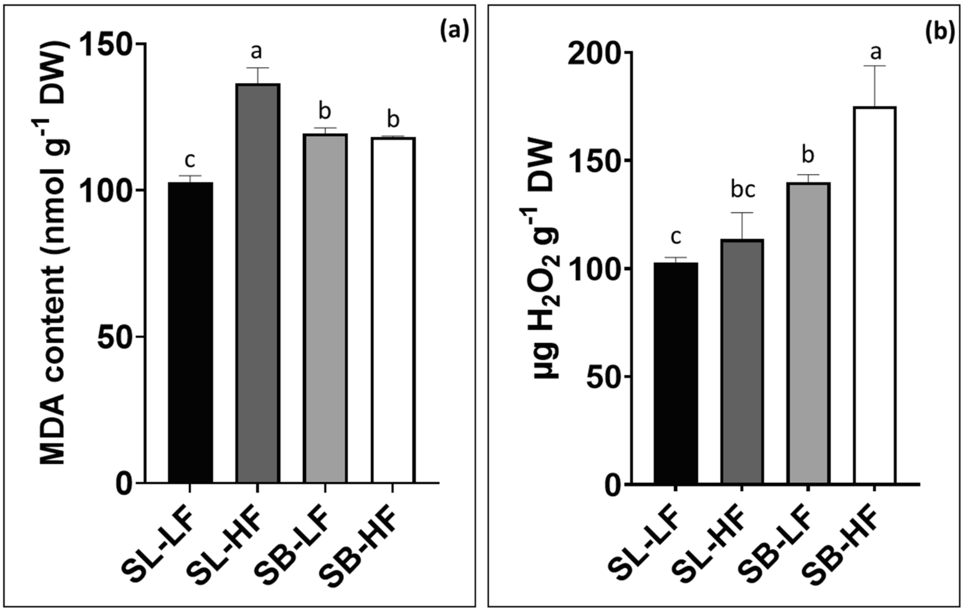

Fig. 4 **a** Malondialdehyde (MDA) and **b** hydrogen peroxide (H_2O_2) contents in wild rocket leaves grown in soil (SB) and soilless (SL), as influenced by high– (HF) and low–input (LF) fertilization program. DW: dry weight. Data represent the mean (± SD) of at least 3 replicates. Means with different lowercase letters indicate significant differences at $P < 0.05$ between different treatments, according to Tukey's test

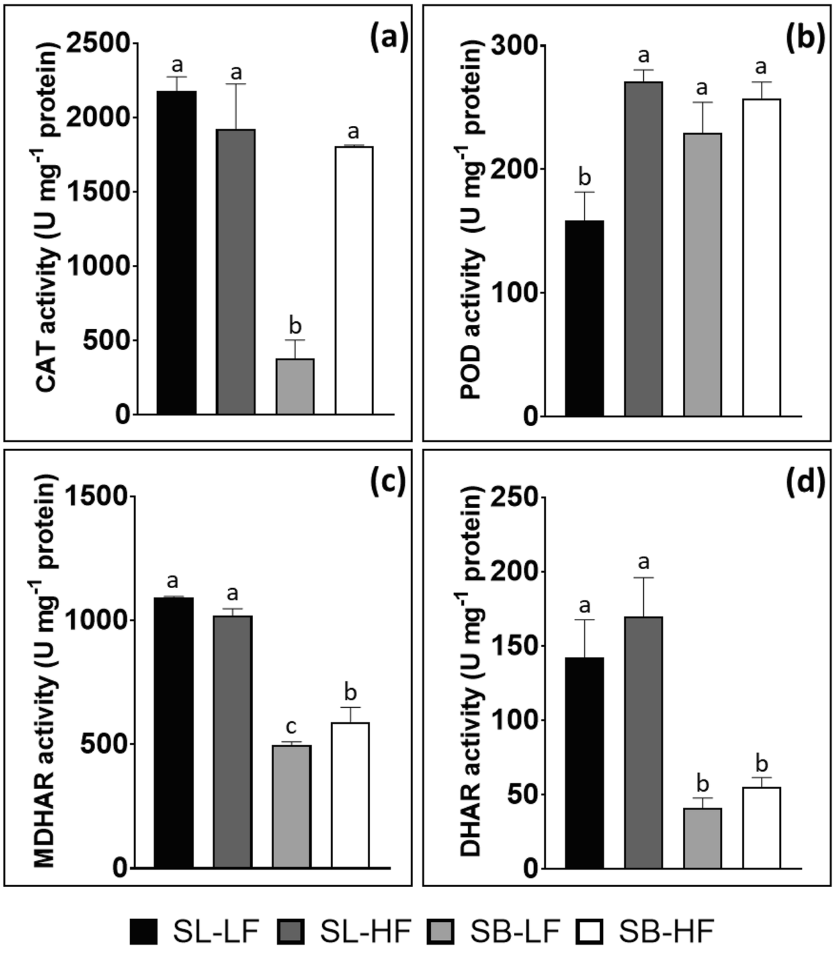

Fig. 5 Activity of the antioxidant enzymes **a** catalase, **b** total peroxidase, **c** dehydroascorbate reductase, and **d** monodehydroascorbate reductase in wild rocket leaves grown in soil (SB) and soilless (SL), as influenced by high– (HF) and low–input (LF) fertilization program. Data represent the mean (± SD) of at least 3 replicates. Means with different lowercase letters indicate significant differences at $P < 0.05$ between different treatments, according to Tukey's test

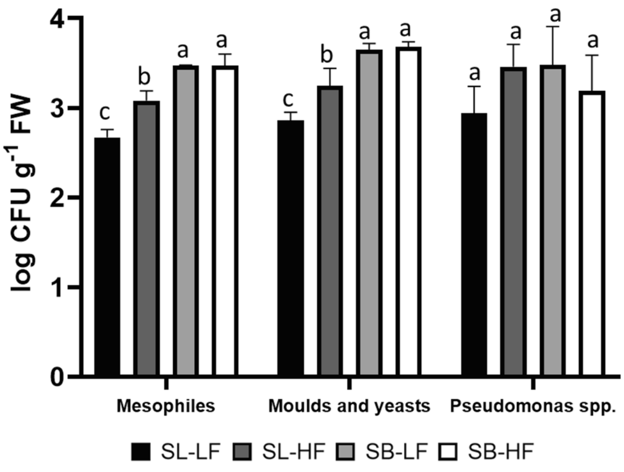

Fig. 6 Microbial loads of **a** mesophiles, **b** moulds and yeasts, and **c** Pseudomonas spp. in wild rocket leaves grown in soil (SB) and soilless (SL), as influenced by high– (HF) and low–input (LF) fertilization program. Data represent the mean (± SD) of at least 3 replicates. Means with different lowercase letters indicate significant differences at $P < 0.05$ between different treatments, according to Tukey's test

N supplementation. This suggests that it is possible to use low levels of N supply in both SB and SL rocket production, avoiding a detrimental NO_3^- accumulation.

NO_3^- content is a crucial parameter to be evaluated, especially in high nitrate–accumulating vegetables like *D. tenuifolia*, to ensure compliance with the maximum imposed limits (EC Regulation No. 1258/2011). In this study, NO_3^- levels slightly exceeded regulatory limits only in SL–HF, whereas in SB cultivation, N supplementation was not followed by a likewise NO_3^- increase, as reported for lettuce by Becker et al. (2015), underlining a differential capacity of using N in the low and high fertilization conditions. The results obtained in this study are in accordance with literature data and explained by the fact that in SL conditions NO_3^- is more available to the plants than in SB, allowing an easier absorption in leaves (Colla et al. 2018; Nascimento 2019) . Interestingly, using low fertigation levels in SL allowed us to reach comparable NO_3^- levels with respect to those registered in both SB–LF and SB–HF, with a slight reduction in plant productivity and comparable leaf area and length.

NO_3^- and Cl^- are important osmoregulatory molecules, which regulate charge balance, turgor, cell volume, and growth. Being monovalent ions and similar physical properties, they share ion transport mechanisms (Corrado et al. 2020). Consistently, NO_3^- and Cl^- contents showed an opposite trend in wild rocket samples. The differences in sulphate content were related to the different cultivation systems, rather than to the level of N fertigation. As reported for NO_3^-, the greater availability of sulphur in hydroponic compared to soil-bound cultivation can explain the results obtained. Accordingly, Di Gioia and colleagues (2018) observed the same trend in hydroponic compared to soil-bound rocket cultivation.

To assess how growing conditions and fertilization dose affect the ascorbic acid system and its redox state, reduced (ASC) and oxidized (DHA) forms of ascorbic acid were analyzed and the relative ratio ASC/ASC + DHA was calculated. Overall, the content of ASC obtained in this study were consistent with those reported for wild rocket by other researchers (Hamilton and Fonseca 2010; Guijarro–Real et al. 2019). Although there is evidence-based literature to support the down-accumulation of some bioactive compounds by increasing nitrogen supply (Albornoz 2016; Kolega et al. 2020), the effect of nutrient solution on ASC content seems not to be linear. Indeed, several other factors may affect the production of ascorbic acid, such as the species and even the cultivar, the growth stage and system, the harvest date, the light intensity, the growing season, biotic and abiotic stress (El–Nakhel et al. 2019; Machado et al. 2020). Furthermore, the lower content of ASC in the SL system may be due to the lower oxidative stress assured by the timely availability of nutrients and water.

The results of this study revealed that TPC was significantly higher in SL compared to SB treatments. Moreover, higher fertilizer doses in SB treatments determined a strong decrease in total phenols levels. Since both the applied growing systems were subjected to the same conditions (temperature, relative humidity, and lighting), the key factors that may have led to such outcomes could have been the nutrient availability and the cultivation system. Our results corroborate previous findings showing a significant increase in phenolic compounds elicited by low concentration of available nutrients (El–Nakhel et al. 2019; Naikoo et al. 2019; Pannico et al. 2020; Sgherri et al. 2010). As wild rockets grown in soil showed greater biomass compared to the soilless treatments, this evidence could be explained by the hypotheses of the protein competition model (PCM) according to which biomass accumulation and secondary metabolites are inversely related (Jones and Hartley 1999; Nybakken et al. 2018). In particular, both protein synthesis and the phenylpropanoid pathways are catalyzed by phenylalanine ammonia-lyase that uses phenylalanine as a precursor. Therefore, an increased protein demand for growth preferentially may convey the phenylalanine into protein synthesis rather than toward the phenolic biosynthetic pathway.

The level of lipid peroxidation follows a pattern similar to that of the nitrate concentration. Although exogenous nitrate nutrition did not affect the level of MDA, its accumulation in leaf tissue seems to have adversely affected the oxidative stress. The results of the current study are consistent with those reported in the literature (Wei et al. 2009; Zervoudakis et al. 2015), showing enhanced MDA content at high N levels in leaves of basil (*Ocimum basilicum* L.) and eggplant (*Solanum melongena* L.), respectively. Moreover, several studies have shown that exists a positive correlation between plant infections and changes in lipid peroxidation and ROS concentration (de Dios 2019; Lanubile et al. 2015). Therefore, the enhanced H_2O_2 and MDA content in soil–bound leaves might be due to the significant increase in microbial biomass (in terms of mesophiles and moulds) observed compared to the SL–LF treatment. The results of our study clearly show that the soilless growing system triggered the induction of H_2O_2 in rocket leaves to a much less extent than the SB system. The enhanced production of H_2O_2 in the SB treatments may act as a signal to activate stress response pathways such as ascorbic acid.

The findings of the present study highlighted that the activity of antioxidant enzymes, including CAT, POD, and two enzymes belonging to the ascorbate–glutathione cycle (DHAR and MDHAR) was modulated by the growing treatments. Interestingly, the POD enzymes, which have antioxidative properties, have been more relevant in decreasing H_2O_2 accumulation and maintaining cell membrane integrity, compared to CAT. Indeed, although CAT is a powerful antioxidant metalloenzyme, enzymes displaying peroxidase activity, work synergistically with catalase to scavenge H_2O_2, even at low concentrations, due to their higher affinity for H_2O_2 (lower K_M) (Palma et al. 2020; Tuzet et al. 2019; Villani et al. 2021). Furthermore, the ascorbate–glutathione pathway has long been considered to play a central role in H_2O_2 scavenging in plants (Ding et al. 2020). Previous studies have investigated and discussed the molecular properties of MDHAR and DHAR and the importance of the latter in coupling the ASC and glutathione (GSH) pools to H_2O_2 metabolism and signaling (Ding et al. 2020; Loi et al. 2020; Rahantaniaina et al. 2017). DHAR catalyzes the reduction of DHA to ASC and it has been considered to be important in maintaining the balanced ascorbic acid pool and its redox state, both in unstressed and in stressful conditions (Ding et al. 2020). Our results support this evidence showing significantly lower levels of DHA in the SL treatments compared to the SB treatments, due to higher DHAR activity.

As expected, the microbial loads of mesophiles, moulds, and yeasts were significantly affected by the growth system and nutrient solution applied. Microbial counts for SL cultivation were lower than those of SB-grown rockets. The range was 3.3–3.7 log cfu g^{-1}, in accordance with or lower than those reported in other papers for rocket and baby leaves (Selma et al. 2012; Sirsat and Neal 2013; Xylia et al. 2021). Accordingly, SL cultivation proved to positively affect the microbiological parameters and reduce the increase of microbiological load also in other fresh-cut produce like lettuce (Pace et al. 2018; Selma et al. 2012; Sirsat and Neal 2013) and table grape (Cefola et al. 2011).

5 Conclusions

This study evaluated for the first time the effect of different culture systems and composition of the nutrient solutions on growth, antioxidant components, quality, and mineral content in *D. tenuifolia* cv Dallas. This cultivar responded strongly to the cultivation method. Soil-bound cultivation led to higher fresh weight, leaves number and area, ascorbate, and chloride levels. Conversely, soilless cultivation showed a higher accumulation of sulphates, total phenols, and increased activity of the antioxidant enzymes dehydroascorbate reductase and monodehydroascorbate reductase, while lower hydrogen peroxide levels and microbial loads were detected. The nutrient solution concentration had little or no influence on the plant growth, the activity of antioxidant enzymes, and the content of sulphate and chloride, while it affected the levels of ascorbate, total phenols, nitrate, hydrogen peroxide, and lipid peroxidation. Overall, in soilless cultivation, the effect of higher nutrient supply on the assayed parameters was generally more pronounced than in soil-bound cultivation, confirming the hypothesis that the low-input fertilization rate improved the nutritional quality of *D. tenuifolia* cv Dallas without eliciting nitrate accumulation. These findings provide useful new information for soil and soilless fertilization and growth management of *D. tenuifolia* cv Dallas and for further research aimed at verifying such responses on a broader selection of wild rocket cultivars and elucidating them more in-depth at transcriptomic and proteomic levels.

Acknowledgements This work was supported by the University of Bari Aldo Moro, grant number H96J15001610005 and by Research for Innovation (REFIN)—POR PUGLIA FESR-FSE 2014/2020—codice progetto 7B942221—CUP H94I20000410008.

Author Contribution All authors have made substantial contributions to the conception and design of the study, acquisition of data, or analysis and interpretation of data as follows. Conceptualization: A.V., M.L., F.S., and C.P. Investigation: A.V., M.L., M.D.I., and S.D.L. Formal analysis: A.V., M.L., and M.D.I. Writing – Original Draft: A.V. and M.L. Writing—Review & Editing: A.V., M.L., M.D.I., F.F.M., F.S., G.M., S.D.L, and C.P. Visualization: A.V. All authors read and approved the manuscript.

Funding Open access funding provided by Università degli Studi di Bari Aldo Moro within the CRUI-CARE Agreement.

Declarations

Conflict of Interest The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Aires A, Rosa E, Carvalho R (2006) Effect of nitrogen and sulfur fertilization on glucosinolates in the leaves and roots of broccoli sprouts (*Brassica oleracea* var. *italica*). J Sci Food Agric 86:1512–1516. https://doi.org/10.1002/jsfa.2535

Albornoz F (2016) Crop responses to nitrogen overfertilization: a review. Sci Hort 205:79–83. https://doi.org/10.1016/j.scienta.2016.04.026

Amtmann A, Armengaud P (2009) Effects of N, P, K and S on metabolism: new knowledge gained from multi–level analysis. Curr Opin Plant Biol 12:275–283. https://doi.org/10.1016/j.pbi.2009.04.014

Aulakh MS, Malhi SS (2004) Fertiliser nitrogen use efficiency as influenced by interactions with other nutrients. In: Mosier AR, Syers JK, Freney JR (eds) Agriculture and the nitrogen cycle: assessing impacts of fertilizer use on food production and the environment. Island Press, Washington, DC, USA, pp 181–191

Aulakh MS, Malhi SS (2005) Interactions of nitrogen with other nutrients and water: effect on crop yield and quality, nutrient use efficiency, carbon sequestration, and environmental pollution. Adv Agron 86:341–409. https://doi.org/10.1016/S0065-2113(05)86007-9

Becker C, Urlić B, JukićŠpika M, Kläring HP, Krumbein A, Baldermann S, Ban SG, Perica S, Schwarz D (2015) Nitrogen limited red and green leaf lettuce accumulate flavonoid glycosides, caffeic acid derivatives, and sucrose while losing chlorophylls, β–carotene and xanthophylls. PLoS ONE 10:e0142867. https://doi.org/10.1371/journal.pone.0142867

Bénard C, Gautier H, Bourgaud F, Grasselly D, Navez B, Caris-Veyrat C, Weiss M, Génard M (2009) Effects of low nitrogen supply on tomato (*Solanum lycopersicum*) fruit yield and quality with special emphasis on sugars, acids, ascorbate, carotenoids, and phenolic compounds. J Agric Food Chem 57:4112–4123. https://doi.org/10.1021/jf8036374

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal Biochem 72:248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Cefola M, Pace B, Buttaro D, Santamaria P, Serio F (2011) Postharvest evaluation of soilless-grown table grape during storage in modified atmosphere. J Sci Food Agric 91:2153–2159. https://doi.org/10.1002/jsfa.4432

Colla G, Kim HJ, Kyriacou MC, Rouphael Y (2018) Nitrate in fruits and vegetables. Sci Hortic 237:221–238. https://doi.org/10.1016/j.scienta.2018.04.016

Corrado G, Formisano L, De Micco V, Pannico A, Giordano M, El-Nakhel C, Chiaiese P, Sacchi R, Rouphael Y (2020) Understanding the morpho–anatomical, physiological, and functional response of sweet basil to isosmotic nitrate to chloride ratios. Biology 9:158. https://doi.org/10.3390/biology9070158

D'Imperio M, Montesano FF, Montemurro N, Parente A (2021) Posidonia natural residues as growing substrate component: an ecofriendly method to improve nutritional profile of brassica microgreens. Front Plant Sci 12:580596. https://doi.org/10.3389/fpls.2021.580596

de Dios Alché J (2019) A concise appraisal of lipid oxidation and lipoxidation in higher plants. Redox Biol 23:101136. https://doi.org/10.1016/j.redox.2019.101136

de Bang TC, Husted S, Laursen KH, Persson DP, Schjoerring JK (2021) The molecular–physiological functions of mineral macronutrients and their consequences for deficiency symptoms in plants. New Phytol 229:2446–2469. https://doi.org/10.1111/nph.17074

Di Gioia F, Avato P, Serio F, Argentieri MP (2018) Glucosinolate profile of Eruca sativa, Diplotaxis tenuifolia and Diplotaxis erucoides grown in soil and soilless systems. J Food Compost Anal 69:197–204. https://doi.org/10.1016/j.jfca.2018.01.022

Ding H, Wang B, Han Y, Li S (2020) The pivotal function of dehydroascorbate reductase in glutathione homeostasis in plants. J Exp Bot 71:3405–3416. https://doi.org/10.1093/jxb/eraa107

Duncan EG, O'Sullivan CA, Roper MM, Biggs JS, Peoples MB (2018) Influence of co–application of nitrogen with phosphorus, potassium and sulphur on the apparent efficiency of nitrogen fertiliser use, grain yield and protein content of wheat. Field Crops Res 226:56–65. https://doi.org/10.1016/j.fcr.2018.07.010

EFSA- European Food Safety Authority (2008) Opinion of the scientific panel on contaminants in the food chain on a request from the European Commission to perform a scientific risk assessment on nitrate in vegetables. EFSA J 6:689. https://doi.org/10.2903/j.efsa.2008.689

El-Nakhel C, Pannico A, Kyriacou MC, Giordano M, De Pascale S, Rouphael Y (2019) Macronutrient deprivation eustress elicits differential secondary metabolites in red and green-pigmented butterhead lettuce grown in a closed soilless system. J Sci Food Agric 99:6962–6972. https://doi.org/10.1002/jsfa.9985

Evans LT, Fischer RA (1999) Yield potential: its definition, measurement, and significance. Crop Sci 39:1544–1551. https://doi.org/10.2135/cropsci1999.3961544x

Ewert F, Rounsevell MDA, Reginster I, Metzger MJ, Leemans R (2005) Future scenarios of European agricultural land use: I. Estimating changes in crop productivity. Agric Ecosyst Environ 107:101–116. https://doi.org/10.1016/j.agee.2004.12.003

Flores P, Navarro JM, Garrido C, Rubio JS, Martínez V (2004) Influence of Ca^{2+}, K^+ and NO^{3-} fertilisation on nutritional quality of pepper. J Sci Food Agric 84:569–574. https://doi.org/10.1002/jsfa.1694

Fritz C, Palacios-Rojas N, Feil R, Stitt M (2006) Regulation of secondary metabolism by the carbon–nitrogen status in tobacco: nitrate inhibits large sectors of phenylpropanoid metabolism. Plant J 46:533–548. https://doi.org/10.1111/j.1365-313X.2006.02715.x

Gorban AN, Pokidysheva LI, Smirnova EV, Tyukina TA (2011) Law of the minimum paradoxes. Bull Math Biol 73:2013–2044. https://doi.org/10.1007/s11538-010-9597-1

Guijarro-Real C, Adalid-Martínez AM, Aguirre K, Prohens J, Rodríguez-Burruezo A, Fita A (2019) Growing conditions affect the phytochemical composition of edible wall rocket (*Diplotaxis erucoides*). Agronomy 9:858. https://doi.org/10.3390/agronomy9120858

Hall M, Jobling J, Rogers G (2012) The germination of perennial wall rocket (Diplotaxis tenuifolia [L.] DC.) and annual garden rocket (Eruca sativa Mill.) under controlled temperatures. Plant Breed Seed Sci 65:15–28 (http://ojs.ihar.edu.pl/index.php/pbss/article/view/328)

Hall MK, Jobling JJ, Rogers GS (2015) Effect of nitrogen supply and storage temperature on vitamin C in two species of baby leaf rocket, and the potential of these crops for a nutrient claim in Australia. J Plant Nutr 38:246–259. https://doi.org/10.1080/01904167.2013.873465

Hamilton JM, Fonseca JM (2010) Effect of saline irrigation water on antioxidants in three hydroponically grown leafy vegetables: *Diplotaxis tenuifolia*, *Eruca sativa*, and *Lepidium sativum*. HortScience 45:546–552. https://doi.org/10.21273/HORTSCI.45.4.546

Hoagland DR, Arnon DI (1950) The water culture method for growing plants without soil. California Agr Expt Sta 347:1–32

Hoque MM, Ajwa H, Othman M (2010) Yield and postharvest quality of lettuce to response to nitrogen, phosphorus, and potassium fertilizers. HortScience 10:1539–1544. https://doi.org/10.21273/HORTSCI.45.10.1539

Jones CG, Hartley SE (1999) A protein competition model of phenolic allocation. Oikos 86:27–44. https://doi.org/10.2307/3546567

Keller M, Hrazdina G (1998) Interaction of nitrogen availability during bloom and light intensity during veraison. II. Effects on anthocyanin and phenolic development during grape ripening. Am J Enol Vitic 49:341–349

Kolega S, Miras-Moreno B, Buffagni V, Lucini L, Valentinuzzi F, Maver M, Mimmo T, Trevisan M, Pii Y, Cesco S (2020) Nutraceutical profiles of two hydroponically grown sweet basil cultivars as affected by the composition of the nutrient solution and the inoculation with *Azospirillum brasilense*. Front Plant Sci 11:1683. https://doi.org/10.3389/fpls.2020.596000

Krouk G, Kiba T (2020) Nitrogen and Phosphorus interactions in plants: from agronomic to physiological and molecular insights. Curr Opin Plant Biol 57:104–109. https://doi.org/10.1016/j.pbi.2020.07.002

Lanubile A, Maschietto V, De Leonardis S, Battilani P, Paciolla C, Marocco A (2015) Defense responses to mycotoxin–producing fungi Fusarium proliferatum, F. subglutinans, and *Aspergillus flavus* in kernels of susceptible and resistant maize genotypes. Mol Plant Microbe Interact 28:546–557. https://doi.org/10.1094/MPMI-09-14-0269-R

Law MY, Charles SA, Halliwell B (1983) Glutathione and ascorbic acid in spinach chloroplasts: the effect of hydrogen peroxide and of paraquat. Biochem J 210:899–903. https://doi.org/10.1042/bj2100899

Li S, Schonhof I, Krumbein A, Li L, Stützel H, Schreiner M (2007) Glucosinolate concentration in turnip (*Brassica rapa* ssp. *rapifera* L.) roots as affected by nitrogen and sulfur supply. J Agric Food Chem 55:8452–8457. https://doi.org/10.1021/jf070816k

Loi M, Liuzzi VC, Fanelli F, De Leonardis S, Creanza TM, Ancona N, Paciolla C, Mulè G (2019) Effect of different light–emitting diode (LED) irradiation on the shelf life and phytonutrient content of broccoli (*Brassica oleracea* L. var. *italica*). Food Chem 283:206–214. https://doi.org/10.1016/j.foodchem.2019.01.021

Loi M, De Leonardis S, Mulè G, Logrieco AF, Paciolla C (2020) A novel and potentially multifaceted dehydroascorbate reductase increasing the antioxidant systems is induced by beauvericin in tomato. Antioxidants 9:435–449. https://doi.org/10.3390/antiox9050435

López-Arredondo DL, Sánchez-Calderón L, Yong-Villalobos L (2017) Molecular and genetic basis of plant macronutrient use efficiency: concepts, opportunities, and challenges. In: Hossain MA, Kamiya T, Burritt DY, Phan Tran LS, Fujiwara T (eds) Plant macronutrient use efficiency molecular and genomic perspectives in crop plants. Academic Press, Cambridge, MA, USA, pp 1–29. https://doi.org/10.1016/B978–0–12–811308–0.00001–6

Machado RMA, Alves-Pereira I, Lourenço D, Ferreira RMA (2020) Effect of organic compost and inorganic nitrogen fertigation on spinach growth, phytochemical accumulation and antioxidant activity. Heliyon 6:05085. https://doi.org/10.1016/j.heliyon.2020.e05085

Mahlangu RIS, Maboko MM, Sivakumar D, Soundy P, Jifon J (2016) Lettuce (*Lactuca sativa* L.) growth, yield and quality response to nitrogen fertilization in a non–circulating hydroponic system. J Plant Nutr 39:1766–1775. https://doi.org/10.1080/01904167.2016.1187739

Martínez-Sánchez A, Marín A, Llorach R, Ferreres F, Gil MI (2006) Controlled atmosphere preserves quality and phytonutrients in wild rocket (*Diplotaxis tenuifolia*). Postharvest Biol Technol 40:26–33. https://doi.org/10.1016/j.postharvbio.2005.12.015

Mozafar A (1993) Nitrogen fertilizers and the amount of vitamins in plants: a review. J Plant Nutr 16:2479–2506. https://doi.org/10.1080/01904169309364698

Naikoo MI, Dar MI, Raghib F, Jaleel H, Ahmad B, Raina A, Khan FA, Naushin F (2019) Role and regulation of plants phenolics in abiotic stress tolerance: an overview. In: Khan MIR, Reddy PS, Ferrante A, Khan NA (eds) Plant signaling molecules: Role and Regulation under Stressful Environments, Elsevier Inc, United Kingdom. pp 157–168. https://doi.org/10.1016/B978–0–12–816451–8.00009–5

Nascimento AL (2019) Nitrate and nitrite in commercial samples of conventional, organic and hydroponic leafy vegetables. Emir J Food Agric 31:812–817. https://doi.org/10.9755/ejfa.2019.v31.i10.2013

Nybakken L, Lie MH, Julkunen-Tiitto R, Asplund J, Ohlson M (2018) Fertilization changes chemical defense in needles of mature Norway spruce (*Picea abies*). Front Plant Sci 9:770. https://doi.org/10.3389/fpls.2018.00770

Pace B, Capotorto I, Gonnella M, Baruzzi F, Cefola M (2018) Influence of soil and soilless agricultural growing system on postharvest quality of three ready–to–use multi–leaf lettuce cultivars. Adv Hortic Sci 32:353–362. https://doi.org/10.13128/ahs-21927

Paciolla C, Ippolito MP, Logrieco A, Dipierro N, Mule G, Dipierro S (2008) A different trend of antioxidant defence responses makes tomato plants less susceptible to beauvericin than to T–2 mycotoxin phytotoxicity. Physiol Mol Plant Patho 72:3–9. https://doi.org/10.1016/j.pmpp.2008.06.003

Palma JM, Mateos RM, López-Jaramillo J, Rodríguez-Ruiz M, González-Gordo S, Lechuga-Sancho AM, Corpas FJ (2020) Plant catalases as NO and H2S targets. Redox Biol. 34:101525. https://doi.org/10.1016/j.redox.2020.101525

Pannico A, Graziani G, El-Nakhel C, Giordano M, Ritieni A, Kyriacou MC, Rouphael Y (2020) Nutritional stress suppresses nitrate content and positively impacts ascorbic acid concentration and phenolic acids profile of lettuce microgreens. Italus Hortus 27:41–52. https://doi.org/10.26353/j.itahort/2020.3.4152

Rahantaniaina MS, Li S, Chatel-Innocenti G, Tuzet A, Issakidis Bourguet E, Mhamd A, Noctor G (2017) Cytosolic and chloroplastic DHARs cooperate in oxidative stress–driven activation of the salicylic acid pathway. Plant Physiol 174:956–971. https://doi.org/10.1104/pp.17.00317

Rajasree G, Pillai GR (2012) Effect of nitrogen nutrition on fruit quality and shelf life of cucurbitaceous vegetable bitter gourd. J Plant Nutr 35:1139–1153. https://doi.org/10.1080/01904167.2012.676127

Renna M, D'Imperio M, Maggi S, Serio, F (2022) Soilless biofortification, bioaccessibility, and bioavailability: signposts on the path to personalized nutrition. Front Nutr 9:966018. https://doi.org/10.3389/fnut.2022.966018

Saini RK, Ko EY, Keum YS (2017) Minimally processed ready–to–eat baby–leaf vegetables: Production, processing, storage, microbial safety, and nutritional potential. Food Rev Int 33:644–663. https://doi.org/10.1080/87559129.2016.1204614

Santamaria P (2006) Nitrate in vegetables: toxicity, content, intake and EC regulation. J Sc Food Agric 86:10–17. https://doi.org/10.1002/jsfa.2351

Schiattone MI, Candido V, Cantore V, Montesano FF, Boari F (2017) Water use and crop performance of two wild rocket genotypes under salinity conditions. Agric Water Manag 194:214–221. https://doi.org/10.1016/j.agwat.2017.09.009

Selma MV, Luna MC, Martínez-Sánchez A, Tudela JA, Beltrán D, Baixauli C, Gil MI (2012) Sensory quality, bioactive constituents and microbiological quality of green and red fresh–cut lettuces (*Lactuca sativa* L.) are influenced by soil and soilless agricultural production systems. Postharvest Biol Technol 63:16–24. https://doi.org/10.1016/j.postharvbio.2011.08.002

Sgherri C, Cecconami S, Pinzino C, Navari-Izzo F, Izzo R (2010) Levels of antioxidants and nutraceuticals in basil grown in hydroponics and soil. Food Chem 123:416–422. https://doi.org/10.1016/j.foodchem.2010.04.058

Sirsat SA, Neal JA (2013) Microbial profile of soil–free versus in–soil grown lettuce and intervention methodologies to combat pathogen surrogates and spoilage microorganisms on lettuce. Foods 2:488–498. https://doi.org/10.3390/foods2040488

Stitt M (1999) Nitrate regulation of metabolism and growth. Curr Opin Plant Biol 2:178–186. https://doi.org/10.1016/S1369-5266(99)80033-8

Tuzet A, Rahantaniaina MS, Noctor G (2019) Analyzing the function of catalase and the ascorbate–glutathione pathway in H_2O_2 processing: Insights from an experimentally constrained kinetic model. Antioxid Redox Signal 30:1238–1268. https://doi.org/10.1089/ars.2018.7601

Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Sci 151:59–66. https://doi.org/10.1016/S0168-9452(99)00197-1

Villani A, Tommasi F, Paciolla C (2021) The arbuscular mycorrhizal fungus glomus viscosum improves the tolerance to verticillium wilt in artichoke by modulating the antioxidant defense systems. Cells 10:944. https://doi.org/10.3390/cells10081944

Wei GP, Yang LF, Zhu YL, Chen G (2009) Changes in oxidative damage, antioxidant enzyme activities and polyamine contents in leaves of grafted and non–grafted eggplant seedlings under stress by excess of calcium nitrate. Sci Hortic 120:443–451. https://doi.org/10.1016/j.scienta.2008.12.009

Wilkens RT, Spoerke JM, Stamp NE (1996) Differential responses of growth and two soluble phenolics of tomato to resource availability. Ecology 77:247–258. https://doi.org/10.2307/2265674

Wink M (2013) Evolution of secondary metabolites in legumes (Fabaceae). S Afr J Bot 89:164–175. https://doi.org/10.1016/j.sajb.2013.06.006

Xylia P, Botsaris G, Skandamis P, Tzortzakis N (2021) Expiration date of ready–to–eat salads: effects on microbial load and biochemical attributes. Foods 10:941. https://doi.org/10.3390/foods10050941

Yang T, Samarakoon U, Altland J, Ling P (2021) Photosynthesis, biomass production, nutritional quality, and flavor–related phytochemical properties of hydroponic–grown arugula (Eruca sativa Mill.) 'standard' under different electrical conductivities of nutrient solution. Agronomy 11:1340. https://doi.org/10.3390/agronomy11071340

Zervoudakis G, Salahas G, Rod M (2015) Nitrogen nutrition effect on aeroponic basil (Ocimum basilicum L.) catalase and lipid peroxidation. Not Bot Horti Agrobo 43:561–567. https://doi.org/10.15835/nbha43210181

Zhao D, Reddy KR, Kakani VG, Reddy VR (2005) Nitrogen deficiency effects on plant growth, leaf photosynthesis, and hyperspectral reflectance properties of sorghum. Eur J Agron 22:391–403. https://doi.org/10.1016/j.eja.2004.06.005

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.